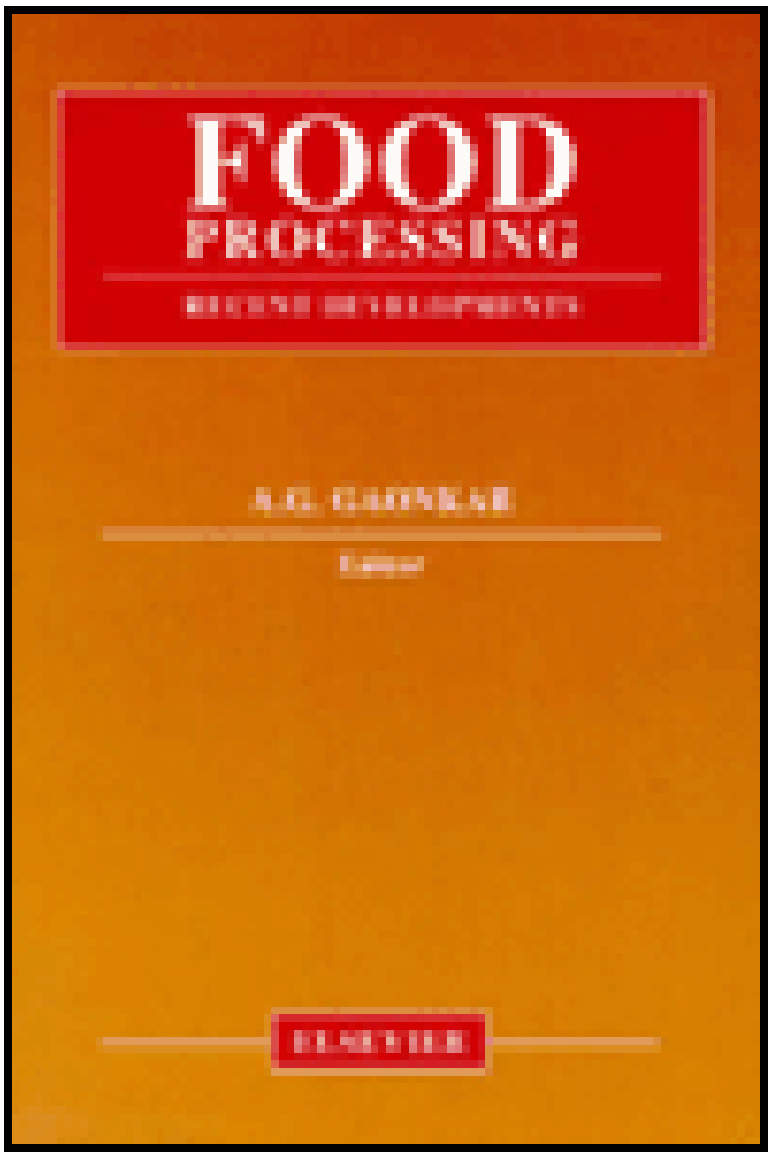
Food Processing: Recent Developments

by Anilkumar G. Gaonkar



- ISBN: 0444815007
- Pub. Date: January 1995
- Publisher: Elsevier Science & Technology Books

Foreword

The overall goal of the food processing industry is to convert raw materials into high valued finished products at the lowest cost. Due to significant variations in raw material properties, the main challenge of the food process engineer is to produce a final product of consistent quality. It is extremely important that the process engineer be aware of the latest scientific advances that would allow for the production of the highest quality products. This book, "Food Processing: Recent Developments" edited by Anilkumar Gaonkar masterfully brings together the most recent concepts in thermal conversions, food component separation and food process sensing and control.

Accurate and rapid methods to measure food product characteristics are crucial for both industrial and research applications. Many new process sensor and control systems are being developed to characterize everything from raw ingredients through to the packaged product. Nuclear Magnetic Resonance (NMR), ultrasonics, and X-rays diffraction have great potential for the sensing and control of food processes. NMR has emerged from the medical field to have eventual widespread use in the food industry. Not only can NMR be used to measure the rates of crystallization, freezing, and diffusion processes but also to monitor flow profiles during heating, cooling and extrusion processes. Currently, NMR is used for experimental applications but in the near future NMR will emerge as a rapid, versatile, and non destructive technique for a wide range of on-line process and quality control applications. Ultrasonic techniques also have enormous potential for use as foreign body detection, temperature, flow, and composition measurements. X-ray techniques have been successfully used to measure rapid and long term physical and chemical changes in foods. Although current equipment is expensive and further research is necessary, major advances are being made that will make on-line sensing and control of a wide range of products and processes possible in the near future.

Selecting the correct separation process is an important part of developing optimal food processes and products. Selective removal of food components can be used to develop higher valued products, at minimal loss during processing. Chromatography, extraction and membrane processes provide some of the most innovative and cost effective methods for separating and concentrating food and biological components. Membrane processes provide gentle separation at low temparature in both aqueous and non-aqueous and in reactive and non reactive situations. Future applications can include altering composition and also microbial removal. Chromatographic methods are important for selective

removed and purification of enzymes and other biological material. Extraction and reverse micell methods are also excellent techniques to remove active constituents and also to remove undesirable constituents. Future advances are expected in the use of aqueous two-phase extraction methods. Major advances are needed in separation systems to lower the final process cost, to develop methods for scaling up to high volume operations and to facilitate complex highly variable raw materials.

Thermal processing methods are the main stay of food preservation. The use of high pressure, radiation, freezing, extrusion and aseptic methods lead to products of unique properties. High pressure is a promising method that has the main advantage of inactivating enzymes and microorganism without the destruction of nutrients and without changing flavor and taste. Bacterial spores can also be killed at high pressures when the product temperature is elevated to 60°C. Aseptic processing through the use of food has advanced from liquids only to particulates through the use of electrical heating methods and through the use of new advances in pump and tubular heat exchange design. Ionizing radiation can be successfully applied to a number of foods without negative effects. However, widespread acceptance has been curtailed by the general public's reluctance to accept the use of "nuclear energy" for human consumption. Freezing of foods has also been successfully used to preserve foods for decades. However, the new freezing technologies will be based on increasing process efficiency and minimizing environmental concerns. Extrusion is the one of the most widely used techniques to change the form and texture of solid foods. By combining heat and pressure to transform raw materials into endless variety of products. Although the thermal methods can be used by themselves to preserve foods, several of the methods are more successful when used in combination. By the same token, not all methods of preservation are applicable to all foods. Therefore, careful study of all methods is necessary.

The complexity of foods demand a wide variety of processing methods to produce products of high quality. The food process engineer must understand and continue to be aware of the latest advances in processing techniques. This book provides an excellent source of information to help maintain the competitive advantage.

Martin R. Okos
Professor of Biochemical
and Food Process Engineering
Purdue University
West Lafayette, IN 47907

Preface

Rapid and continued developments in various branches of science and technology led to considerable improvements in food processing methods. The new processing technologies contributed to enhancement in the quality and acceptability of foods.

The aim of this book is to assemble, for a handy reference, new developments pertaining to selected food processing technologies. The book contains invited chapters contributed by scientists actively involved in research, most of whom have made notable contributions to the advancement of knowledge in their field of expertise.

It is not possible to cover all the technologies for processing foods critically and systematically in a single volume. Food processing methods covered in this book include: NMR imaging, on-line NMR, on-line sensors, ultrasonics, synchrotron radiation to study fast events, membrane processing, bioseparation, high pressure processing, aseptic processing, irradiation, freezing, extrusion and extraction technologies.

This book, adequately referenced and illustrated with numerous figures and tables, is a valuable reference for scientists/engineers/technologists in industries and government laboratories involved in food processing, food research and/or development, and also for faculty, advanced undergraduate, graduate and postgraduate students from the Food Science, Food Engineering, and Agricultural Engineering departments.

I wish to thank all the contributing authors for their dedication, hard work and cooperation and the reviewers for valuable suggestions. Last, but not least, I would like to thank my family, friends, relatives, colleagues and the management of Kraft Foods Research for their encouragement.

April 1995

Anilkumar G. Gaonkar
Kraft Foods, Inc.
Glenview, IL 60025

Contributors

Celso O. Bejarano Wallens
Kraft Foods, Inc., 801 Waukegan Road, Glenview, IL 60025, USA.

A.E. Blaurock
Kraft Foods, Inc., 801 Waukegan Road, Glenview, IL 60025, USA.

R. Chinnaswamy
Midwest Grain Products, Inc., 1300 Main Street, Atchison, KS 66002-0130, USA.

D.R. Gray
Midwest Grain Products, Inc., 1300 Main Street, Atchison, KS 66002-0130, USA.

Rikimaru Hayashi
Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kitashirakawa, Sakyo-Ku, Kyoto 606-01, Japan.

Frank W. Henrikson
Center for Advanced Food Technology, Rutgers- The State University of New Jersey, New Brunswick, NJ 08903-0231, USA.

Kazuhiko Kandori
School of Chemistry, Osaka University of Education, Asahigaoka 4-698-1, Kashiwara-Shi, Osaka-Fu 582, Japan.

Robert Kauten
Department of Food Science and Technology, University of California, Davis, CA 95616-8598, USA.

Michael McCarthy
Department of Food Science and Technology, University of California, Davis, CA 95616-8598, USA.

D.J. McClements
Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA.

P.J. McDonald
Department of Physics, University of Surrey, Guildford, Surrey GU2 5XH, United Kingdom.

Wassef W. Nawar
Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA.

Gordon W. Niven
Department of Biotechnology and Enzymology, Institute of Food Research, Reading Laboratory, Early Gate, Whiteknights Road, Reading RG6 2EF, United Kingdom.

Thomas E. Szemplenski
Aseptic Resources, Inc., 10008 W. 120th Street, Overland Park, KS 66213, USA.

K.K. Tiwari
Department of Chemical Technology, University of Bombay, Matunga Road, Bombay 400 019, India.

Gun Trägårdh
Department of Food Engineering, Lund University, P.O. Box 124, S-22100 Lund, Sweden.

Jim Venetucci
Liquid Carbonic, Freezing Systems Division, 135 South La Salle Street, Chicago, IL 60603-4282, USA.

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Chapter 1

Applications of NMR Imaging in Processing of Foods

Robert Kauten[a,b] and Michael McCarthy[a,c]

[a]Department of Food Science and Technology, University of California, Davis, California

[b]Department of Neurosurgery, University of California, Davis, Medical Center, Sacramento, California

[c]Department of Agricultural Engineering, University of California, Davis, California

1. INTRODUCTION

Magnetic resonance imaging (MRI), originally developed for medical applications (Morris, 1986), has recently been exploited for observation and characterization of foodstuffs and their manufacture (McCarthy and Kauten, 1990; Schmidt and Lai, 1991; Schrader et al., 1992). MRI and its related techniques have already proven useful in non-invasive observations of fruit and vegetable quality, e. g., ripening (Chen et al., 1989 & 1993) and fruit defects (Wang et al., 1988; Wang and Wang, 1989; Chen et al., 1989). Lipid/water content has been visualised in beef and pork (Groeneveld et al., 1984), salad dressing (Heil et al., 1990), and fish (Winkler et al., 1991). The value of MRI has been demonstrated in several processes involved in cheesemaking: syneresis (Özilgen and Kauten, 1993); formation of eyes during ripening of Swiss cheese (Rosenberg, et al., 1991, 1992); fat droplet size determination in the finished product (Callaghan et al., 1983); and diffusion of salt into cheese (brining). MRI observations of water and oil phase changes include drying (Perez et al., 1988; Ruan et al., 1991; Song, et al., 1992; Ruan, et al., 1992; Schrader and Litchfield, 1992), fat crystallisation (Simoneau et al., 1991 & 1992), and freezing (McCarthy et al., 1989; Özilgen et al., 1993). Transport phenomena evaluated by MRI range in mobility from mm/day in a creaming emulsion to as fast as 3 meters per second: foam stability (German and McCarthy, 1989); diffusion of water and oil (Callaghan et al., 1983; Ruan et al., 1991; Watanabe and Fukuoka, 1992) and its relation to temperature variations (Sun et al. 1993); creaming of emulsions (Kauten et al., 1990); and flow in aseptic processing (McCarthy et al., 1992a) and extruder models (McCarthy et al., 1990b). Mobility serves both as the origin of many aspects of the magnetic resonance phenomenon, and as an MRI-observable attribute of the fluids within foods. After a brief introduction to the theory underlying the exploitation of the magnetic resonance phenomenon, the discussion moves from low to high mobility processes, from crystallisation, through diffusion,

creaming emulsions and syneresis, and concludes with more rapid flow in aseptic processing and extrusion.

1.1. Theory of magnetic resonance imaging

The magnetic resonance (MR) phenomenon, and its utilisation in exploring the properties of food materials, depends on the inherent magnetic properties of certain atomic nuclei in a magnetic field. The hydrogen nucleus, a proton, behaves as a spinning charged particle; it possesses angular momentum and generates a polar field. In the earth's weak magnetic field, incoherent motion caused by thermal energy prevents any significant population of protons from aligning with the magnetic field. Little difference exists between energy levels of the numbers of protons aligned with or against the field; thermal energy equalizes the two populations.

Placing hydrogen nuclei in a stronger magnetic field imposes a larger difference in energy levels between protons aligned with and those aligned against the field. Thermal motions still tend to equalize the populations, but the population of nuclei aligned with the magnetic field will very slightly outnumber those aligned against it. A sudden pulse of energy at a frequency and amplitude precisely adjusted for a given nucleus will move the protons from the lower energy level (aligned with) to the higher level (aligned against). Energy depends on frequency:

$$E = \frac{h}{2\pi}\,\omega, \tag{1}$$

h denoting Planck's constant and ω frequency in radians per second. As mentioned above, a stronger magnetic field imposes a larger energy difference between the aligned and opposed populations, and the relationship is linear:

$$\omega = \gamma B_0, \tag{2}$$

where γ is the gyromagnetic ratio, a constant unique for each MR-sensitive nucleus, and B_0 is the strength of the applied field in gauss or Tesla. Equation 2 forms the basis for understanding the behavior of nuclei in any magnetic field, whether uniform or nonuniform.

Perturbing the alignment of hydrogen nuclei in a magnetic field allows observation of the state of the nuclei; molecular structure, temperature, mobility, and even position influence the properties of the signal emitted by the nuclear system as it relaxes, or returns to equilibrium (aligned with the magnetic field). The signal decay, or free induction decay (Figure 1), contains all of the information available by MR. The signal of Figure 1 consists of two components, water resonating at 25 Hz and oil at 100 Hz. The reference frequency of 21.4 megahertz has been subtracted, leaving audio frequencies to represent the small differences (parts per million) between the transmitter frequency and the resonance frequencies. Oil and water resonate at somewhat different frequencies due to the variation in their electronic environment; protons have bonded to oxygen to form water, and to carbon chains to form oils. Chemical shift results from shielding by

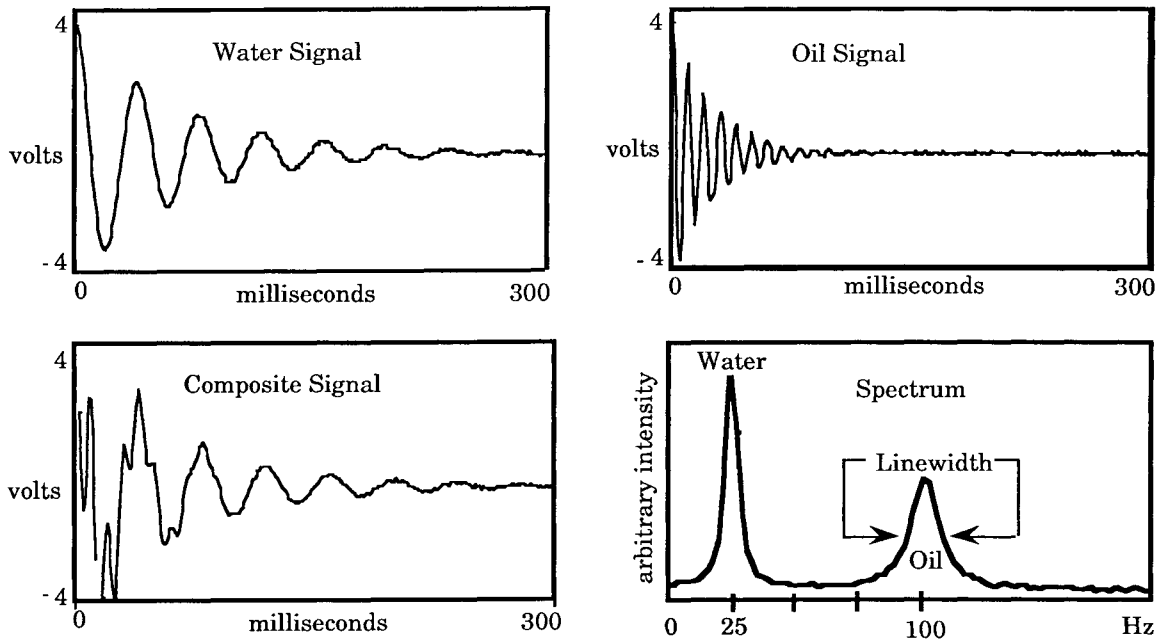

Figure 1. Hypothetical proton magnetic resonance signals from water and oil differ in frequency and relaxation times. Frequency varies linearly with magnetic field strength; the higher electron affinity of oxygen in water as compared to carbon in oil provides less electronic shielding for water protons than for oil protons. For a given magnetic field, protons of water experience a stronger local field and resonate at a higher frequency. Relaxation times vary inversely with line width. The relatively long decay constant of water protons (67 ms) results in a line width of 4.75 Hz, while the shorter relaxation time of oil (25 ms), yields a line width of 12.7 Hz.

electrons of protons from the effect of the applied magnetic field. The actual field experienced by protons in a molecule decreases with electronic shielding, thereby requiring a lower radiofrequency (rf) for resonance:

$$\omega = \gamma(B_0 - \sigma) \tag{3}$$

where σ denotes shielding field strength in gauss or Tesla. The greater electronegativity or electron affinity of oxygen compared to carbon provides less electron shielding (higher frequency) to water protons than to oil protons (lower frequency) at a given magnetic field strength.

The decay of the MR signal results from relaxation; in its absence, the perturbed nuclei would emit no signal. Temperature influences molecular mobility, which in turn induces changes in relaxation rates, manifested in the rate of signal decay. Viscosity, another quality related to mobility, affects relaxation rates in the same way. Molecular structure also determines mobility; larger or more hindered structures move less easily than do smaller or more open structures. This discussion will deal with two mechanisms of MR relaxation, spin-lattice, or T_1 relaxation, and T_2, or spin-spin relaxation. T_1 relaxation occurs as energy transfers from the high-energy spin state nucleus to the surroundings, or lattice, usually as heat, manifested as molecular rotation close to the resonance frequency. T_1, the reciprocal of the exponential relaxation rate, denotes the time constant of the return of perturbed magnetisation to its equilibrium state in alignment with the external field (Farrar, 1989):

$$M_z(t) = M_0(1 - e^{-t/T_1}) \tag{4}$$

where $M_z(t)$ and M_0 denote the magnetisation aligned with the magnetic field at time t and at equilibrium, respectively. T_2 relaxation occurs via exchange of spin states with nearby nuclei, thereby dephasing the signal and averaging it to zero:

$$M_t = M_0(e^{-t/T_2}), \tag{5}$$

where M_t denotes the observed signal at time t. Only fast processes (molecular motion frequencies near that of resonance) affect T_1 relaxation, while both fast and slow processes (less than resonance frequency) affect T_2 relaxation (Farrar, 1989). For this reason the T_2 relaxation rate ($1/T_2$) often exceeds $1/T_1$. Mobility affects relaxation rates in a non-linear manner. Efficiency of relaxation increases with mobility until the correlation time, τ_c (roughly the expected duration the nucleus remains in one position) equals the period corresponding to the resonant frequency:

$$\omega\tau_c \approx 1 \tag{6}$$

Any deviation in correlation time from this value decreases the relaxation rate; at low frequencies (low magnetic field strength), the high mobility and short correlation time of bulk water slows its relaxation. In contrast, hydrogen atoms in an oil molecule, attached to the chain of carbons, rotate relatively slowly with the

molecule, lengthening the correlation time and increasing the relaxation rate of oil relative to water. Relaxation rate differences allow discrimination between signals originating from oil and water. The magnetisation of oil recovers its alignment with the external magnetic field more quickly than does bulk water; a rapid succession of rf pulses may prevent the magnetisation of the water from recovering to equilibrium and diminish the water signal with respect to that of the oil. Conversely, the water signal also decays more slowly than does the oil signal, allowing water signal enhancement simply by delaying acquisition. Figure 1 illustrates a similar situation, in which the water signal at a chemical shift of 25 Hz decays to zero with an exponential rate constant of 0.015 t, while the relaxation of the oil signal at a chemical shift of 100 Hz proceeds with a time constant of 0.04 t. A wait of 100 ms before acquisition of the composite signal would nearly eliminate the contribution from the 100 Hz component (Figure 1). The term "T_2 weighting" refers to an image or spectrum acquired in this way. The resonance at 100 Hz in the frequency spectrum has a wider spread of frequencies, or line width, than does the resonance at 25 Hz; ideally, the line width at half height of the resonance (Figure 1) varies inversely with T_2:

$$\text{L. W.} = \frac{1}{\pi T_2} \tag{7}$$

Our very hypothetical water and oil resonances have T_2 relaxation times of 67 and 25 ms, respectively, yielding line widths of 4.75 and 12.7 Hz. Line widths this narrow would occur only in a very uniform magnetic field. The large bore (>12 cm) magnets suitable for imaging of food samples have spatially varying fields ; that and the relatively large sample size (several centimeters) usually produce line widths sufficient to partially overlap resonances from oil and water. In recognition of the actual situation, T_2^* (T_2 star) denotes the combined effect of T_2 and field irregularities:

$$\text{L. W.} = \frac{1}{\pi T_2^*} \tag{8}$$

Nonuniformities in the magnetic field cause spatial variations in the resonant frequency as gradients do, but in an undesirable, non-linear fashion. Resolution decreases as T_2^* increases.

1.2. Gradients

The foundation of magnetic resonance imaging lies in the response of resonance frequency to changes in the magnetic field; difference in frequency or phase due to application of magnetic field gradients provides information on position, diffusion rates and flow velocity. In general, imposition of a linear field gradient upon a uniform magnetic field (B_0) influences resonance frequency by a modification of equation 2,

$$\omega = \gamma(B_0 + G_x x), \tag{9}$$

where G_x and x denote a linear gradient in the x dimension and position on the x axis, respectively. Equation 9 describes the phenomenon which permits slice selection, frequency encoding and phase encoding.

Slice selection in a spin-echo experiment (Figure 2) allows excitation of a relatively narrow band of frequencies, which, in the presence of the magnetic field gradient described above, corresponds to a limited volume of resonating nuclei. Excitation of nuclei from a designated volume necessarily restricts the origin of the acquired signal to nuclei from that volume, and facilitates non-invasive observation of the interior of objects.

The phenomenon described by equation 9 also allows encoding of position according to frequency, aptly named frequency encoding (Figure 2). After Fourier transformation (a mathematical method for converting a mixture of frequencies, varying with respect to time, to a frequency map, or spectrum, with variation with respect to frequency; Figure 1) of a signal received from an object within a field gradient, the resulting profile or projection portrays spatial information versus intensity. The resonances of Figure 1 at 25 and 100 Hz could also originate from two objects 1 cm apart in a 75 Hz/cm gradient. Acquisition of the signal proceeds during imposition of the gradient, in contrast to phase encoding. Phase encoding involves application of the linear gradient for a short time, imposing a temporary frequency change (Figure 2); upon removal of the gradient, the resonance frequency returns to its previous value, but its accumulated phase ϕ will depend on the strength of the gradient G_x at position x and the duration of application, t:

$$\phi = \gamma G_x x t. \tag{10}$$

If no reversal of this gradient effect occurs during the experiment, the acquired signal contains phase encoded positional information; all gradients used in a spin-echo imaging sequence must be reversed to avoid imparting undesired phase shifts to stationary nuclei (Figure 2).

MRI technology can quantify any condition which confers a coherent phase shift to the MR signal from an ensemble of nuclei. Application of two gradients, the first inducing phase, the second, opposite in sign, reversing phase, encode motion. Setting the phase accumulation to zero in this way erases any positional information from stationary nuclei; any nucleus moving to another position in the gradient field, different in magnetic field strength, will accumulate phase, and its phase difference depends upon the distance moved during time t. The time frame of the motion determines the design of the experiment. Slow motions such as diffusion or perfusion, involving translations at 10^{-5} cm^2/s, require relatively long (e. g., 40 ms), high amplitude gradient pulses, separated by durations of sufficient length to allow motion and accumulation of phase (Figure 2). Velocity measurements, though similar in general structure, demand particular gradient durations and separation times for investigation of each velocity distribution (Figure 2). Mobility of nuclei determines the behavior of the NMR signal, and analysis of the NMR signal can in turn define the mobility of the nuclei, ranging from slow to fast, from the slow molecular movements of crystallisation to flow rates of meters per second.

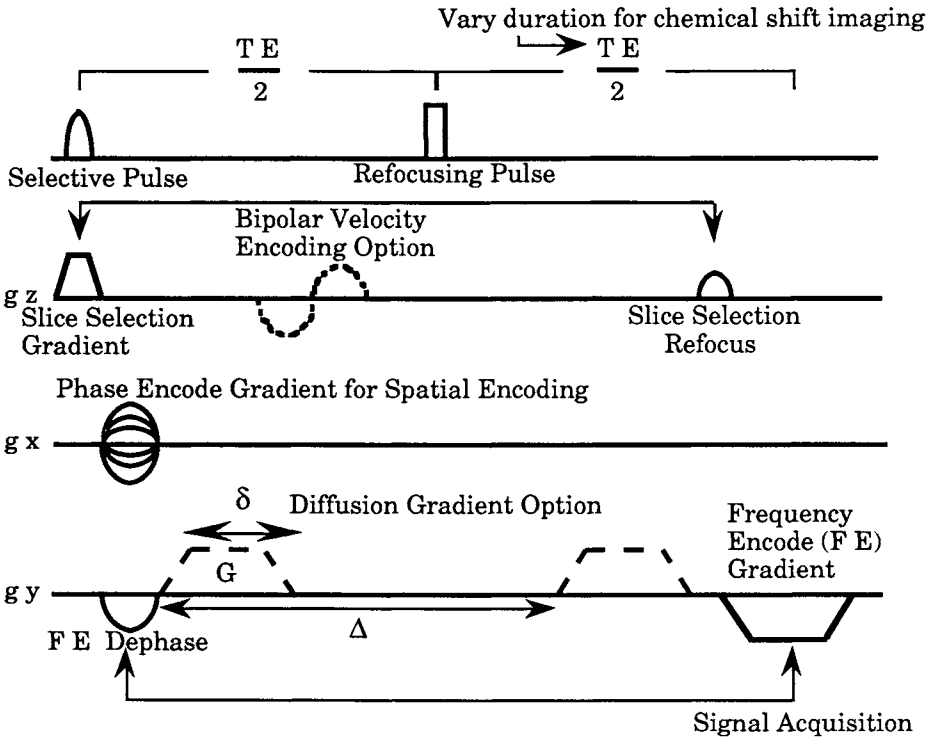

Figure 2. The spin-echo imaging pulse sequence may be modified to phase encode position or velocity, to observe chemical shift vs. position, or to observe signal attenuation due to diffusion in the presence of a gradient. Solid lines signify features present in standard 2-dimensional imaging experiments, dashed lines represent optional gradients for velocity and diffusion experiments. The multiple lobes of the Phase Encode Gradient refer to the variation of gradient amplitude with each scan, starting at negative, through zero to positive. The Bipolar Velocity Encoding gradient varies in the same way. Gradients connected by brackets (F. E. Gradient, F. E. Dephase) illustrate that use of any gradient results in phase accumulation unless balanced by another gradient opposite in sense. The Refocusing Pulse reverses the sense of any phase previously accumulated. Interchanging components of gradient time lines gx, gy and gz allows acquisition of spatial, diffusion and velocity information from 3 orthogonal planes.

2. CRYSTALLISATION AND FREEZING

Decrease in mobility during freezing or crystallisation greatly attenuates MR signal amplitude, providing a means of following these related processes. Intensity of the MR signal depends on the relaxation rate of the interrogated nuclei; the very fast relaxation rates found in solids prevent observation by use of liquid MR techniques. This inability to observe nuclei in a solid matrix presents a method for observation of kinetics of the liquid-solid phase transition. Disappearance of signal from a volume containing liquid may signify crystallisation or amorphous glass formation. Observation of the movement of the intensity interface during solidification has provided kinetics information for crystallisation of fat/water emulsions and freezing of meats and vegetables.

2.1. Crystallisation

Magnetic resonance imaging has enabled the direct observation of crystallisation kinetics of fats in bulk or in fat/water emulsions containing one or more species of triglyceride. Initially, images and spatially-localized spectra acquired during crystallisation of warm (60-80° C) trilaurin:water and trimyristin:water (2:3) emulsions during cooling to 20° C validated the method (Simoneau, et al., 1991). Later, the same imaging techniques illustrated the slowing of crystallisation kinetics resulting from "poisoning" of the emulsion with a mixture of fats. Calorimetry data permitted correlation of enthalpy with the crystallisation kinetics (Simoneau, et al., 1992). Images acquired during the crystallisation of bulk lipids and emulsions during cooling resemble those acquired during freezing; the decrease in mobility merely occurs in a different material at a lower temperature.

2.2. Freezing

Freezing, whether involving crystallisation or amorphous glass formation, decreases molecular mobility, thereby increasing relaxation rates and attenuating the MR signal. Movement of the freezing interface has been observed in food samples such as beef, chicken, potatoes, peas and corn (Figure 3). In meat and potato samples, imbedded thermocouples, visible in the images, permitted simultaneous measurement of temperature and freezing front progression (McCarthy, et al., 1993; Özilgen, et al., 1993). Magnetic resonance observations of extent of freezing have also been correlated with enthalpy using calorimetry. These studies indicated that use of MRI for monitoring enthalpy of products leaving the freezer would allow adjustment of freezer temperature to quickly reduce enthalpy of frozen products to their storage enthalpy. The authors calculate that on-line monitoring of enthalpy to avoid under- or overcooling before storage could save 17% in total freezing energy expenditures (Özilgen, et al., 1993). Lack of molecular mobility, leading to attenuation of the MR signal, confers an advantage in freezing applications.

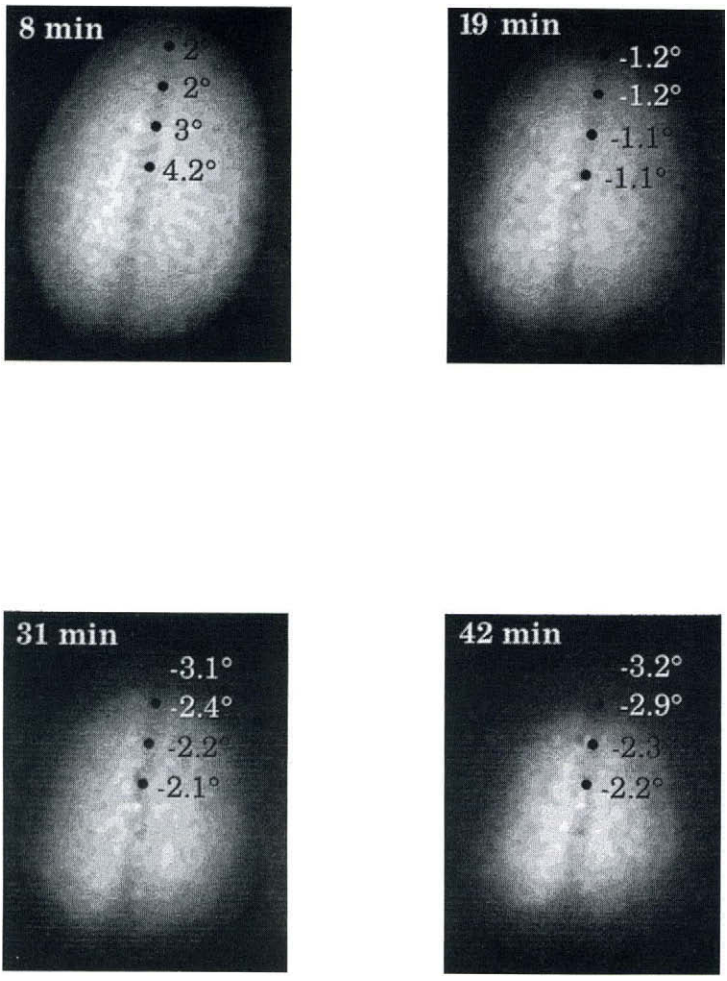

Figure 3. Thermocouples (black dots) imbedded in a potato allow correlation of freezing interface position with temperature during freezing at -30° C within the magnet of an MRI spectrometer. Air velocity was 2.1 m/s. Calorimetry measurements accompanied identical freezing experiments without thermocouples to correlate freezing front progression with enthalpy.

3. DIFFUSION

As in freezing, diffusion studies have exploited attenuation of the NMR signal; in contrast to freezing, this attenuation results from molecular mobility, and not its absence. Most diffusion imaging experiments make use of the spin-echo pulse sequence (Figure 2): briefly, an rf pulse disturbs the magnetisation of the sample nuclei; the nuclei dephase by T_2 mechanisms, diffusion and magnetic field nonuniformities; a refocusing pulse is applied; and the signal regains coherency (a spin echo). The refocusing pulse can only reverse dephasing due to a non-uniform magnetic field, not that due to T_2 relaxation or diffusion. Diffusion usually produces negligible attenuation under these circumstances. One can enhance attenuation due to diffusion by sequentially imposing two gradients of equal amplitude and opposite polarity during the time between the first rf pulse and acquisition (echo time). The first gradient confers phase upon nuclei dependent on their position; the second, opposite gradient reverses and restores the original phase of stationary nuclei. In the experiment of Figure 2, the refocusing pulse reverses the polarity of magnetisation, hence the second identical gradient reverses the effect of the first gradient. Any nuclei which have moved during the time between the diffusion gradients will retain a phase difference, and the spectrometer receives an out-of-phase, attenuated signal from these nuclei. For a series of experiments varying only the amplitude of the rectangular gradient pulses, the ratio of signal intensities from diffusion-weighted (S_w) and non-weighted (S_n) experiments becomes (Le Bihan, et al., 1988):

$$\frac{S_w}{S_n} = e^{-\gamma^2 G^2 D \delta^2 \left(\Delta - \frac{\delta}{3}\right)} \tag{11}$$

where γ, G, D, δ, and Δ denote the gyromagnetic ratio, gradient amplitude, self-diffusion coefficient, duration of the gradient, and time between opposing gradients, respectively (figure 2). Relaxation affects each experiment equally and may be ignored. In the series of experiments proposed above, all parameters except G in the exponential term remain constant; plotting $\ln(S_w/S_n)$ vs. $-\gamma^2 G^2 \delta^2(\Delta-\delta/3)$ for various gradient amplitudes produces a line whose slope yields D, the self-diffusion coefficient.

Diffusion imaging shows promise for mapping of internal temperatures of foods. Changes in temperature alter the apparent self-diffusion coefficient (D_{app}) of water, and conversely, knowledge of D_{app} provides an estimate of temperature. Temperature mapping by diffusion imaging has already been demonstrated in a medical hypothermia model (Le Bihan et al., 1989) and recently in a model food gel (Sun et al., 1993).

Diffusion imaging can also determine particle size in water/lipid emulsions, by incrementally increasing the time between opposite gradients (Δ) and noting the interval at which the signal attenuation ceases. In the case of restricted diffusion, a short Δ will result in the signal attenuation expected in bulk fluid; an appropriately longer Δ (dependent on particle size) will show less signal

attenuation than in bulk, due to restriction of movement of intraparticle fluid by particle walls. Simply put, small particle size impedes the travel of molecules to areas of varying field strength. Varying the Δ between gradients will usually result in variation of the echo time (Figure 2), therefore attenuation of the signal requires correction for T_2 dephasing. Manufacture of margarines, dressings and cheese would benefit from the non-invasive particle size determination provided by diffusion imaging. Indeed, Callaghan et al. (1983) have estimated fat droplet size in cheese using diffusion spectroscopy.

Sodium imaging allows the non-invasive observation of the progress of sodium chloride diffusion into a milk protein gel, a model of cheese brining during ripening. A simple spin-echo sequence produced the images of Figure 4 (middle and right) without use of diffusion gradients; the spectrometer observed only sodium nuclei, which have a gyromagnetic ratio, hence frequency, different from hydrogen. Here, changes in sodium nuclei density quantify diffusion.

4. EMULSIONS

Food emulsions, including margarine, ice cream, mayonnaise and salad dressing, represent another economically important system observable by MRI. The potential for measurement by MRI of oil/water ratios in separated salad dressings emerged early on (Heil, et al., 1990). Manufacturers of these products and of non-food emulsions must understand the affect of emulsifiers on phase separation of emulsions, and control particle size distribution. Magnetic resonance imaging can quantify phase separation kinetics by exploiting relaxation and chemical shift differences, and estimate particle size through quantifying restriction of diffusion.

In contrast to previous methods, MRI techniques for rapidly determining relaxation rates of oil and water permit non-invasive measurement of separation kinetics of emulsions. Many previous methods for quantifying phase separation require disturbing the emulsion and thereby accelerating separation (Dickinson and Stainsby, 1988). Ultrasound techniques, while non-invasive, require the absence of air bubbles, often present in homogenized samples. Ultrasound offers no spatial selectivity and demands separate measurements at each point (Povey, 1987). A technique developed for rapid T_1 relaxation rate determination (Canet, et al., 1988; Fanni, et al., 1989) proved useful for rapid determination of oil and water volume fractions. When modified with imaging techniques, the technique offered the volume fraction ratio at all points simultaneously along a vertical emulsion profile (Kauten, et al., 1991). The apparent T_1 at each point along the profile obeyed the equation:

$$\frac{1}{T_{1\,obs}} = \frac{\phi_{water}}{T_{1\,water}} + \frac{\phi_{oil}}{T_{1\,oil}} \qquad (12)$$

with ϕ denoting the volume fraction. Using this method, Pilhofer et al. (1993) observed rates of separation of milkfat/water emulsions and compared the data to predictions from Stoke's law,

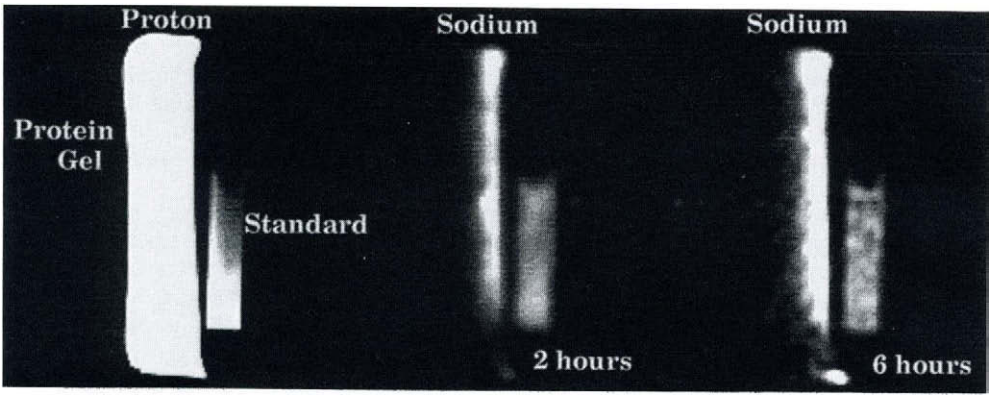

Figure 4. Sodium MRI illustrates diffusion of sodium into a milk protein gel as a model of brining in cheese manufacture. A proton image (left) shows the form of the gel, which lies in an overturned beaker, and the 1% (w/v) sodium chloride/water standard, in a 1 cm cuvette. In this experiment, water saturated with sodium chloride was poured over the gel in the upright beaker. After durations of 2 and 6 hours, the salt water was decanted and the standard was replaced for sodium imaging. The lower MR-sensitivity and abundance of sodium results in much smaller signal-to-noise ratios compared to the proton image.

$$v = \frac{g\Delta\rho d^2}{18\eta} \qquad (13)$$

where d is the droplet diameter, g is acceleration due to gravity, η is the viscosity of the continuous phase, and $\Delta\rho$ the difference in phase densities. Creaming rates of milkfat emulsions proved much slower than predicted on the basis of particle sizes determined by a laser diffraction particle-size analyser.

Another method for non-invasive measurement of oil/water ratios in emulsions exploits the chemical shift phenomenon. Deliberate non-refocusing of dephased signals during a spin-echo imaging experiment permits spatial mapping of chemical shift (Majors et al., 1990). Incrementing the second, refocusing period by a small duration (another method of phase encoding) produces a dataset composed of two superimposed frequencies resolvable by Fourier transformation. An emulsion of hexadecane in water thus appears as two bands of intensity, proportional to the content of each component; chemical shift on one axis, positional information on the other (Figure 5).

In addition to volume fraction determination, MRI diffusion techniques allow calculation of particle size distribution, important in resulting texture and emulsion stability. The same principles and techniques apply to discerning emulsion particle size as to cheese oil droplet size, discussed in the diffusion section.

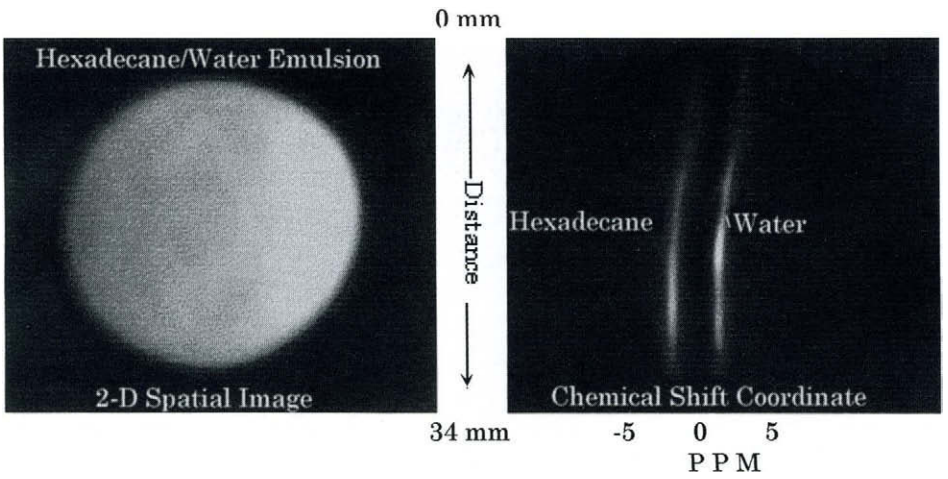

Figure 5. Chemical shift imaging permits the estimation of oil/water ratios versus position in a hexadecane/water emulsion. The hexadecane/water chemical shift image (right) basically consists of stacked oil/water spectra like those of Figure 1, observed from above.

5. SYNERESIS

Our discussion now moves from the very slow movement of separating emulsions to the faster flow of syneresis, an earlier process in cheesemaking. Syneresis, the expulsion of whey from shrinking cheese curd, begins immediately after disturbance of the curd by cutting. Control and understanding of conditions affecting rates of syneresis would aid in the reproducible manufacture of quality cheese. Until recently, all methods for measuring syneresis involved mechanical disturbance of the curd (Berridge and Scurlock, 1970; Geurts, 1978; Marshall, 1982; Green, 1987), accelerating whey expulsion and leading to varying results (Walstra et al., 1985). Differences in T_2 relaxation rates between entrapped and expelled whey allow the non-invasive estimation of volume of expelled whey and shrinkage of curd (Özilgen and Kauten, 1993). Free whey has a much slower relaxation rate than does whey within the curd; an echo time of 300 milliseconds allows the signal from sequestered whey to relax significantly, while leaving the signal from expelled whey relatively intense (Figure 6). Characterisation of whey expulsion may not require complete 2-dimensional images if syneresis occurs at the same rate throughout the curd volume. Projections, one-dimensional representations of intensity along one spatial axis, display the quantities of curd and whey accurately, and require orders of magnitude less time (1 second) for acquisition. Thermocouples monitoring temperature at several locations in the curd do not significantly degrade the data. This system permits non-invasive observation of syneresis under varied conditions of temperature, calcium chloride concentration, pH and grade of milk. Addition of chemical shift imaging techniques

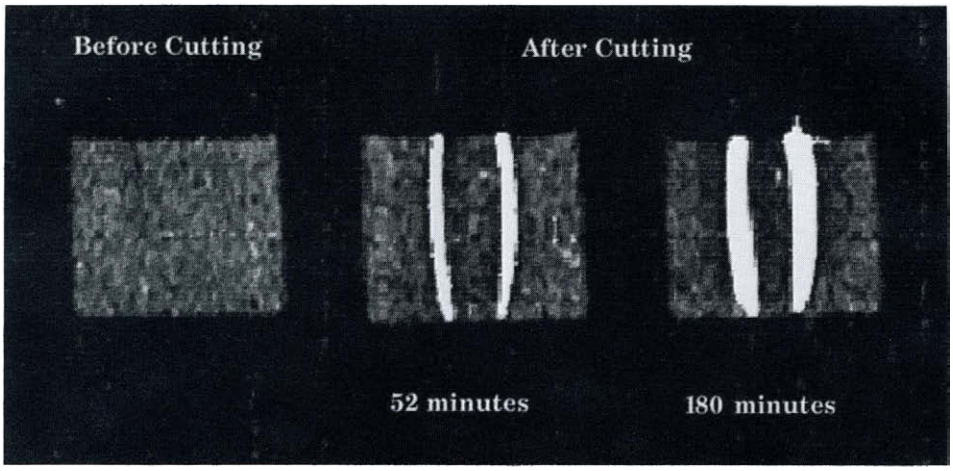

Figure 6. T_2-weighted images of cheese curd before (left) and after cutting demonstrate syneresis, the expulsion of whey from shrinking curd. An echo time, or pause before acquisition (Figure 2) decreases the signal contribution from the faster relaxing curd associated whey, and enhances the relative intensity of slower relaxing expelled whey. Addition of cuprous sulfate to the circulating water surrounding the incubation chamber greatly increases the relaxation rate of the water, rendering it invisible.

(see Emulsion section) can also illustrate relative quantities of fat contained in curd and whey during syneresis.

6. FLOW

Magnetic resonance imaging flow techniques allow the non-invasive observation of velocity profiles and hence residence times during aseptic processing and extrusion. MRI techniques useful in determining velocities and residence times fall under two broad categories: time-of-flight and velocity-encoded techniques (Listerud, 1991). Currently, time-of-flight techniques have seen more exposure in food science journals (McCarthy, et al., 1992a; McCarthy, et al., 1992b), and allow easier comprehension by non-physicists.

6.1. Time-of-Flight Techniques

Simply put, time-of-flight (TOF) methods mark or tag nuclei in a fixed volume at time zero and later interrogate the same or another volume to determine whether the nuclei have moved. TOF can characterize any pattern of motion, from flow in straight pipes (Caprihan and Fukushima, 1990; McCarthy, et al., 1992a) to complex heart muscle contractions (Zerhouni, et al., 1988; Axel and Dougherty, 1989) and radial motions in extruders (McCarthy, et al., 1992b). The method of tagging nuclei varies from simple slice selection to imposing a grid or a single black line. The TOF technique employing slice selection excites nuclei in a plane perpendicular to the direction of flow (tagged volume, Figure 7), pauses to allow development of the flow pattern, then interrogates the same volume or fluid volumes downstream from the initial slice. In the case of laminar flow, slices acquired from sequential volumes downstream will reveal concentric rings of intensity, corresponding to elements of the initial tagged fluid forming a paraboloid due to their increase in velocity with distance from the pipe wall. A three dimensional dataset representing the fate of a selected volume of fluid has thus been acquired. Another variation of TOF, patterned nulling of the intensity of a volume of fluid or tissue, allows observation of more complex motions by following the distortion of the selected pattern over time. Specific configurations of radiofrequency pulses in the presence of magnetic field gradients produce the desired motif of lines (see Extruder discussion). Imposing a pattern on a slice of fluid during pipe flow could also reveal radial or transverse mixing between layers, caused by turbulence.

6.2. Velocity-Encoding Techniques

The other MRI method for observing flow patterns involves movement of excited nuclei in a gradient, resulting in phase encoding of velocity. The basic technique resembles diffusion imaging, in that two gradients of opposite sign cancel spatial phase encoding of stationary nuclei; only nuclei which have moved during the encoding period accumulate phase in proportion to their displacement. Velocity-encoding differs from diffusion imaging in that while determination of a diffusion coefficient relies only on signal attenuation due to gradient-induced dephasing, velocity encoding actually quantifies phase differences by Fourier transformation of the cyclic pattern of intensity variation. The frequency of the cyclic variation, produced by the inversely coordinated incrementing of two field gradients from, e. g., negative through zero to positive, proves proportional to velocity. A two dimensional image designed in this way portrays spatial information on its frequency encode dimension, versus velocity along the phase encode dimension (Figure 8). The experiment can provide two dimensional positional information by including spatial phase encoding by a gradient orthogonal to the frequency encoding gradient, producing, in the case of laminar flow, a paraboloid similar to TOF datasets. The primary difference between the paraboloids lies in the flow dimension: TOF expresses the dimension as time; flow-encoding maps the third dimension as velocity. Both flow-encoding and time-of-flight techniques can aid in the design and control of extrusion and aseptic processing.

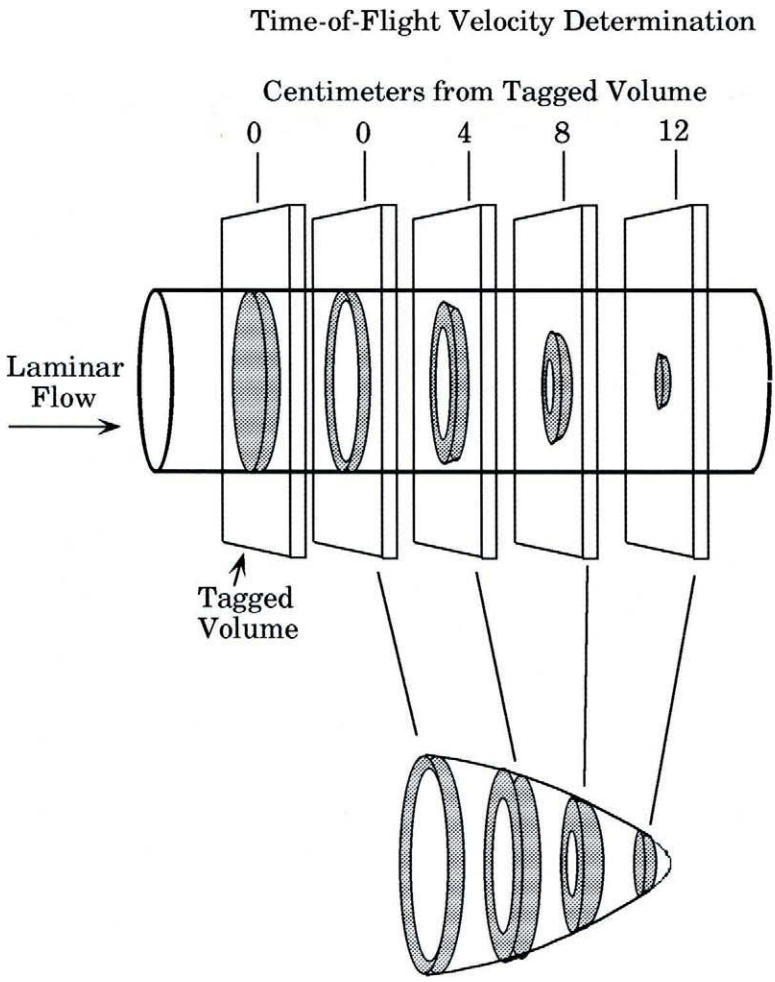

Figure 7. The most common time-of-flight velocity determination technique consists of selectively exciting (tagging) the nuclei within a plane (here 2 mm thick) perpendicular to the direction of flow. After allowing the flow pattern of the volume to develop, slices of fluid downstream from the tagged volume reveal intensity, and therefore velocity, dependent on radial position. Stacking the slices forms a paraboloid, the pattern of laminar flow.

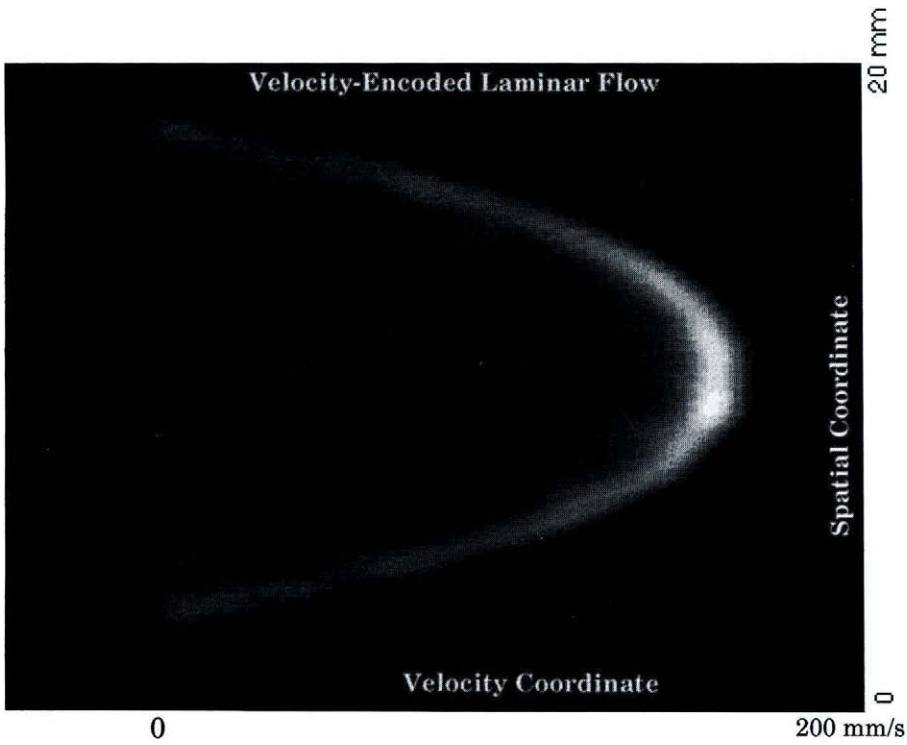

Figure 8. Phase-encoded velocity techniques yield two-dimensional velocity profiles of laminar flow in models of aseptic processing. Phase-encoded velocity images result from Fourier transformation of gradient-induced cyclic phase iterations whose frequency depends on velocity (Figure 2). This velocity profile illustrates laminar flow of water through a 15.3 mm pipe at an average velocity of 79 mm/s.

6.3. Aseptic Processing Applications

Use of velocity profiles provided by MRI could prevent either under- or overcooking of foods during aseptic processing. Aseptic processing requires the heating of food at a prescribed temperature, for time sufficient to sterilize it, as it moves through a pipe. Packaging and sealing while still hot protect the product from spoilage. Undercooking, leading to spoilage, and overcooking, resulting in loss of desired sensory attributes (taste, texture) present a problem addressable by MRI flow techniques. Obviously the solution involves ensuring that all of the food material remains in the heating pipe for a time sufficient to sterilize, but no longer, to preserve quality. Velocity profiles of the food material, whether obtained by velocity encoding or time-of-flight techniques, can reveal velocities at any position in the pipe (McCarthy, et al., 1992a), leading to accurate calculations of residence time.

6.4. Extrusion Applications

Residence time as well as shear rate govern product quality in food extrusion; consequently velocity profiles provided by MRI flow techniques could assist in the construction and adjustment of extruders. As in aseptic processing, residence time determines cooking time, and often some portions of a batch cook less than others. This condition results in undesired quality variations. As shear rate influences texture, the data supplied by velocity profiles allow adjustment in screw speed and design to optimise shear rate and therefore quality and reproducibility. Knowledge of shear rates and residence times could verify or modify existing mathematical models of extrusion (McCarthy, et al., 1992b). Both phase encoded velocity and time-of-flight experiments produce velocity profiles, but a TOF technique yielded the first published MR images of fluid motion in an extruder.

The TOF technique used by McCarthy, et al. (1992b) to observe fluid velocities in an extruder imposed a single dark band across the barrel diameter; subsequent distortion of the band revealed fluid displacement, permitting calculation of radial velocities and comparison to theory. A radiofrequency pulse in the presence of a gradient dephased the magnetization of a 2 mm plane of fluid perpendicular to the image slice; the resulting dark band (Figure 9) persisted, decaying slowly by T_1 mechanisms. An adjustable interval between formation of the band and acquisition of the image provided time for the band to stretch in the direction of fluid movement. Acquisition of images at several intervals depicted evolution of velocity profiles, illustrating linear drag flow (die open) and the combination of drag and parabolic pressure flow (die closed, no net flow). Velocity calculations from the fluid displacements compared well to theoretical predictions based on an analysis by Harper (1981), and modified by the authors to reflect an aspect perpendicular to the screw axis:

$$v_p = V - v_z \cos\theta + v_x \sin\theta \tag{14}$$

where

$$V \text{ (screw velocity)} = \pi ND. \tag{15}$$

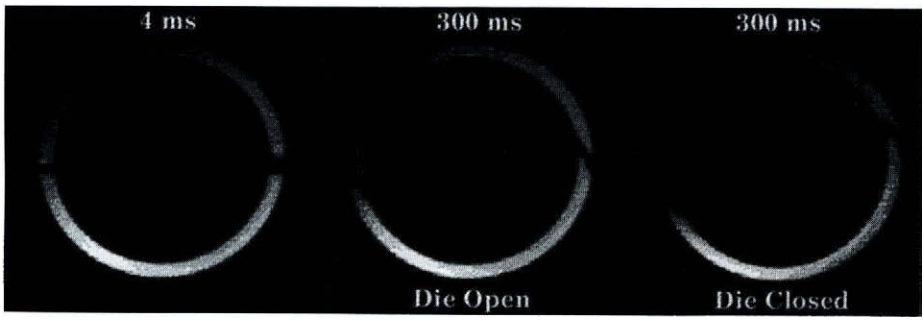

Figure 9. Distortion of a dark band by the motion of the helical screw of an extruder illustrates fluid flow patterns. A radiofrequency pulse in the presence of a vertical gradient dephases the magnetisation of a 2 mm thick band across the extruder, while the screw rotates counterclockwise at 10 rpm. Data acquisition occurs once per revolution, at exactly the same position. Adjusting the time between band formation and acquisition allows observation of flow pattern development from 4 ms until decay of the band by T1 relaxation. Opening the terminal die of the extruder allows outflow, and the flow pattern appears linear from the barrel wall (outer edge) to the screw surface (middle). This illustration of drag flow contrasts with the flow pattern observed with a closed die (no net outflow), in which drag flow balances pressure flow, a parabolic back pressure, to form a velocity maximum 1/3 of the radial distance from the screw to the barrel (right).

In the case of an open die (no back pressure, drag flow), the equation simplifies to

$$\frac{v_p}{V} = 1 - \frac{y}{H} + 3\sin^2 q \left[\frac{y}{H} - \left(\frac{y}{H}\right)^2\right] \tag{16}$$

and in the case of a closed die (no net flow, both drag and pressure flow), the equation simplifies to

$$\frac{v_p}{V} = 1 + 2\frac{y}{H} - 3\left(\frac{y}{H}\right)^2 \tag{17}$$

where v_p denotes the velocity perpendicular to the screw axis, x, y and z are the horizontal, vertical and screw axis directions, respectively, θ is the helix angle, D is the inner diameter of the barrel, N is the screw speed, H is the height of the flight. In the authors' extruder rotating at 20 rpm (4 cm/s) with a closed die dictating pressure flow, theory predicts the point of maximum velocity (5 cm/s at 1/3 H), exactly as found from the velocity profiles.

7. SUMMARY

MRI offers a versatile, noninvasive, nondestructive experimental technique to study food processing. Its advantages stem from the ability to study and quantify any phenomenon which generates contrast, i.e., change in signal intensity. Magnetic resonance imaging derives many of its attributes from mobility, and not coincidentally MRI techniques have found use in quantifying dynamic processes from the molecular to the macro level. This discussion has touched on MRI food applications for the study of freezing and crystallisation, diffusion, emulsions, syneresis, and flow, and hopefully supplied enough theory to allow an intuitive grasp of the possibilities. Realisation of the potential of MRI techniques for non-invasive observation and characterisation of food processes has only begun.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 2

The use of nuclear magnetic resonance for on line process control and quality assurance

Peter J McDonald

Department of Physics, University of Surrey, Guildford, Surrey GU2 5XH, UK

1. INTRODUCTION

Nuclear magnetic resonance (NMR) is now a mature spectroscopic technique and is widely recognised as being one of the most powerful spectroscopies available for the study of the structure and dynamics of condensed matter. Its origins are in the pioneering experiments of Bloch and Purcell in the mid 1940's (Bloch et al, 1946; Purcell et al, 1946). These experiments were followed by the discovery of the chemical shift (Proctor and Yu, 1950; Dickinson, 1950) which opened up the field of high resolution spectroscopy of the liquid state. Today high resolution spectroscopy is a routine analytical technique in almost every major chemistry laboratory and many others besides. The widespread introduction of pulsed techniques (Torrey, 1949; Hahn, 1950) led to the field of relaxation time analysis and so to the investigation of molecular dynamics on timescales covering at least eight orders of magnitude. These simple techniques are now widely used for bench top laboratory characterisation of the physical state and composition analysis of heterogeneous systems, including foodstuffs. As will be seen, they form the basis of most on line NMR applications. Subsequently, magic angle spinning (Andrew et al, 1958) and multiple pulse line narrowing (Waugh et al, 1968) extended the range of high resolution spectroscopy to the solid state. The first magnetic resonance images were published in 1973 (Lauterbur, 1973; Mansfield and Grannell, 1973) and over the subsequent years it has become possible to spatially resolve virtually all magnetic resonance parameters, initially from liquid samples but increasingly from solids as well. Imaging times have become progressively shorter so that 'real time' imaging is now possible and the scales of application have been extended from single cells to human bodies.

Throughout its development, magnetic resonance has remained largely a laboratory based technique. One reason is the common perception that magnetic resonance is 'complicated' or 'difficult'. Whilst the plethora of measurement techniques and possible outcomes and explanations requires that extreme care is exercised in interpreting new results, in many cases simple interpretations are possible. Another major reason is that many scientists continue to think of high resolution spectroscopy when they think of magnetic resonance. However, a high resolution spectrum is not the most obvious measurement to make for process control applications. It requires a very high magnetic field homogeneity and

preferably also a high magnetic field in order to resolve the narrow resonance lines and hence chemical shifts and spin-spin couplings which serve to fingerprint the sample. In solids or in samples containing bound liquid fractions, or in porous samples with spatial heterogeneity of the magnetic susceptibility the observed resonance is broad and relatively featureless. Chemical shifts cannot be resolved without resource to complex line narrowing techniques which are not always applicable and rarely amenable to on line implementation. After spectroscopy, imaging comes to mind but there are problems with imaging as well, even if the magnetic field requirements can be met. Most standard imaging procedures take several minutes to acquire the three dimensional data sets necessary to inspect a sample in three dimensions. For most mass production purposes this is too long and in any case poses the problem of how to display, analyze and interpret the very large volume of generated data sufficiently quickly. Lower dimensional imaging, such as one dimensional profiling, is very much faster and generates much less data. Under suitable circumstances it can be applied usefully. This is most likely to be the case when looking for changes in density or solid content, which is invisible to conventional magnetic resonance imaging, such as stones in pitted soft fruits. In essence, profiling is no more than a sophisticated variant of free induction decay analysis discussed below, albeit that the signal is acquired in the presence of an applied magnetic field gradient.

By far the most appropriate technique for on line analysis is a measurement based on the characterisation of free induction decay signals and on relaxation time analysis, and in particular on spin-spin relaxation time analysis. These measurements are sometimes called low resolution NMR. Unlike high resolution spectroscopy where state of the art spectrometers use 15 T magnets, low resolution measurements can be made in low magnetic fields, say 0.15 T using magnets with relatively poor spatial homogeneity. Small, low field permanent magnets are ideal and the rapid and continuing advances in magnetic materials science and magnet design technology make these increasingly viable. Moreover, whereas high field spectrometers and imagers may be priced in millions of dollars, low field systems can be just a few tens of thousands of dollars. Other advantages of low resolution NMR are that the measurements can be made very quickly, typically within 1 s, and that in favourable circumstances simple interpretation is possible.

2. LOW RESOLUTION NUCLEAR MAGNETIC RESONANCE

When a sample containing magnetic nuclei such as hydrogen protons is placed in a static magnetic field, B_0, the nuclei align with the field and a bulk nuclear magnetisation develops. This process occurs exponentially in a characteristic time T_1, the spin lattice relaxation time such that

$$M(t) = M_0(1-\exp(-\frac{t}{T_1}))$$

where M(t) is the magnetisation at time t and M_0 is the equilibrium magnetisation. In almost all food systems commonly encountered T_1 values are of order 1.0 s. If

the magnetisation is disturbed from the equilibrium direction, it precesses about the static magnetic field at the Larmor frequency given by

$$\omega_L = \gamma B_0$$

where γ is the magnetogyric ratio of the nuclei in question. For hydrogen protons, $\gamma/2\pi$ = 42.57 Mhz/T. A suitable stimulus to initiate precession is a resonant radio frequency pulse called a 90° pulse applied via an excitation coil around the sample. The precessing magnetisation induces a transient response in the coil which is detected and demodulated and known as the free induction decay (FID) signal. The initial intensity of the signal, I_0, is proportional to the number of nuclei in the sample.

Since the individual nuclear magnetic moments experience not only the applied field but also local magnetic fields, B_{loc} due to their molecular environment they precess at the local frequency

$$\omega_{loc} = \gamma(B_0 + B_{loc}).$$

Variations in B_{loc} cause different nuclei to precess at different rates and the initial coherence of the nuclear magnetisation is lost. With the loss of coherence (dephasing) goes the loss of observed signal. For all but the very fastest decays which usually exhibit complex decay functions, this generally occurs exponentially in a characteristic time T_2, the spin-spin relaxation time so that

$$I(t) = I_0 \exp(-\frac{t}{T_2})$$

where I(t) is the signal intensity at time t. Usually the largest local fields encountered in hydrogen NMR originate from magnetic dipolar interactions between neighbouring magnetic nuclei, followed by much smaller electron nuclear interactions responsible for chemical shifts. Variations in magnetic field brought about by variations in susceptibility are also important. For hydrogen in solids, dipolar field strengths are of order a milli Tesla and the corresponding spin-spin relaxation time is a few micro seconds, say 10-30 μs. The dipolar interaction tends to swamp all others. In liquids, the much greater molecular mobility ensures that dipolar interactions are effectively averaged to zero on the timescale of the experiment. In consequence, dipolar interactions do not contribute significantly to spin-spin relaxation and other effects are seen. The spin-spin relaxation time may be a few seconds in a very pure liquid but more typically values in the range 5-500 ms are observed. Between these limits (10 μs - 500 ms) is a whole continuum of relaxation times reflecting a continuum of degrees of mobility and interaction strengths. Multiple component exponential decays are usually observed from heterogeneous solids. Table 1 gives an indication of the range of spin-spin relaxation times commonly encountered in food science.

Another major source of apparent spin-spin relaxation is inhomogeneity of the applied magnetic field. This leads to signal loss in a characteristic time T_2^*, so that

$$\frac{1}{T_2^{obs}} = \frac{1}{T_2^{sample}} + \frac{1}{T_2^*}.$$

If T_2^{sample} is much greater than T_2^* then T_2^{obs}, the observed value, is independent of the sample and is only a measure of the quality of the magnet. In order to remove systematic errors resulting from a measurement of T_2^*, it is usual to observe long T_2^{sample} FID components in the form of a spin echo train. So called 180° pulses are applied at regular intervals after the initial 90° pulse throughout the decay. The additional pulses serve to refocus dephasing due to the magnetic field inhomogeneities but not B_{loc}. The CPMG (Carr and Purcell, 1954; Meiboom and Gill, 1958) pulse sequence for this is well established, experimentally robust and straightforward to implement.

Table 1
Typical ^1H low resolution NMR T_2 relaxation times of food constituents.

Constituent	T_2
Solid protein / carbohydrate	10-20 μs
Solid / semi solid / liquid lipid	10-20 μs / 100-200 μs / 10-20 ms
Edible oils	100-200 ms
Ice / bound moisture / free water	10 μs / 500 μs / 500 ms

3. POTENTIAL ON LINE MEASUREMENTS

3.1. Techniques

A common bench top low resolution NMR measurement is a solid to liquid ratio determination. Figure 1 illustrates the general concept. It is drawn for a hypothetical two phase system with a solid (66.6%) with a T_2 of 20 μs and a liquid (33.3%) with a T_2 of 1 ms. The magnetisation intensity immediately following the 90° pulse, at time t=0, is proportional to the total number of hydrogen atoms in the sample (I_0) and is therefore a measure of the solid plus liquid content. The signal due to the solid part of the sample (S) decays rapidly whereas the signal due to the liquid part (L) does not. The intensity S+L is measured as quickly as possible. The magnetisation intensity remaining, say, 70 μs after the pulse originates only from the liquid. If the liquid decay time is sufficiently long so that negligible decay occurs during the 70 μs then the ratio of these signals is proportional to the fractional liquid content as follows:

$$\text{Liquid content(\%, w/w)} = \frac{L}{L + fS} * 100.$$

The calibration factor, f, accounts for decay of the solid signal occurring in the spectrometer dead time, shown hashed in figure 1, between the pulse and the first measurement opportunity, typically 5-10 μs, and also the difference in the number of hydrogen atoms per unit mass in the two phases. If a detailed hydrogen atom

count per unit mass in the two phases is known as is the solid phase relaxation time then a precise ratio can be calculated from S and L. Often, however, it is sufficient merely to calibrate the liquid content measured some other way (say drying) against this NMR parameter with f set empirically. In practice, it is often preferable to measure L from a spin echo intensity. The method is adaptable to the measurement of water, oil and fat. A related method involves observation of just the liquid signal, at say 100 μs and comparison of its intensity against a known standard. It has been pointed out that low magnet homogeneities can be an advantage in this measurement since the dominant cause of spin-spin relaxation for the liquid may be the magnet, T_2^*, and not the sample so that variations in liquid T_2 are unimportant (Tiwari et al, 1974).

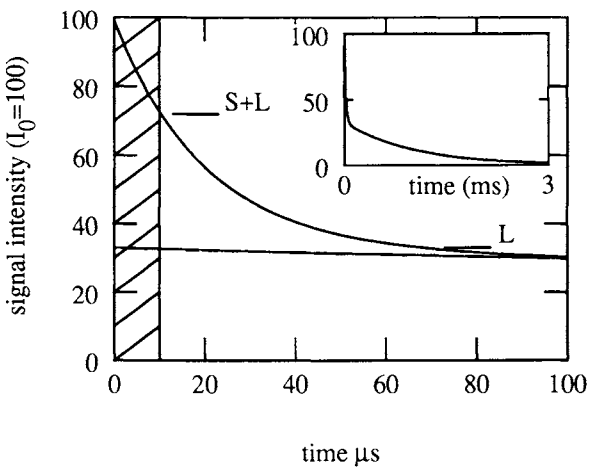

Figure 1. A schematic LR NMR signal showing the solid (S) and liquid (L) intensities (main figure) used to determine the solid / liquid ratio of foodstuffs. The inset shows the full signal.

In simple systems in which there is rapid exchange averaging of nuclei between free and bound liquid phases, the observed relaxation rate is given by

$$\frac{1}{T_2^{obs}} = \frac{P^b}{T_2^b} + \frac{P^f}{T_2^f}$$

where P^b and P^f are the fractional contents of bound and free liquid respectively and T_2^b and T_2^f are the individual relaxation times of the bound and free liquids (Zimmerman and Brittin, 1957). Hence, T_2^{obs} increases with the free liquid content. The relaxation time can be rapidly and accurately measured in a low homogeneity magnetic field using a CPMG sequence. However, most food systems are not as simple as this and the reader is referred to the work of Belton and co-workers (Belton et al, 1992 and references therein) for a more complete discussion.

3.2. Applications

Laboratory low resolution NMR measurements have been carried out on a wide variety of systems, including many food systems and the results reported in the literature, (Padua et al, 1991; Lazaros et al, 1990; Guillou and Tellier, 1988, Rutledge et al, 1988; Defour, 1985; Tiwari and Burk, 1980). An excellent review of NMR applied to food systems in general and including low resolution measurements has been made by Belton, Colquhoun and Hills (Belton et al, 1992). As an example, Nicholls and De Los Santos have used low resolution NMR to study moisture content in corn gluten (Nicholls and De Los Santos, 1991). Figure 2, taken from their work, shows the ratio of the second echo intensity of a CPMG echo train (the liquid signal) to the full FID intensity immediately following the 90° pulse (the liquid plus solid signal) against moisture content determined from oven drying. An excellent correlation, typical of low resolution NMR moisture determinations is observed.

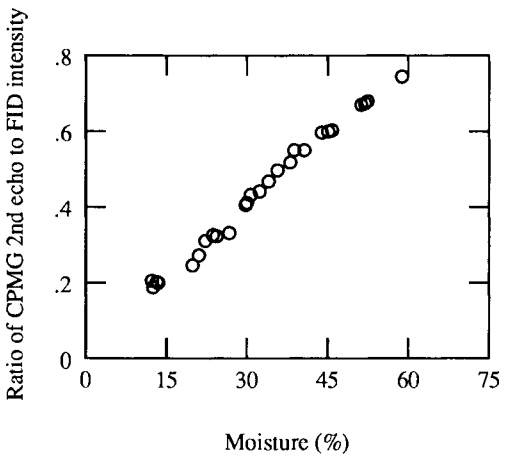

Figure 2. The ratio of the CPMG 2nd echo to FID intensity versus moisture content for corn gluten. (adapted from Nicholls and De Los Santos, 1991)

Other application areas for low resolution NMR include the monitoring of dehydration and rehydration and of freezing and thawing (Monteiro Marques et al, 1991; Zagibalova et al, 1979). In these cases it should be noted that as well as the total liquid / solid content, it is sometimes possible to infer information about the molecular environment of the liquid from the T_2 relaxation curve and hence information about the true reversibility of the process. The detailed shape of the very short T_2 decay part of the relaxation curve due to solids is generally non exponential. Although it is hard to measure accurately, the shape is sensitive to the degree of crystallinity, so that amorphous and crystalline phases can, in principle, be separated. In favourable circumstances, the long T_2 component is inversely proportional to liquid viscosity giving a further parameter which can be

inferred (Mora-Gutierrez and Baianu, 1989). The application of magnetic field gradients leads to the NMR signal being sensitive to flow and diffusion (Watanabe and Fukuoka, 1992) and NMR flowmeters have been developed (Pryakhin et al, 1989). Droplet size in emulsions can also be monitored because the T_2 relaxation of a liquid is sensitive both to restricted self diffusion and to variations in magnetic susceptibility. Finally, NMR can be used as a foreign body detector. However, the sensitivity depends very much on the existence of distinctly different relaxation behaviour in the sample and foreign body. Metals, which are usually an anathema to NMR spectroscopists, inhibit the working of the spectrometer by destroying the sample coil tuning and are easily detected.

3.3. Advantages and disadvantages

Four particular advantages of the low resolution NMR method as depicted by figure 1 over other non-NMR methods are generally recognised. Firstly, the NMR measurement is from the bulk sample rather than the sample surface, as is the case with infrared for example. Consequently, the measurement is unaffected by surface effects such as drying. Secondly, the NMR method is non invasive and non destructive and is thus suitable for on line analysis. Indeed it is not even necessary to touch the sample with a probe as with a capacitance test and so the sample may be packaged or in a pipe. Thirdly, no separate sample weighing is required since the solid to liquid ratio is determined directly. Finally, the method is very rapid. However, the sensitivity of the NMR signal to so many parameters (both sample related and experimental) implies that careful bench top characterisation of the product is required before on line use is begun. Moreover, it means that product formulation must be maintained for long periods without change if the technique is not to require regular recalibration. This may be a disadvantage. Under favourable circumstances the method is very accurate and moisture measurements to 0.4% accuracy have been suggested (Gribnau, 1992). NMR intensities and relaxation times are temperature sensitive and control, or at least monitoring, of the sample temperature greatly increases the accuracy of the NMR measurement. This may itself be done by NMR means. Appropriate methods include cross correlating two simultaneously measured NMR parameters and use of a calibrated NMR phantom in the spectrometer beside the sample.

4. ON LINE INSTRUMENTATION

4.1. Design considerations

In a few cases moisture measurements have been carried out on line, or at least beside a production line. In order to build an on line system a number of special spatial and temporal considerations have to be considered (Bjorkstam and Listerud, 1985). Most of these relate to the design of the NMR magnet and the radio frequency excitation coil. The sample must pass through both components simultaneously. Both must clearly be sufficiently large to accommodate the product but an overlarge magnet is unduly expensive and an overlarge excitation coil leads to excessive radio frequency power requirements and, due to a poor filling factor,

poor signal to noise. The design is severely constrained by a basic rule of NMR which states that the static and excitation magnetic fields must be orthogonal. Therefore, whilst a simple approach is to pass the production line axially through a solenoidal excitation coil it is not possible to use a solenoidal electromagnet for the static field as well. However, the production line may be passed between the poles of an iron yoke double E electromagnet or of a permanent magnet whilst using a solenoidal excitation coil. If this is not possible, it may be necessary to use other excitation coil geometries such as birdcage resonators or surface coils, both of which are well established for medical imaging applications. Birdcage resonators can be constructed around a cylinder with the excitation field parallel to one diameter. Generally, it is found that the excitation field is more uniform although a little weaker and the tuning more stable in birdcage resonators compared to similarly sized solenoids. Surface coils are flat and can be laid over the sample. They suffer from the disadvantage of small excitation volumes. So called one sided magnets which can be placed below a production line are also available but these suffer from the disadvantage that the homogeneous field volume is small and generally flat. It may not be representative of the whole sample if, for instance, the product settles and stratifies on a conveyor belt. Magnetic field strengths used in on line applications are generally of order 0.25 T corresponding to a hydrogen proton resonance frequency of just over 10 MHz. This limit is brought about by size and cost of the magnet and generally decreases with increasing sample volume.

A major design and cost factor relates to radio frequency power. In order to observe the short T_2 components of the FID, essential for a solids determination, it is necessary to have a very short and very intense excitation pulse. Apart from having large and expensive radio frequency amplifiers (and powers in excess of 1 MW have been reported (De Los Santos, 1994)), intense radio frequency pulses can be obtained by using highly tuned (i.e., high Q) excitation coils. However, equally necessary is a rapid recovery of the receiver system following the pulse and in particular a short ring down time for the coil. This generally requires lower Q. One option is to incorporate Q switching and active damping technology, another is to use separate receive and transmit coils (Fukushima and Roeder, 1981). Increasing the NMR frequency helps and also dramatically improves the signal to noise ratio of the experiment. However, the cost of the magnet can increase unacceptably fast. As a guide, for a fast recovery system with a sample approximately the size of a loaf of bread the required transmitter power will be of order 10-100 kW.

The required length of the magnet is determined by the speed of the production line and by the relaxation times T_1 and T_2. For a sample with a spin lattice relaxation time of 1.0 s and a production line moving at 0.2 m/s, a polarising magnet 0.6 m long is needed in order to produce an initial magnetisation which is 95% (three decay constants) of maximum. If the sample T_2 is 0.5 s then in order to see two decay constants of the FID the subsequent measurement magnet will need to be a further 0.2 m long. To this must be added the length of the product, say 0.2 m, giving a total length of 1 m. One large magnet may be used. If two separate magnets are used, the first can be of much lower homogeneity and can be set at a higher field strength so as to increase the magnetisation polarisation

and hence the signal to noise ratio of the subsequent measurement. The second needs to be of significantly greater homogeneity. Permanent magnets of this size, whilst relatively inexpensive, are notoriously heavy. Electromagnets can give greater field strengths but considerations of electrical power consumption and physical bulk tend to favour the permanent magnets. If the signal to noise ratio of the measurement permits, and only reproducible signal amplitudes are of interest, then it is possible to use shorter magnets. For instance, if 50% of the equilibrium magnetisation provides sufficient signal then the polarization magnet in the above example can be reduced in length to just 0.14 m. Moreover, if it is not required to sample the magnetisation beyond 100 μs of the FID, then the measurement magnet need be barely longer than the product.

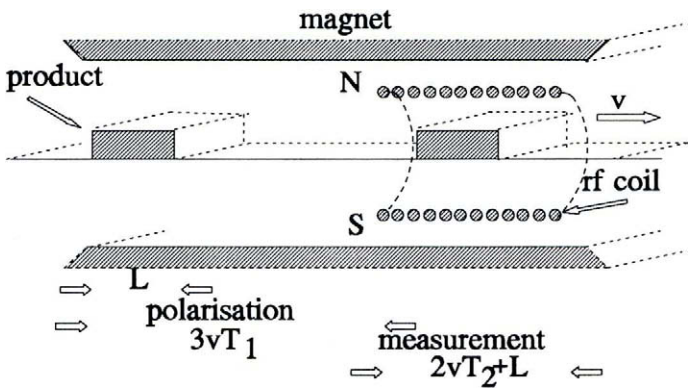

Figure 3. A summary of the design features for an on line low resolution NMR system around a conveyor carrying discrete products.

A number of the points discussed above are summarised in figure 3 which shows a schematic of a hypothetical low resolution NMR spectrometer around a conveyor carrying discrete products. Apart from the magnet and radio frequency coil, the main parts of an NMR spectrometer are the radio frequency receiver and transmitter and control computer. A small radio frequency amplifier is rack mountable, larger amplifiers are free standing. The remaining components are now usually housed in a desk top computer which also serves for data analysis and the output of feedback control signals to the process.

Environment presents another problem. Temperature stabilisation of the magnet is likely to be required in order to ensure field homogeneity and strength remain constant. This is more of a problem for magnets made from rare earth materials than iron but even these must be controlled in some way. Often this is done by having a frequency lock signal derived from a test sample in a small NMR coil near the main coil and sample in the magnet. The lock provides a signal for

feedback control to magnet. The homogeneity of the magnet is spoilt by the introduction of extraneous ferrous material. Fixed installations can usually be shimmed out by the addition of a set of shim coils carrying carefully preset small electric currents. However, these will not cope with other ferrous objects brought near the system and a ferrous exclusion zone needs to be set up around the magnet. Depending on the size and design of the installation, this may be 1 m or more in radius. Workers in the vicinity of the apparatus may require screening for heart pacemakers and other metallic implants. Some commercially available magnets have substantially reduced the surrounding fringe magnetic field by the incorporation of active shielding. Actively shielded magnets can be placed almost anywhere in the production line. The radio frequency irradiation will not penetrate through metal. Thus, for instance, metal pipes containing flowing product must be replaced by non metal pipes in the vicinity of the sensor coil if the coil is to be external to the pipe. However, the presence of a metallic shield around the sensor coil dramatically improves signal to noise by reducing the influence of external electrical noise pick up and a sensor coil within the pipe may be advantageous, especially as it leads to an excellent filling factor. For hydrogen NMR, hydrogen rich conveyor or pipe material gives a background signal which must be calibrated out if the material cannot be changed. Glass and PTFE are often useful construction materials.

Technical and economic considerations often suggest that in line sampling may be more appropriate than on line analysis of the total product being produced. Sampling can take various forms of which two are particularly favoured. The first is a scheme which momentarily stops a fraction of the product, normally by removing it from the line. The second is a scheme which analyses a more slowly moving fraction of the product on a thief or side line. With both these options magnet size, radio frequency excitation power and general engineering difficulties associated with the NMR are reduced and so, therefore, is cost. Moreover, more time is available for the measurement leading to increased accuracy and sensitivity of the measurement. However, a suitable sampling procedure must be developed if the results of the measurement are to be extrapolated to the whole line. In some cases (e.g., foreign body detection) sampling is clearly impossible.

4.2. Experimental systems

Only a very few specific on line and large industrial applications of NMR have been developed and a subset of these have been documented in the literature and are in the public domain. They have been developed for a variety of applications not all of which are in the food industry. What many of them have in common, however, is the acceptance of effective sample characterisation according to an NMR derived signal without undue detailed interpretation of the information being obtained. As yet, no manufacturer offers a standard large scale on line system off the shelf, indeed to do so would be very difficult as production environments vary greatly. However, larger scale modular systems should become available in the foreseeable future and this should reduce costs. Two groups have made significant contributions. The first is based at Southwest Research Institute, San Antonio, Texas, USA. The second is associated with RM Pearson now at Tri Valley

Research, Pleasanton, California, USA. With both groups a number of systems have been tested and successfully used in the field.

The first published references to the use of NMR as a process control technique were made relatively early by Nelson of Varian Associates and Reilly and Savage of Shell Development Company in 1960 (Nelson et al, 1960; Nelson, 1964) and discussed a spectrometer constructed five years earlier and installed in a pilot plant in 1956. This was before the availability of pulsed techniques. The system used continuous wave NMR to monitor the chemical shift spectrum from a liquid stream with the entire spectrum being recorded once every 6 s. To overcome the problem of polarising the nuclei a small sample reservoir was included within the magnet immediately before the sensitive volume. The sample tube was 3 mm in diameter and the sample flow rate a few centimetres per second. Faster flow rates were not possible as the sample was not then in the magnet sufficiently long to prevent broadening of the high resolution lines. Analogue electronics was used to lock the system to one line in the spectrum and automatic gain control was used to keep it at constant amplitude. Thereafter, changes in a second line of the spectrum related to compositional changes in the liquid. The equipment worked at 30 MHz. More recently, Tellier and co-workers have modified a high resolution NMR sprectrometer to monitor the ratio of water and CH_2 resonances in flowing fine meat pastes in order to determine the fat content (Tellier et al, 1990).

The development of a series of small nuclear magnetic resonance spectrometers for process control has been carried out by RM Pearson and colleagues, first at Kaiser Aluminium and more recently at Tri Valley Research (Pearson and Job, 1992; Pearson et al, 1987; Pearson and Parker, 1984). Initially, three small Bruker / IBM bench top systems were adapted for installation on line in Kaiser Aluminium oxide plants. They were able to satisfactorily measure the moisture in aluminium oxide on line although the instruments were far from user friendly. Subsequent spectrometers were better. A major problem for these systems was temperature stabilisation. Working at Tri Valley and with the aid of more modern computing systems and more advanced magnet technology a range of systems has been developed including one for measuring moisture and oil in cereals. As with the other applications already discussed, the systems are small and based on sampling a side stream of the main production line. The latest use Halbach magnets which are cubic with a side of approximately 225 cm and a sample access tube of over 3 cm diameter and are being developed for process control in a hot asphalt mix plant.

Much of the work at Southwest Research Institute is reviewed in a paper by Nicholls and De Los Santos (Nicholls and De Los Santos, 1991). They describe a system which encloses a side stream of the production line, a system based on a magnet which resides below the production line using one sided magnet and coil geometries and a system which resides below the line but samples from it into a more conventionally shaped spectrometer via a piston. A schematic, reproduced from the original paper, of this last system which has been built and successfully tested in the field, is shown in figure 4. It was developed under a US Department of Energy contract as a means for improving energy conservation in agricultural drying plants. A mechanical piston is lowered and product from the line falls into

a sample cell where it is measured. After the measurement the sample is returned to the line by the piston and a fresh sample taken. It is suggested that this has several advantages, notably the compactness of the spectrometer and the minimal changes required to the production line (drilling a hole in the bottom). No side streaming is required. The procedure is particularly suitable for dry products. It is possible to build standard reference samples into the piston which can be measured as the piston rises / falls so that temperature drifts and other extraneous problems are greatly alleviated. The one sided system they describe suffers from the disadvantage that only a small volume of the product is sampled and that it is always taken from the same area of the production line. The authors estimate that the cost of a sensor is of order $30-50,000 (1991) and that the payback period in a large gluten drying plant could be of order 1 year.

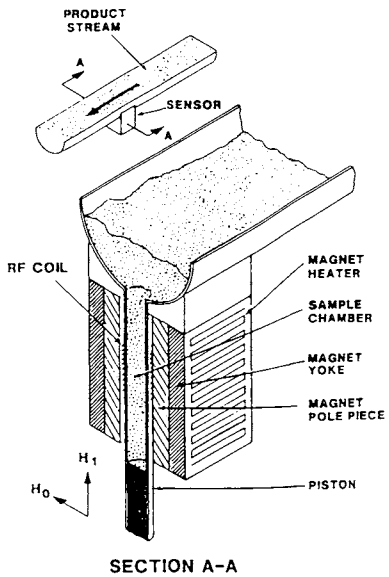

Figure 4. A schematic diagram of a piston sampling LR NMR sensor. The design is the subject of US patent No. 5129267, 1992. (adapted from Nicholls and De Los Santos, 1991)

It is worth mentioning, if only for curiosity, that workers at Southwest Research Institute have also developed an NMR sensor which can be transported on the back of a tractor (Paetzold RF et al, 1985). This machine has been successfully used to measure sub surface soil moisture at a depth of a few cm. Other large spectrometers developed at Southwest Research Institute include a baggage handling system designed for detecting narcotics and plastic explosives in suitcases. (De Los Santos, 1994).

Bore hole NMR logging tools (Kleinberg et al, 1992) are another excellent example of NMR spectrometers developed to work in extremely hostile

environments. Complete excitation coil and magnet assemblies are dropped several kilometres down bore holes in the search for oil by the petroleum industry. The sensitive volume is developed outside the sensor so that actual sampling of the liquid in the rock around the hole is possible.

A small transportable NMR system for control applications is now marketed by ATI Instruments. This system uses a condensed field magnet weighing only 45 kg, about 20% that of the equivalent electromagnet, and operates at the relatively high field strength of 1.4 T (60 MHz). It offers 1 cm sample diameter access. The applicability of this system has been demonstrated for applications in the food, petroleum, pulp and paper industries. However, most activity has concentrated on applications to polymers including additive concentrations, conversion rates, %polymer in recycled monomer and process stream composition. Snoddy (Snoddy, 1993) has used the spectrometer to determine the viscosity of polymer samples in a simulated production environment. The polymer viscosities were in the range 3300 to 5000 cP and the viscosity could be determined in less than 1 minute with a standard error of 112 cP.

5. CONCLUSION

NMR undoubtedly has the potential to be used successfully for on line process control and quality assurance applications in a number of environments and for various industries including food. The basic low resolution NMR techniques which can be used to determine, for example, moisture content are well established. The means of building large and unusually shaped magnets and coils for use in hostile environments has been demonstrated. The cost, although expensive, need not be exorbitant and pay back can be in just a few years. This potential has been recognised many times before. It remains to be seen if at last some of the prejudices which have prevented NMR being widely taken up will at last be overcome.

ACKNOWLEDGEMENTS

The author is grateful to Dr EG Smith of Unilever Research Port Sunlight Laboratories UK and Dr CI Nicholls, now at SMIS Ltd UK for helpful discussions relating to the preparation of this manuscript.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 3

On-Line Quality Control: Advances in Sensor Technology

Frank W. Henrikson

Center for Advanced Food Technology (CAFT), Rutgers-The State University of New Jersey, New Brunswick, New Jersey, 08903-0231, USA

1. INTRODUCTION

The importance of high quality and efficiency in food processing is growing as consumer demand for new and better products at lower prices expands. The success of new product introductions lies with consumer acceptance of key food attributes such as texture, color, flavor, freshness, and nutrition. Automation in food processing allows for control of the consistency of these attributes by measuring a specific property, adjusting processing conditions to maintain the attribute associated with the property, comparing the measurement to predefined specifications and through computer control, adjusting the process to maintain overall consistency. Such automation is essential to the establishment of dynamic, flexible, and competitive manufacturing technologies. Well positioned, on-line[+] sensor technology is at the core of efficient process control and assurance of high product quality. Measuring physical and chemical attributes of food materials in real-time must be achieved to meet process control targets and ensure product quality.

Traditional measurements, done off-line in a laboratory, are performed under controlled conditions. Since these situations do not mimic actual processing conditions, such measurements will obviously be of limited usefulness for real-time process control. In addition, with off-line measurements, a significant time lag will often occur for results to be translated into process adjustments, thereby creating the possibility that significant production runs will be compromised.

In-process measurements, in contrast, allow real-time determination of chemical and physical properties thus allowing immediate feedback for process control leading to optimization of attribute quality. On-line measurements, therefore, save money in terms of both loss of product and efficiency of process.

[+] The term "on-line" is used to refer to real-time measurement during processing. In this sense, it includes both in-line measurement (within a processing system) and on-line measurement which may use a side-stream bypass of material for measurement.

This chapter will focus on the types of sensors currently available for use in food processing systems and the new technologies emerging from research laboratories throughout the world.

The importance of on-line sensors to the food industry was noted by the U.S. Department of Energy (DOE) in a January, 1990, report entitled "Assessment of Sensors Used in the Food Industry", prepared by the National Food Processors Association [1]. The DOE supported this study because of its interest in activities to improve the efficiency of energy conservation and utilization systems. The DOE believed that in-line sensors in food processing could achieve these goals. The study was based on interviews with more than 50 representatives of food companies, food equipment manufacturers, and representatives of sensor manufacturers and suppliers and 13 industry, academic and consortia sensor research and development organizations. These people were selected to determine the current status of food industry process control sensor technology. Instrument manufacturers were contacted to obtain information about current capabilities and market status; food processors were contacted to identify available technology and assess its strengths and weaknesses.

A consensus emerged from the DOE survey as to characteristics of an ideal on-line sensor. Table 1 lists the most important of these characteristics. Other studies of industrial needs found support for similar requirements [2-5]. For example, Kress-Rogers [2] also found that an ideal sensor should be free of catalysts that might enhance oxidative processes in the food. In addition, total costs including capital, maintenance, and operating, should be low in relation to the benefits of overall process control.

Table 1
Characteristics of an ideal on-line sensor

- Accurate
- Reliable
- Low cost
- Easily maintained
- Tolerant of harsh food processing plant conditions such as vibration, environment laden with moisture, dust, or particles, high cooking temperatures, wide temperature cycles, chemical sanitizing and cleaning, exposure to oils and solvents, and abrasion and impact from passing product

The DOE survey also examined the most important in-process measurements to the food industry addressing process control as well as quality assurance. They are listed in Table 2 [1-6]. Giese [4] provides details about various tests and measurements that can assist in quality assurance during food processing for production of safe, wholesome foods. These tests include immunoassays, near infrared spectroscopy, chemical sensing, and color measurement.

Table 2
Priority sensing needs for food processing

- Moisture in solids
- Humidity
- Chemical composition
- Rheological properties
- Presence of foreign matter
- Soluble solids

Scientific understanding of material properties and sensor technological developments are emerging to address the needs identified by food processors. The food industry has generally lagged behind other process industries in the use of sensors and related microprocessor control instrumentation. Among the more important reasons for this are the variability in raw ingredients used in food processing, the complexity of food materials, the lack of availability of on-line sensing systems that can perform in harsh food processing environments, and the clean-in-place needs for safe food processing systems [2, 3, 7].

New sensors are being developed in areas such as moisture determination to meet these identified needs, by a variety of research laboratories and research centers worldwide [1]. At consortia such as the Center for Process Analytical Chemistry (CPAC) at the University of Washington (Seattle, Washington, USA); the Center for Advanced Food Technology (CAFT) at Rutgers, the State University of New Jersey (New Brunswick, New Jersey, USA); the Japanese Research and Development Association for Sensing in the Food Industry (Tokyo, Japan); the Leatherhead Research Association (Surrey UK); and Campden Food and Drink Research Association, (Campden, UK); sensors are being developed through partnerships of material and sensor technology researchers, sensor manufacturers, and food processors.

For example, at the Center for Advanced Food Technology, faculty in food science and a variety of engineering disciplines work with sensor manufacturers to place the faculty research and technology developments in sensing systems which meets the needs described above. These systems are then tested in the food processing plants of Center industrial members. This overall effort allows the development of new sensors which address industrial needs, with shared technology development risk by the companies and sensor manufacturers, offset by government funds.

Many other contributions from linkage of individual research organizations and instrument and sensor manufacturers are also bringing new sensor technology forward. It is important to remember that it is only in recent times that sensor manufacturers developed the capability to manufacture sensors which can be used in food processing environments. This is due to the emergence of new materials, new micro fabrication and miniaturization strategies, and the

development of knowledge-based computer technologies such as fuzzy logic [8] and expert system neural networks [9, 10] which help accommodate for the variability in process measurements.

2. TYPES OF ON-LINE SENSORS

A new generation of sophisticated analytical sensors is beginning to allow real-time on-line information on the plant floor of food manufacturing operations. There are a number of different ways to classify and describe on-line sensors for processing systems. Sensors may be invasive or non-invasive. Due to the harshness of food processing environments, it is always desirable to use sensors that do not contact the food system. However, in many situations including some of those involved in measurements of food ingredient properties or chemical reactions of food components, currently available sensors must come into contact with the food material [11].

In choosing which sensor to use for a particular application, selectivity, sensitivity, or cost may not be the only factors which must be taken into consideration. The sensor technology, whether optically, electrically, or acoustically based, is also a factor . In certain processing systems, for example, signal interference would preclude the use of an electrically-based sensor. If a sensor was to be used in a microwave oven environment to measure moisture, the components of an electrically-based system would be unacceptable. Even with an optically-based sensor, all measuring components within the oven cavity must contain no metallic components, including the protective sleeves used to shield the fragile optical fibers.

Sensor selection can also be made based on the active material used in the sensor and how it will react with the food materials in the system within specific temperature ranges. For example, in a high temperature baking process, ceramic materials may be a better choice for use in making moisture sensors than polymer-based materials since they can withstand much higher temperatures without being damaged.

Physical sensors and analytical sensors will measure different food characteristics and may, therefore, be used under different circumstances. For example, if the total weight of a product is the only measurement of interest, a classic load cell would be an appropriate choice. However, if the ratio of solid to liquid were desired, an ultrasonic sensor or one of the emerging microwave sensor technologies would be more appropriate. Kress-Rogers [2] illustrates these differing types of measurement capabilities and sampling techniques.

Sensor selection will also be based on consideration of the location in the process a measurement is needed. On-line measurements can be made at varying points during a process as shown in Figure 1. To select the ideal sensor for a given use, therefore, one must consider the selectivity for the property to be measured, the process operating conditions including the potential for electromagnetic interference caused by the process, the temperature, and potential interfering chemical species.

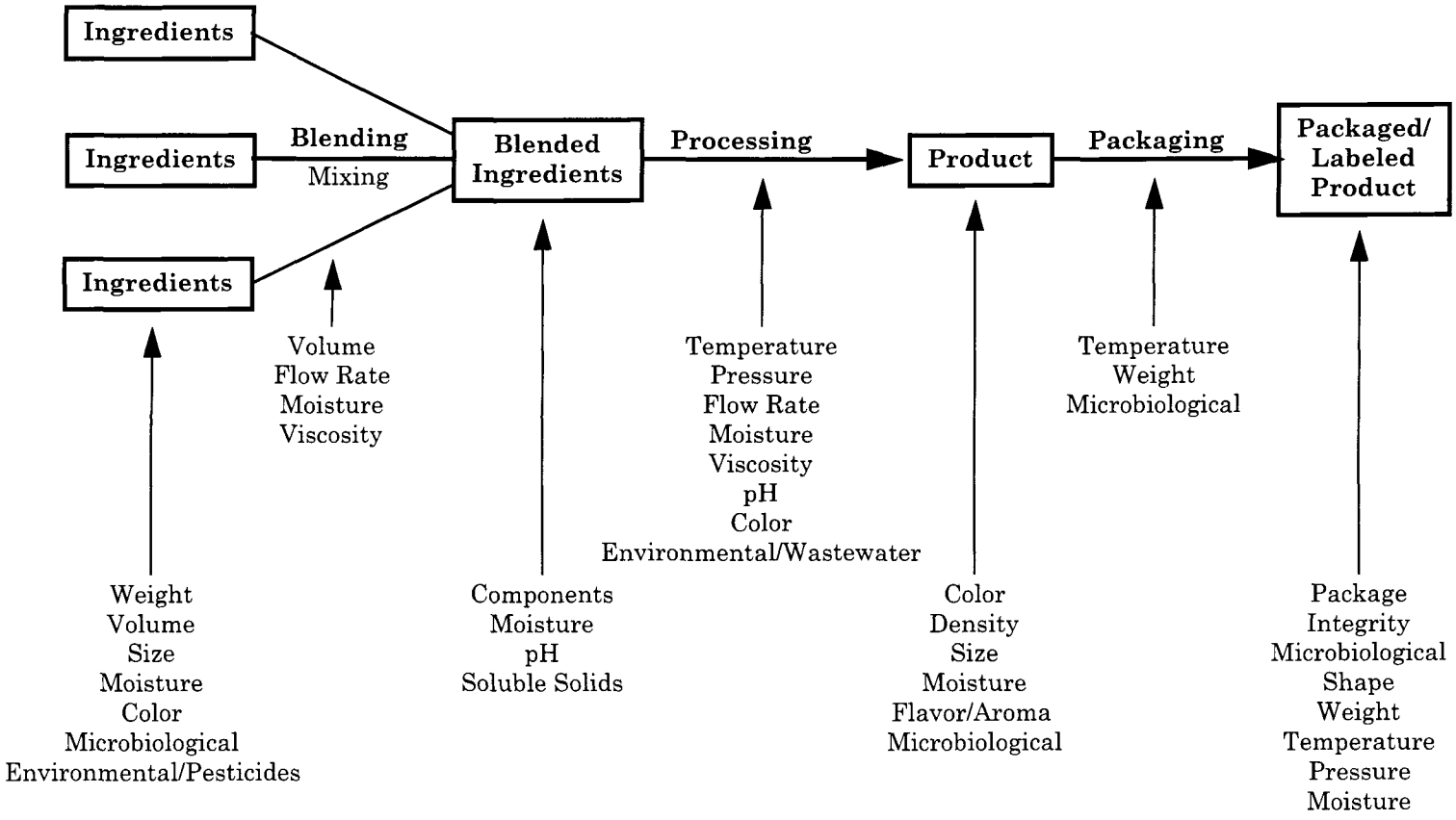

Figure 1. Important applications for sensors in a generic food manufacturing operation

2.1. Sensing technologies

The various sensing technologies in use today correlate a specific property or molecular signature of the measured system with a fundamental property of the sensing technology or sensing material [10]. For example, if one were measuring the amount of moisture in a food process with a moisture absorbing thin polymer film, electrical impedance of the film might be measured with no water present as a base line. Moisture absorbed by the polymer would change its impedance. A good sensing material would come close to showing a linear change with the moisture. Such new materials are emerging from research laboratories. Many of these are not yet patented. A great need for further understanding of new sensor materials remains because the sensing properties are extremely dependent upon their surrounding environment. For instance, some good polymer-based moisture sensing materials are seriously affected by other chemicals, particularly alcohols, and they can temporarily or permanently lose their measuring capabilities in the presence of such chemicals.

2.1.1. Optically-based sensing

Optically-based sensing was identified at the beginning of the nineteenth century. The scientific principle has taken a long time to be adapted to a viable sensing technology. Considerable information about the optical properties of the measured systems is required to develop such technologies; much of this is still the subject of research studies [12]. Optical spectroscopy operates according to the following principle, schematically illustrated in Figure 2. A light source is focused into a fiber optic cable which transmits the light with very little loss of strength to a sensor. The sensor interacts with the environment or food system to be measured either physically by absorption, or chemically with altered properties. This change in the sensor usually absorbs and reduces the light signal. After continuing through the fiber to a detector, the incoming and detected signals are compared and the change in signal correlated with the attribute environment being measured.

Optical sensing technology became recognized as a valuable measurement tool in the flour, grain and forage industry in the mid 1960's. Its background and principles are well described by Scotter [13]. Improved spectral discrimination of instrument output as well as the development of new optical performing materials have led to on-line application in determining fat, protein, alcohol and moisture [4, 13]. The technology has continued to develop and more specific capabilities have begun to emerge in practical sensing systems [14, 15].

For example, specificity of optical sensing has been expanded using the electromagnetic spectrum from the visible light range, to near infrared, to far infrared. Also, new optical materials have been developed that reduce signal loss and extend the useful signal transmission range making on-line use very attractive. For example, the Fiber Optic Materials Research Program at Rutgers University has developed porous fiber optic materials which can be used in conjunction with specific chemically sensitive compounds to sense a range of properties including moisture, pH, NO_x, SO_2, and H_2S [14, 16, 17]. Others have investigated temperature and thermal profiling [18]. Optical sensing technologies

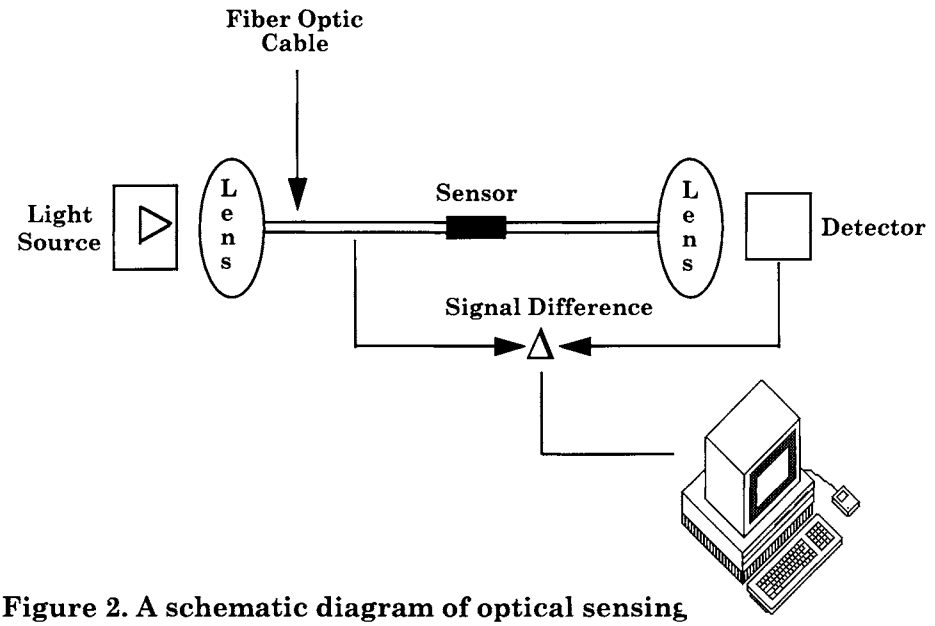

Figure 2. A schematic diagram of optical sensing

are being developed to meet needs in the processing of cereals, breads, cookies, dairy products, and fruits, as well as to measure properties of flour and other ingredients [13, 19-21].

One of the more significant areas of development that has aided in new use of optical sensing is the source of light. The light emitting diode (LED) and laser diode (LD) have made it possible to obtain much stronger light signals in narrow frequency ranges. This has helped overcome selectivity and signal to noise issues which affect the ability to effectively correlate measurements.

Several other optical sensing technologies should be noted. Using optical sensing, the fluorescence properties of certain materials can be exploited. On-line moisture sensing developments using a polymer based fluorescent film have been reported by Pedersen, et. al. [22].

Color monitoring with infrared technology and color machine vision also have new on-line quality measurement capabilities to determine, for example, the effects of thermal processing on peas and carrots [15, 23]. Machine vision provides new techniques for inspection and placement of packaged food placeables, and certain inspections for product packaging [24].

2.1.2. Electrically-based sensing

Electrically-based sensing often uses ceramic or polymer materials that specifically correlate emf (voltage), impedance, or dielectric constant with the desired measurement property. Such sensing operates according to the following principle, shown schematically in Figure 3. A sensor, placed into an electrical circuit, reacts with moisture, heat, pressure, or some other property in the food

environment, or by chemical reaction with the food system, causing a perturbation in the electrical properties of the sensing material. This change can be measured and correlated with the attribute of interest.

There are three major types of electrochemical sensors being used for process measurement: ion-selective electrodes (ISE), ion-selective field effect (ISFET) transistors and metal oxide gas sensors [25]. The most commonly used ion-selective electrode is the glass pH sensor, an off-line device. This device cannot be used on-line in food processing operations due to its fragile glass structure [26].

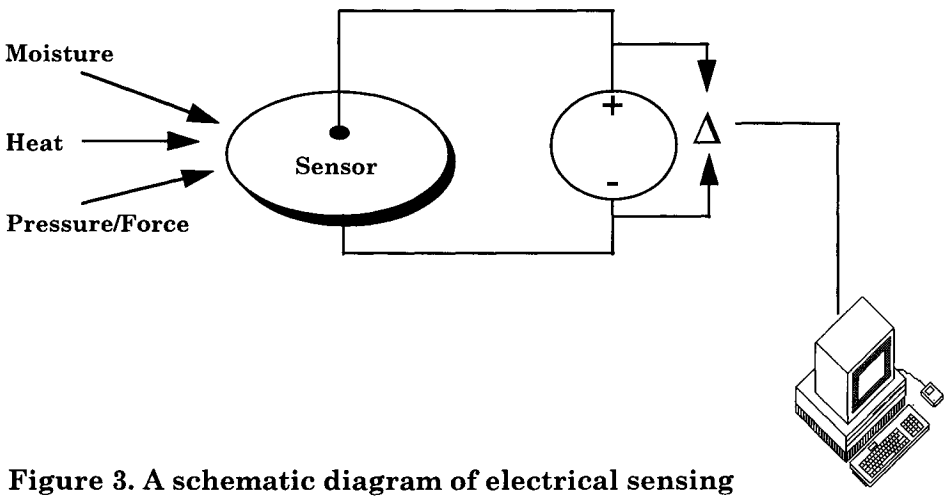

Figure 3. A schematic diagram of electrical sensing

To overcome this problem, ISFET sensors became important. The development of an ISFET sensor, particularly for pH measurement, was aimed at overcoming the hazard of the fragile membrane of the ion-selective electrode. Various insulating oxide films such as SiO_2 and Al_2O_3 have been investigated to optimize performance of the ISFET over a wide range of processing conditions since this type of sensor is influenced by a variety of ionic materials (e.g., Na^+, Ca^+), and temperature. The drawback to these for on-line use is that the adhesives used in their manufacture generally do not withstand the higher temperatures used in some food manufacturing processes [27].

Metal oxide gas sensors may hold the most promise for use in high temperature food processing applications. They are widely used for measuring ethanol during fermentation processes [28]. These sensors react with specific chemicals in the environment resulting in a change in conductivity or impedance. The complexity of most food systems makes understanding ion selectivity key for use of such sensors. Again, the research community continues to investigate improved materials for potential applications to other gases that may be related to product formulation (e.g., butanol, acetone, formaldehyde, etc.) [29, 30].

Polymer-based sensors represent another major class of electrically-based on-line measurement tools. Recent developments have given rise to a variety of commercial sensors based on the measurement of conductivity or impedance of a polymer film as it reacts with the food processing system environment. Early applications of polymer sensors were developed using piezoelectric materials to measure force [31]. Both piezoelectric and other thin film polymers are under study for use in higher temperature on-line moisture measurement [32, 33].

One area where thin film polymers have recently begun to see utilization is in the electronic nose. Using arrays of sensors with either a lipid analogue or a tin oxide membrane supported on a polymer base, the response pattern to electric potential is determined. Multivariant analysis and statistical evaluations with techniques like the chemometric technique developed at the Center for Process Analytical Chemistry are applied to develop and correlate pattern recognition [34, 35]. The concept of using flavor/odor analysis for product quality determination has great appeal. Currently, research is occurring toward discrimination of coffee blends and roasts. Considerable application development is still required. Advanced sensors in this area to measure flavor/aroma would find many opportunities for use including peanut roasting and snack foods.

2.1.3. Other sensing technologies

The use of microwave sensing to date has been primarily associated with bulk moisture determination [28]. Dielectric constant and loss factor are properties of food materials ideally suited for microwave determination of moisture content. Since, microwave instruments are dependent on sample density, their broad application is limited due to the difficulty of measuring density on-line. More recently, a new two-variable technique has permitted development of a more compact and inexpensive instrument. In addition, on-board microprocessors and data storage have enhanced data acquisition and processing capabilities [36, 37]. A better understanding of food dielectric properties coupled with microwave advances provides opportunities for further on-line exploitation. The principle advantage of this instrument is its non-intrusive capability. The cost/benefit ratio may still be too high relative to other developing moisture sensors to see utilization broadly increased.

Nuclear magnetic resonance (NMR) must be mentioned as a potential on-line process sensing tool. While NMR has been extensively used in the laboratory to study the relaxation time of food matrices leading to determination of bound and unbound water, its cost is prohibitive for on-line use. However, new magnet designs that are far less costly as well as development of innovative food transfer systems through the magnet are under study [1, 3, 38]. Chapter Two of this book focuses on the use of NMR for on-line quality assurance.

The use of thermal diffusivity/thermal conductivity to correlate with food moisture content has been developed into a new sensor for highly viscous materials such as doughs, cheeses, and emulsions and for measurements in powder materials. The sensing system, measuring the rate of heat absorption to the food sample, provides a correlation with moisture content and thermal conductivity of the sample from its calibrated database [39-42].

Another sensor technology receiving increasing attention and investigation is ultrasonics. Ultrasonic measurement in food systems is of high interest, like NMR, because of its non-intrusive nature. Ultrasonic technology is well established for determining fill levels and for in-process metering and has been adapted to determination of solids content in simple solutions. These sensing techniques use the principle of determining sound absorption, reflection, and phase shift to correlate with properties of the system attenuation [43]. As more complex food systems are investigated, particularly nonhomogeneous media such as doughs or cheeses, signal interpretation becomes limiting. Nevertheless, a number of applications of ultrasonic technology to emulsions, milk, temperature measurement, and determining the quality of fresh products, have been reported [44-47]. Chapter Four of this book provides more information about ultrasonics.

2.2. Sensing methods

2.2.1. Physical sensing

Clearly, beginning with the receiving and storage of ingredients, through blending, processing and packaging, measurements of temperature, pressure, weight (continuous and discrete), size, shape, fluid flow of gas and liquids, viscosity, and liquid level, are important for process and product quality assurance. Most of these sensors are currently in use by the food industry. New developments in computer-automated control will assist in fine tuning their use [48]. In addition, food industry application of on-line rheometry for viscous materials like doughs is also emerging [49-51].

2.2.2. Chemical sensing

Chemical sensors are used to measure a variety of chemical and biochemical components in food systems. Physicochemical sensors are emerging as important to address the measurements of water (bulk moisture, gaseous moisture, and humidity); fat; protein; carbohydrate; chemical composition; soluble solids; and color. Measurement of some combination of these parameters often opens up a new control strategy toward optimizing product quality.

Companion to physical measurements, there are number of chemical parameters of significant influence to food quality. Their measurement will provide the specificity for good control of product manufacture. Chemical measurements include pH, ionic species (e.g., chloride), odors, flavors, dissolved oxygen, developed gases, and alcohols, as well as measures of freshness and ripeness. While most of these parameters can be measured off-line, new on-line sensors are emerging [19].

Biosensors are often defined as sensors which use a biologically sensitive material producing a biochemical signal that is converted by a transducer into an electrical response. The biomaterial is generally placed on or into a membrane. An important advantage of a biosensor is selectivity and the capability to detect very small amounts of such compounds as glucose. Sterilization, or exposure to harsh conditions in a food process are often fatal to a biosensor. Enzyme-based

biosensors used in food measurements generate signals from chemical reactions. Immunosensors are based on antigen-antibody reactions. Currently, the main application of these latter biosensors is in the pharmaceutical industry. Enzyme-based biosensors are commercially available; glucose and lactate are the two main analytes for which instruments are available and routinely used [52].

2.2.3. Microbiological sensing

Rapid microbiological determination increases production efficiency by reducing the time and interim storage needed until food can be considered safe enough for distribution. Again, off-line technology is capable and accurate but inadequate for the increasing throughput of modern food processing. Rapid measurement is needed, including bacteria, mold, and pesticides [15].

3. FOOD PROCESSING SYSTEM SENSOR NEEDS

As the research and development community of the food processor, academia, and the instrument manufacturer responds to the challenge of achieving more efficient processing and better product quality, novel on-line sensor technologies are emerging. The following sections offer suggestions for application of previously described sensing technologies to a variety of food processing systems. While any particular on-line sensor may not be applicable to a proprietary industrial process, the generic sensing suggestion may offer directions to the system and process engineers charged with improving production efficiency and product quality.

Besides the on-line measurements that a sensor can provide, smart sensor packages can improve the collected data and suggest better control strategies for the processor. Smart sensors are instruments that generally combine two or more measurements interpreted by a computer or microchip to improve the information output [55]. For example, if pH were the critical parameter in the process, multiple pH sensors might be desirable to assist in accurate process control. pH sensors can be multiplexed together and computer statistical analysis performed. In most on-line instruments today, a computer or computer chip are an integral part of the system to analyze, correlate, or make temperature corrections for the measured data.

3.1. Baking

Different types of measurements are important at different points in the baking process as indicated in the generic process shown in Figure 1. In particular, there are some unique requirements because of the high temperatures encountered during the actual baking. This is also the time when the ability to control processing parameters can influence final product color, texture, and flavor, so feedback to control earlier process steps can be important. For example, in a baking process, it might be important to know the moisture and protein content in the flour because of the critical effect on structure of the product. While other components are also of significance to the quality of baked products,

protein has the principle effect on loaf volume. The accurate weight and volume of mixed ingredients and the viscosity of the blend are also important to quality [56]. Other important factors include knowledge of the stage of fermentation, moisture content, and color of the product.

There are a number of on-line sensors that are likely to be of use to the baking industry with its higher temperature requirements. For example, moisture and humidity measurement are of interest in many of the processing steps. Research at the Center for Advanced Food Technology is beginning to result in on-line sensor prototypes that address moisture with new sensors [57-59].

Other moisture sensors are in development to address the higher temperatures encountered in ovens. Infrared systems are being used to examine on-line product moisture at various process stages. Scotter describes the use of NIR for determinations of fat, protein, and carbohydrate [13]. To assist in making these measurements, instrument manufacturers have been developing unique on-line test cells and increasing the measuring distance from the test cell to the remainder of the instrumentation with better fiber optics and microelectronics to allow on-line use [60].

Other moisture sensor research efforts are aimed at developing lower temperature porous fiber optical sensors, fluorescence optical sensors suitable for food environments, and polymer-based thin film sensors capable of operating in environments to 180°C [58].

3.2. Meat/fish processing

In meat processing, the most significant areas of interest concern meat aging, decomposition, and bacterial inspection as part of overall quality assurance programs. In fish processing, moisture content and fat content are also significant. The Japanese Research and Development Association for Sensing Systems in the Food Industry, a partnership of food processors and sensor manufacturers, has undertaken studies in a variety of areas including meat and fish. They have reported significant progress in developing quality assurance sensors which promise to improve food processing operations [61].

For example, a multisensor biosensing system was developed that measures two diamine compounds, putrescine and cadaverine, as indicators of meat decomposition. For investigating bacterial growth, the Japanese R&D Association identified a fluorescence method that detects coliform colonies within six hours. Sensitive instruments are under development.

Kress-Rogers, et. al. [62] report on measurements of meat freshness using a prototype knife-like probe with a biosensor array that measures glucose concentration at depths of two to four mm below the meat surface. It is reported that the probe is ready for consideration for commercial development.

In fish processing, fat and water content are of interest. A compact microwave instrument to measure the fat content of a variety of fish species relies on a microstrip sensor which has been demonstrated with herring [63].

The Japanese R&D Association reports high interest in processing of surimi products. pH was shown to be a good measure of quality and the development and use of an ISFET type probe provides good data in short time periods [61].

3.3. Dairy processing

The ability to examine the composition of milk and milk products such as cheese and ice cream is of interest to meet standardization requirements. Determination of total fat and solids in milk, moisture and fat in butter and margarine, and moisture content in processed cheese are among important characteristics to be measured.

Wide variations in butterfat content of milk have been reported [60] and the production and cost savings derived from using a process control system utilizing on-line NIR measurement described. The Japanese R&D Association also describes the development of special sampling systems together with the use of NIR to establish on-line process control [61].

Two developments related to cheese manufacture are of significance. A fiber optic sensor was developed to measure the changes in diffuse reflection of coagulating milk using NIR reflectance. Time correlations between enzyme addition and signal pattern were developed to predict cutting time, defining feasibility of the technology [64]. Moisture measurement in cheese using NIR [60] and a new thermomoisture probe [39] were also reported.

Aseptic milk packages provide an interesting application for non-invasive sterility control using ultrasonic measurement. By transmitting an ultrasonic beam into the package inducing acoustic flow streaming, it was shown that the velocity spectrum measured by the Doppler shift of reflected sound decreased in packages with microbial growth [65].

3.4. Fermentation

Several organizations have reported sensor developments aimed at automation in fermentation processes. The Japanese R&D Association investigated techniques for quality improvement in yogurt production by lactic acid fermentation processes. A key part of the system includes the development of a fully automated pH sensing system [61].

Guenneugues, et. al. [66] report that an NADH fluorescent biosensor has been demonstrated to indicate the end of the process in lactic yeast fermentation of deproteinized whey substrates. Tests in batch processing were precise; continuous processing disturbances require further study related to each specific process.

In another research study, different types of biomass sensors were examined and their performance compared with the NADH fluorosensor [67]. Results were inconclusive but the researchers suggest that a knowledge-based computer system might be feasible to better interpret the biosensor information.

3.5. Beverages

The beverage industry includes a wide variety of products with attendant varying measurement needs. The use of refractometers has been well accepted for determining component concentrations, for example, sucrose in water (for soft drinks) solids in orange juice, and solids in milk [28]. The potential for color sensing in conjunction with knowledge-based intelligent computer software has been reported in coffee processing [68].

More recently, the application of an electronic nose using an array of twelve tin oxide sensors was shown to discriminate between both the blend and roasting level of coffees. This technique depends on multivariant analysis of the sensor data for specific classification [34]. A multisensor array with lipid membranes supported on polymer films was also shown to discriminate between different beers and different coffees [35].

An interesting approach for screening fruit juice authenticity uses NIR and multidimensional discriminant analysis [69]. A new enzyme biosensor has been developed to measure aroma of green tea. Screening was of interest because of the value-added aspect of certain high quality green teas in Japan. The amino acid oxidase and oxygen electrode were found to be rapid and easy to use [70].

3.6. Fats and oils

Fats and oils are of interest as product ingredients as well as for use in frying processes. Studies conducted at the Leatherhead Food Research Association investigated monitoring of frying oil quality to ensure wholesome fried products and adequate shelf life. Kress-Rogers, et. al. [71] discuss the development of a prototype probe to monitor viscosity of frying oil. The probe incorporates two vibrating rods where dampening of the vibrations is indicative of viscosity. The use of NIR has also been reported for determination of fat and oil content [61].

3.7. Packaging

Packaging and package seal integrity directly affect the shelf life of products. On-line determination of integrity could significantly improve the efficiency of food packaging operations since off-line evaluation might cause rejection of an entire batch of a packaged product. The Center for Advanced Food Technology has surveyed leak detection technology and reported a need for new developments [72]. Other developments in this area include a new ultrasonic detection techniques for aseptic milk packaging [65].

3.8. Fruit and vegetable processing

Several new studies have been reported on the use of NIR and ultrasonic examination of fruits and vegetables for identification and freshness. The NIR sensor study showed that apple cell walls have a characteristic spectrum that indicates the capability of authentication of fruit-containing product using this technique [73]. Discriminant analysis was used for positive quantitative identification. Scotter and Legrand [69] also showed NIR screens of fruit juice authenticity using discriminant analysis.

An ultrasonic study of color and ripeness of melons indicated differing attenuation correlating with these characteristics. Further studies of this concept were recommended [47].

3.9. Pasta processing

In pasta processing, controlled drying is very important to ensure product quality. Moisture sensors that can be used on-line in production dryers will impact on processing capability. The Japanese R&D Association reported the development of a ceramic zirconia oxygen type moisture sensor capable of

measuring moisture up to 150°C [61]. The Center for Advanced Food Technology has also reported development of a fiber optic sensor that can measure moisture to 300°C and is useful in both dryers and baking ovens [59].

4. CONCLUSIONS

On-line sensor technology is currently emerging to address both process efficiency and quality improvement in food processing operations. Processing of food products is complex, involving many steps which, when controlled, can have a direct effect on the quality of the product as perceived by the consumer. To control a food process, sensor technology can be coupled with process control systems. This technology can take many forms, from simple physical measurements of material weight and ingredient size, to complex analytical chemical and biosensors which can measure chemical reactions as they are occurring during food production.

The challenge for food processors, therefore, is to select the best sensor and overall process control strategy for a given process. This involves knowledge of sensor technologies and types, as well as knowledge of the process and the steps which need to be monitored and controlled.

At the research level, identification and synthesis of new materials is a key factor in the development of new sensors for on-line use. Cooperation between the material scientist, the food scientist, the physical chemist, the physicist, and the engineer is important to be able to understand and tailor sensor performance for the on-line processing environments of the food processor. Such research studies are giving rise to new sensors including polymers, ceramics, and new optical systems.

It is hoped that this chapter will provide readers with the tools necessary for selection of sensors for targeted applications. Although further research and development is necessary to bring many of these emerging sensor technologies to the market, successes like those described from laboratories at the Center for Advanced Food Technology, the Center for Process Analytical Chemistry, the Japanese Research and Development Association for Sensing in the Food Industry, the Leatherhead Research Association, and the Campden Food and Drink Research Association, illustrates that sensing technology is coming of age in today's food processing industry.

ACKNOWLEDGMENTS

This is publication No. F 10550-1-95 of the New Jersey Agricultural Experiment Station supported by State funds and the Center for Advanced Food Technology (CAFT). The Center for Advanced Food Technology is a New Jersey Commission on Science and Technology Center. The author wishes to thank Gayle K. Stein for technical writing assistance and Johanny Muniz for assistance in manuscript preparation. Secretarial assistance of Valentina Rahalewicz is also acknowledged.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 4

Ultrasonics in Food Processing

D.J. McClements

Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA

1. INTRODUCTION

Ideally, a food manufacturer would like to take a combination of raw materials and convert them into a high quality product at the lowest possible cost. This conversion is achieved by subjecting the raw materials to a number of processing conditions, e.g., heating, cooling, pressure, shearing or mixing. Inherent variations in the raw materials and of the processing conditions mean that the properties of the final product vary in an unpredictable manner. To control and minimize these variations food manufacturers need to characterize the properties of the raw materials, and to monitor the food at each stage of processing. Food processing operations are becoming increasingly sophisticated and are often computer controlled. Traditional chemical and gravimetric techniques are time consuming and laborious to carry out, and so there has been considerable motivation for the development of rapid analytical sensors for monitoring the properties of foods.

Ultrasound utilizes interactions between high-frequency sound waves and matter to obtain information about the composition, structure and dimensions of materials through which it propagates. The power levels used in ultrasonic testing are so low that the properties of the material are not altered, thus the technique is *non-destructive*. This is in contrast to high energy ultrasonic applications which are sometimes used in the food industry for cleaning, cell disruption, heating, sterilization or emulsification purposes [1].

A wide variety of different applications of ultrasound to foods have been developed over the past 50 years or so, reflecting the diversity and complexity of food materials, and the versatility of the ultrasonic technique [2-6]. Even so, ultrasound has still not found wide spread use in monitoring food processing operations. This situation will almost certainly change in the near future. Advances in microelectronics have made available sophisticated electronic instrumentation capable of making accurate ultrasonic measurements at relatively low-cost. The interaction between ultrasound and many microheterogeneous materials is fairly well understood, and there are mathematical formulae available for interpreting ultrasonic measurements in a number of systems relevant to the food industry. Finally, ultrasound offers a number of advantages over alternative techniques used to monitor food processing operations: it is capable of rapid and precise measurements, it is non-intrusive and non-invasive, it can be applied to systems which are concentrated and optically opaque, it is relatively inexpensive and it can easily be adapted for on-line measurements.

The basic concepts of ultrasonic propagation in materials, and methods used to carry out and interpret measurements in food systems have been reviewed elsewhere [2-6]. In this chapter applications of ultrasound relevant to food processing are discussed.

2. ULTRASONIC PROPERTIES OF MATERIALS

The use of ultrasound for monitoring food processing operations relies on the knowledge or measurement of the ultrasonic properties of the material being tested. The three most

important ultrasonic properties are the *velocity* at which an ultrasonic wave propagates through a material, the extent to which the wave is *attenuated*, and the *acoustic impedance* (which determines the amount of ultrasound reflected from a boundary between two materials).

Ultrasonic velocity. The velocity *(c)* at which an ultrasonic wave travels through a material is related to its physical properties by the following equation.

$$c^2 = \frac{E}{\rho} \tag{1}$$

Here E is the appropriate elastic modulus (which depends on the physical state of the material and the type of wave propagating) and ρ is the density. The less dense a material or the more resistant it is to deformation the faster an ultrasonic wave propagates. Usually, differences in the elastic moduli of materials are greater than those in density and so the ultrasonic velocity is determined more by the elastic moduli than by the density. This explains why the ultrasonic velocity of solids is greater than that of fluids, even though fluids are less dense [7]. The modulus used in the above equation depends on the physical state and dimensions of the material being tested. For bulk solids the appropriate modulus is K+4G/3, where K is the bulk modulus and G is the shear modulus, for solid rods it is Young's modulus, Y. (A rod is a material which has a diameter much smaller than the wavelength of ultrasound, i.e., $d << \lambda/20$). For liquids and gasses the appropriate modulus is the bulk modulus, which is the reciprocal of the adiabatic compressibility κ. Shear waves will propagate through solids (E=G) but are highly attenuated in liquids and gasses and do not usually travel far enough to be detected. The ultrasonic velocity is determined by measuring either the wavelength of ultrasound at a known frequency ($c = \lambda f$), or the time taken for a pulse of ultrasound to travel a known distance ($c = d/t$).

Attenuation coefficient. As an ultrasonic wave propagates through a material its amplitude decreases, i.e., the wave is attenuated. The major sources of attenuation by a material are *adsorption* and *scattering*. Adsorption is due to mechanisms which convert some of the energy stored as ultrasound into other forms and ultimately into heat, e.g., viscosity, thermal conduction and molecular relaxation [8]. Scattering is important in heterogeneous materials, and occurs when an ultrasonic wave encounters a discontinuity (e.g. a particle, crack or void) and is scattered in directions which are different from that of the incident wave. Unlike adsorption the energy is still stored as ultrasound, but it may not be detected by a receiver in the forward direction because its propagation direction and phase have been altered. The attenuation coefficient α of a material has units of Nepers per meter (Np m^{-1}) when defined by the following equation:

$$A = A_0 e^{-\alpha x} \tag{2}$$

Here A is the amplitude of the wave, and x is the distance traveled. The attenuation coefficient is determined by measuring the dependence of the amplitude of an ultrasonic wave on distance and fitting the measurements to the above equation. The attenuation is often given in units of decibels per meter (dB m^{-1}) where 1 Np = 8.686 dB.

Acoustic Impedance. The acoustic impedance ($Z = \rho c$) determines the proportion of an ultrasonic wave reflected from a boundary between two materials. When a plane ultrasonic wave is incident on a plane interface separating two materials of different acoustic impedance it is partly reflected and partly transmitted (Figure 1). The ratios of the amplitudes of the transmitted (A_t) and reflected (A_r) waves to that of the incident wave (A_i) are called the transmission (T) and reflection coefficients (R), respectively.

$$T = \frac{A_t}{A_i} = \frac{2Z_1}{Z_1 + Z_2} \tag{3}$$

$$R = \frac{A_r}{A_i} = \frac{Z_1 - Z_2}{Z_1 + Z_2} \tag{4}$$

The greater the difference in acoustic impedance between the two materials the greater the fraction of ultrasound reflected. This has important consequences for the design and interpretation of ultrasonic experiments. For example, to optimize transmission of ultrasound from one material to another it is necessary to chose two materials with similar acoustic impedance. To optimize reflection materials with very different acoustic impedance should be used. The acoustic impedance of a material is often determined by measuring the fraction of ultrasound reflected from its surface.

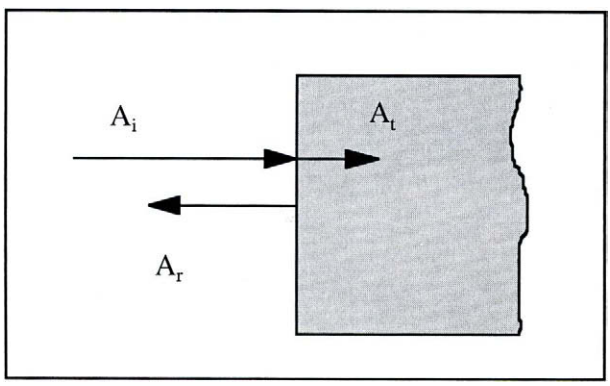

Figure 1. Reflection and transmission of an ultrasonic wave from a boundary between two materials.

Solids usually have larger ultrasonic velocities and acoustic impedances, than liquids, which have larger values than gasses. Air has a very low acoustic impedance compared to liquids or solids which means that ultrasound is almost completely reflected from an interface between air and a condensed medium. This can be a problem when ultrasound is used to test dry materials, e.g., biscuits or egg shells. A small gap of air between an ultrasonic transducer and a sample can prevent ultrasound from being transmitted into the material. For this reason coupling materials (often aqueous or oil based) can be placed between the transducer and sample to eliminate the effects of the air gap, or alternatively ultrasonic transducers with soft-polymer faces can be used.

3. EXPERIMENTAL MEASUREMENTS

At present there are few ultrasonic instruments which can be purchased off-the-shelf which are specifically designed for food processing applications. This is the main reason why ultrasound has not been used more frequently in the food industry. This situation is already changing, and a number of instrument manufacturers have recently developed ultrasonic sensors for application to food materials (e.g., Cygnus Instruments, Dorchester, Dorset, UK;

Nusonics Inc., Tulsa, OK, USA). These sensors are suitable for simple applications which rely on measurements of the ultrasonic velocity at a single frequency, e.g. concentration determinations, flow rates, thickness measurement, detection of foreign bodies. More sophisticated instruments are likely to be developed in the near future which will open up a wider range of applications, e.g., particle sizing. The ultrasonic properties of materials can be determined in one of two ways: either the wavelength and amplitude of ultrasound is measured at a known frequency, or the time-of-flight and amplitude of a pulse of ultrasound which has traveled a known distance are measured [9, 10]. The latter technique is by far the most popular and useful technique for food processing applications, because the experimental configuration is simple to design and operate, measurements are rapid, non-invasive and non-intrusive, there are no moving parts and the technique can easily be automated.

The simplest and most widely used technique is called the *pulse-echo* technique. More sophisticated pulsed methods have been developed to improve the accuracy of measurements [9, 10], however, the operating principles are basically the same as those of the pulse-echo technique. For this reason, only the pulse-echo technique is described and some of the modifications are mentioned in passing.

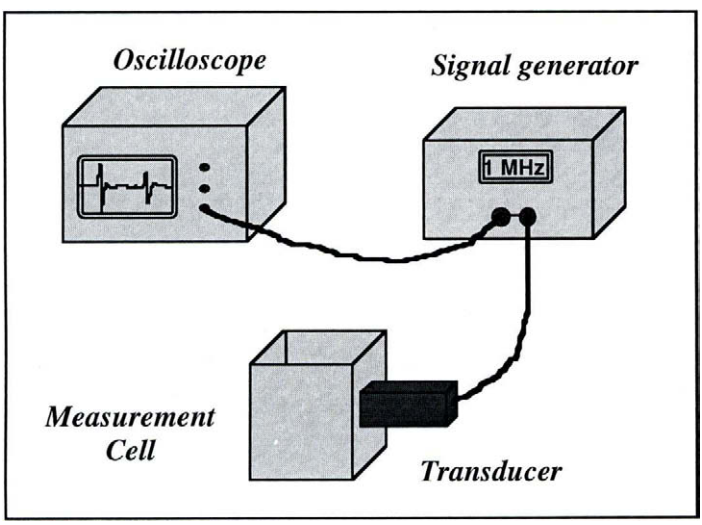

Figure 2. Schematic diagram of the experimental configuration for an ultrasonic *pulse-echo* experiment.

A typical experimental configuration consists of a measurement cell which contains the sample, a signal generator, an ultrasonic transducer and an oscilloscope (Figure 2). The signal generator produces an electrical pulse of an appropriate frequency and amplitude. This pulse is converted into an ultrasonic pulse by the transducer. It then propagates through the sample until it reaches the far wall of the cell where it is reflected back to the transducer (Figure 3). The transducer now acts as a receiver and converts the ultrasonic pulse back into an electrical pulse which is displayed on the oscilloscope. Because each pulse is partially transmitted and partially reflected at the cell walls a series of echoes are observed on the oscilloscope (Figure 3). The velocity and attenuation coefficient are determined from these echoes.

Each echo has traveled a distance twice the cell length d further than the previous echo and so the velocity can be calculated by measuring the time delay t between successive echoes: c

= $2d/t$. The cell length is determined accurately by calibration with a material of known ultrasonic velocity, e.g. distilled water: $2d = c_w.t_w$ (where the subscripts refer to water). Alternatively, if the ultrasonic velocity in the sample is known its thickness can be determined. The attenuation coefficient is determined by measuring the amplitudes of successive echoes: $A = A_0e^{-2\alpha d}$, and comparing them to the values determined for a calibration material. A number of sources of errors have to be taken into account if accurate measurements are to be made, e.g., diffraction and reflection [6].

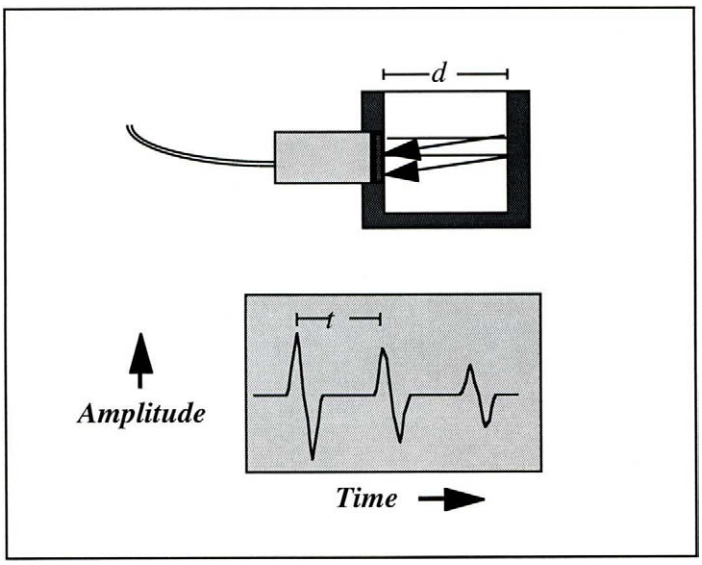

Figure 3. An ultrasonic pulse travels back and forth across the measurement cell so that a series of echoes is observed on the oscilloscope.

4. APPLICATIONS OF ULTRASOUND IN FOOD PROCESSING

4.1. Presence/absence detection

The presence or absence of an object between a pair of ultrasonic transducers (or a single transducer and a reflector plate) can be detected by measuring the amplitude of the received signal [11]. If an object is present the amplitude of the received signal will be reduced. This technique is useful for counting the number of objects passing a certain point on a conveyor belt. If the speed on the conveyor belt is known the size of the objects can also be determined.

Ultrasound can also be used to detect the presence or absence of a material in a container, e.g., a pipe, can or tank. An ultrasonic transducer is placed on the outside wall of a container and the amplitude of an echo reflected from the inside wall is measured (Figure 4). This amplitude depends on the acoustic impedance of the material in the tank (equation 4). If no material is present in the container (i.e., low acoustic impedance) the amplitude of the received echo will be greater than if a condensed material is present. This type of sensor could be used to determine whether a liquid in a tank had fallen above or below some critical level, or to determine whether their was some material remaining in a pipe. It is particularly useful for

application to closed containers where visual observations are not possible. The information from the sensor could be part of a control loop, so the system could be fully automated.

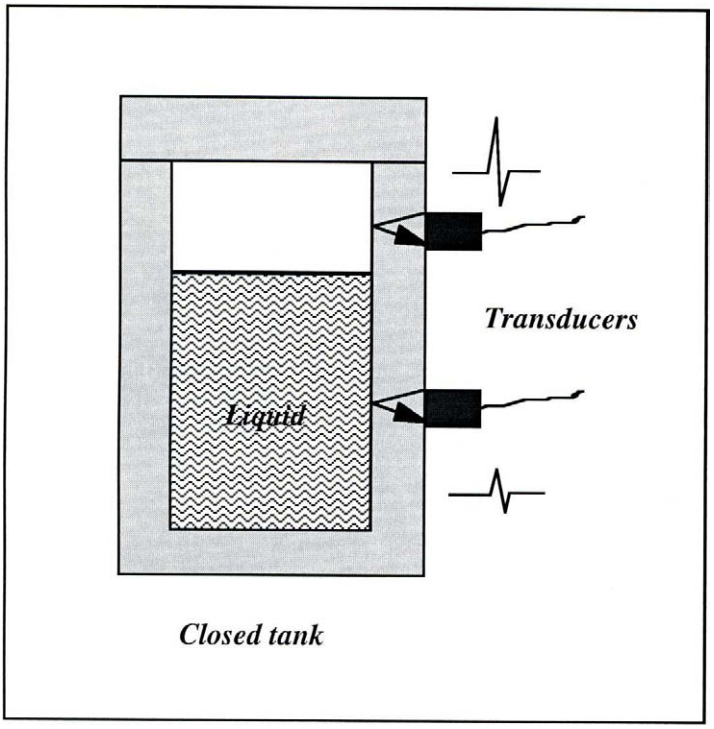

Figure 4. Determination of the presence/absence of a liquid in a container. The amplitude of the echo increases in the absence of liquid.

4.2. Thickness and Level detection

Ultrasound is commonly used in industrial applications for making precise measurements of the thickness of materials [11]. An ultrasonic transducer is pressed against the side of a material and the time taken for a pulse to travel across the material and back is measured (Figure 5). If the velocity of ultrasound in the material is known then the distance can be calculated: $2d = ct$. Ultrasound is particularly useful for measurements on materials which are difficult to access by conventional methods, e.g., the determination of the thickness of a pipe when access is only available to the exterior of the pipe. It can also be used to measure the thickness of individual layers in multilayer systems (Figure 5).

The determination of the thickness of the layers of fat and lean tissue in animal flesh is the most popular use of ultrasound in the food industry at present [6]. In fact there are over a hundred references pertaining to this application of ultrasound in the *Food Science and Technology Abstracts* (1969-1993). In contrast to most other applications of ultrasound in the food industry, which have rarely developed further than use in the laboratory, there are a number of commercial instruments available for grading meat quality [6]. This application is based on measurement of time intervals between ultrasonic pulses reflected from boundaries between layers of fat, lean tissue and bone (Figure 5). Ultrasonic techniques have the advantage that they are fairly cheap, easy to operate and give predictions of meat quality of

live animals. Other examples of thickness determinations include: liquid levels in cans or tanks, thickness of coatings on confectioneries, egg shell thickness. Thickness can usually be determined to better than 1% on samples with overall thicknesses ranging from 0.02 mm to 1 m [11].

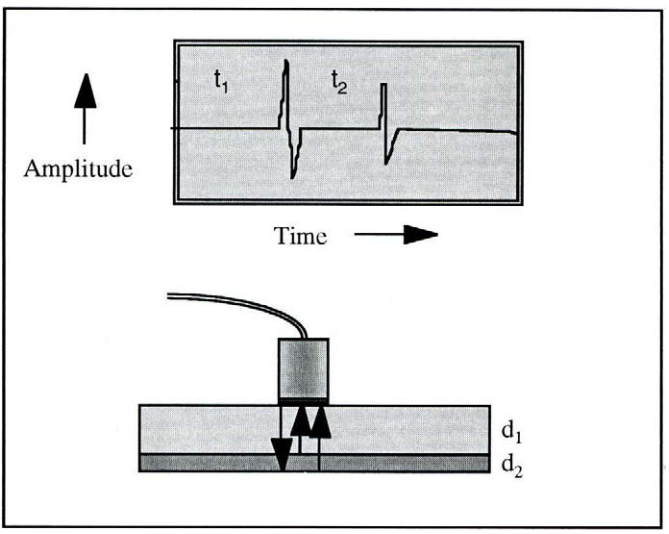

Figure 5. Ultrasonic pulse-echo technique for determining the thickness of layers in multilayer materials.

4.3. Foreign body detection

Undesirable foreign materials such as pieces of metal, glass, wood, plastic or other debris may contaminate foods during processing. Many foods are optically opaque and so methods which utilize light cannot be used. If an ultrasonic pulse is propagated into a sample it will be reflected from any boundaries it encounters, providing there is a large enough difference in acoustic impedance between the food and the foreign body, which is usually the case. The distance of the foreign body from the surface of the can is determined by measuring the time-of-flight of ultrasonic pulses reflected from the foreign body and from the can wall (Figure 6): $d_2=d_1.t_2/t_1$. By moving an ultrasonic transducer around the sample it is possible to determine the size and location of the foreign body [11]. This technique is a simple example of the imaging techniques used in medicine to determine the health and sex of fetus' in the womb.

4.4. Flow rate measurements

Measurement of the flow rate of materials through pipes during processing is important in many areas of the food industry. There are a number of different types of ultrasonic sensor available which can be used to measure the flow rate of liquids, the three most important being those based on transit time, Doppler and cross-correlation measurements [11]. Ultrasonic flow meters are capable of determining flow rates up to a few meters per second, on systems which have dimensions ranging from less than a millimeter (i.e., blood flow in veins) to greater than a kilometer (i.e., flow of water in rivers).

Transit time. The transit time for a pulse of ultrasound to travel a distance d through a static fluid is given by the equation: $t = d/c$, where c is the velocity of ultrasound in the fluid. If the fluid is flowing with a velocity V the transit time will be modified. When the ultrasonic wave

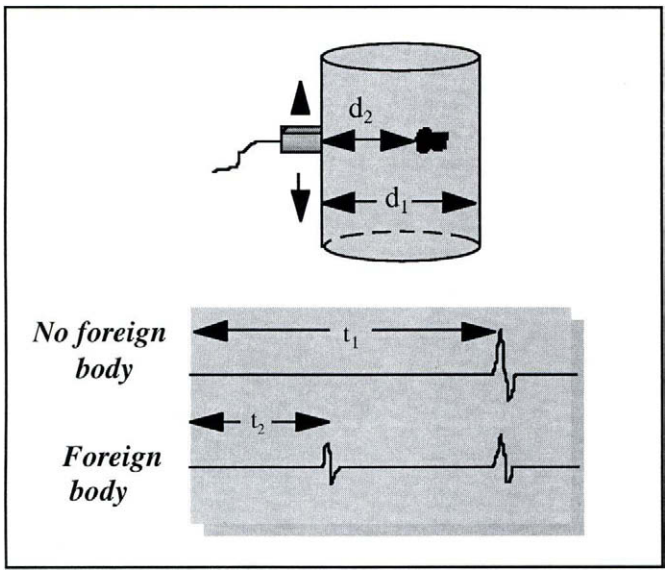

Figure 6. Detection of a foreign body in a can using the ultrasonic pulse-echo experiment: $d_1=ct_1$, $d_2=ct_2$.

travels in the same direction as the fluid is moving the overall velocity is increased *(c+V),* and the transit time is reduced $t_1=d/(c+V)$. If the ultrasonic wave travels in the opposite direction to the fluid its overall velocity is decreased *(c-V),* and the transit time is increased $t_2=d/(c-V)$. The velocity of the fluid can therefore be determined by rearranging these two equations:

$$V = \frac{d(t_2 - t_1)}{2t_1t_2} \tag{15}$$

The determination of flow rate is therefore independent of the ultrasonic velocity of the fluid, which is important as this may vary with the composition of the fluid or the temperature. To make measurements upstream and downstream the transducers can be placed across a bend in a pipe, or fixed at an angle to one another [11]. In the latter case it is necessary to correct the measurements for the angle.

Doppler. Doppler flow meters measure the frequency shift which occurs when an ultrasonic wave is reflected from a moving object [7]. The frequency shift is related to the velocity of the particles and so their flow rate can be determined [11]. In the food industry these objects may be particles suspended in the fluid or density fluctuations in the liquid due to flow.

Cross correlation. Cross correlation flow-meters also rely on the presence of inhomogeneities in the fluid to determine the flow rate. Two transducers are fixed to a pipe at a known distance *d* apart, and the pulses reflected from the flowing liquid are measured. The signals received from the transducers are compared using a technique called cross-correlation which looks for similar patterns in the two signals corresponding to reflections from the same particle (or set of particles) passing by the different transducers. This permits the time interval *t* for the inhomogenitity to travel between the transducers to be calculated: $V = d/t$.

The ultrasonic flow meters described above measure the average flow velocity of the fluid, in practice there will be a flow profile across the pipe. More sophisticated flow meters have been developed which can be used to measure flow profiles [11]. It should also be pointed out that

the Doppler and cross-correlation flow meters measure the flow of the particles which may be different from that of the fluid itself.

4.5. Temperature measurements

The ultrasonic properties of materials are sensitive to temperature and so ultrasound can be used to provide information about temperature. Ultrasonic thermometers have been developed which consist of a rod of material with a piece of another material of different acoustic impedance bonded to the end (Figure 7). An ultrasonic pulse propagating along the rod is partly reflected and partly transmitted at the boundary between the two materials. The reflected part travels back to the transducer, whilst the transmitted part propagates through the end-piece before it travels back to the transducer. The difference in time *(t)* between the two echoes is the time it takes the pulse to travel twice the length *(d)* of the end-piece. This time depends on the ultrasonic velocity of the end-piece material and its length, both of which vary with temperature. The thermometer is calibrated by measuring t in a series of liquids at known temperature. Careful design of the thermometer is needed to avoid interference from side wall reflections, and reverberations in the end-piece.

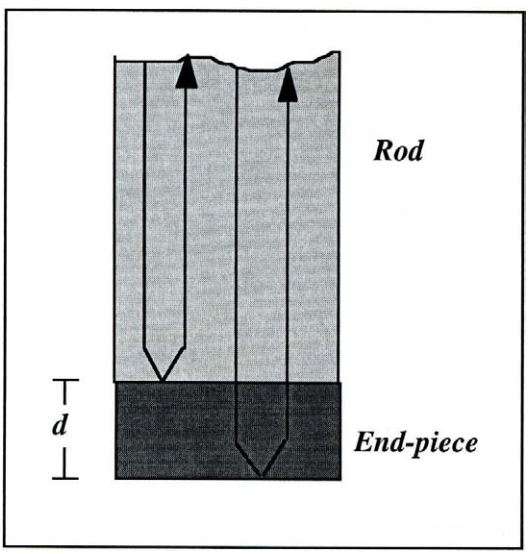

Figure 7. An ultrasonic thermometer.

It is also possible to use the material whose temperature is to be measured as it's own thermometer, by utilizing the temperature dependence of its ultrasonic properties. The ultrasonic velocity of water increases by about 4 m s^{-1} K^{-1} at room temperature, so the temperature of aqueous-based foods could be measured to a fraction of a degree once their velocity-temperature profile had been established [2]. This will depend on the composition of the material, which may be a problem if the composition is variable. Ultrasonic sensors will prove useful in situations where it is inconvenient to use conventional temperature sensors, e.g., in microwave environments or at high temperatures.

4.6. Determination of composition and microstructure

Ultrasound has been used to measure the composition of a wide variety of different foods over the past half century or so, e.g., fat:lean ratio of meats, oil content of fatty foods, solid fat

contents, milk composition, sugar concentration, alcohol content of drinks, triglycerides in oils, air in aerated foods, salt concentration of brine and biopolymer concentrations in gels and aqueous solutions [6].

This application of ultrasound relies on their being a significant change in the ultrasonic properties of a material as its composition changes. Figure 8 shows the variation of ultrasonic velocity with sugar content for a series of glucose/water mixtures. The accuracy of the concentration determination depends on how accurately the velocity can be measured and the magnitude of the change in velocity with composition: the greater the change the more accurately the concentration can be determined. The ultrasonic velocity increases by about 4 m s^{-1} per 1% increase in sugar concentration. There are commercial instruments which can measure the ultrasonic velocity to better than 0.2 ms^{-1} and so the sugar content can be measured to better than 0.1% Similar figures can be obtained for solid fat content determinations and concentration determinations in aqueous solutions of salts, proteins and carbohydrates.

The ultrasonic properties of microheterogenous materials, such as emulsions and suspensions, depend on the size of the particles, thus it is possible to used ultrasound to obtain information about microstructure. Measurements of the ultrasonic velocity and attenuation as a function of frequency are used to determine the particle size distribution [6]. Ultrasound has a number of important advantages over other techniques used for microstructure and composition determinations: it is capable of rapid and precise measurements, it can be used in opaque systems, it is non-destructive and it can be used on-line.

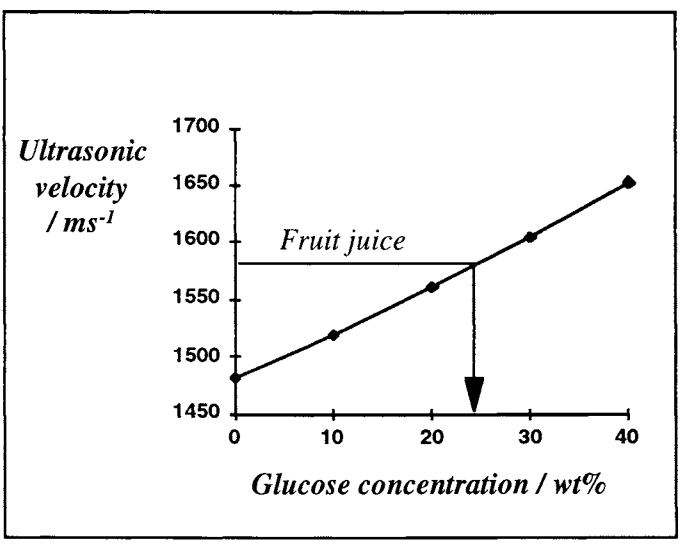

Figure 8. Dependence of ultrasonic velocity on the tristearin concentration of tristearin/paraffin oil mixtures at 18°C.

4.7. On-line measurements

One of the most promising applications of ultrasound in the food industry is as an on-line sensor for measuring the properties of food materials during processing. There are a number of important attributes which any on-line sensor must have. It must be capable of rapid and reliable measurements, be non-invasive and non-destructive, be robust, low cost, easily

automated and hygienic [12]. Sensors based on ultrasound have all of these attributes. A typical on-line ultrasonic sensor system is shown in figure 9.

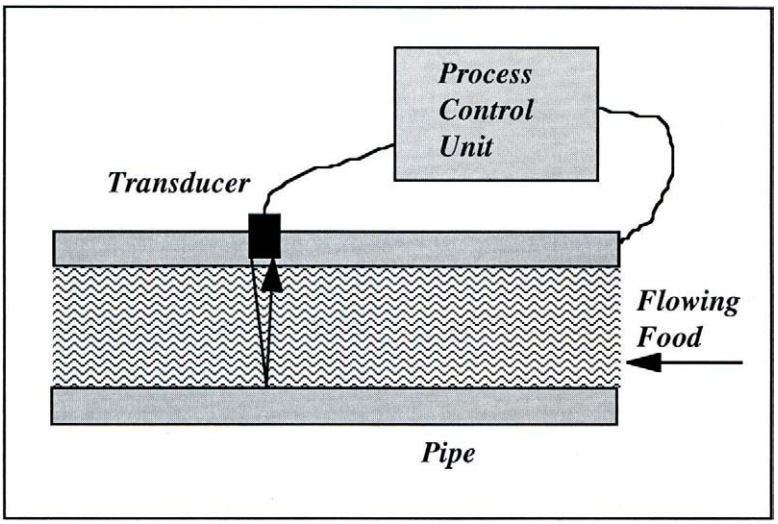

Figure 9. On-line sensor for measuring the ultrasonic properties of foods flowing through pipes.

The sensor consists of an ultrasonic transducer set into the wall of a pipe through which the sample flows. The time taken for a pulse to travel across the sample (t) is measured using a digital timing device, and the ultrasonic velocity is calculated from a knowledge of the inside diameter of the pipe (d): $c = 2d/t$. The velocity is then related to some physical property of interest, e.g. sugar concentration, solid fat content, or particle size. This device can be fitted into the existing pipe work of a factory. Because the sensor is set into the wall of the pipe and does not contact the food there are no problems with hygiene or cleaning-in-place. The output from an on-line sensor can be used in a process control loop to optimize the processing conditions in real-time. A number of sensors placed along a production line can be used to monitor the properties of a food at different stages of manufacture.

4.8. Limitations and advantages

It is useful to give a brief overview of some of the major advantages and limitations of ultrasound as a tool for monitoring food processing operations. Ultrasound is fairly inexpensive to purchase and operate, it is robust and can therefore be used in factories, it is capable of rapid ($<<$ 1 second) and reliable measurements, in a non-destructive and non-invasive manner. In addition, measurements can easily be automated and so the technique is suitable for on-line measurements as well as an analytical instrument in the laboratory. The major disadvantages are: there are few commercial instruments specifically designed for application to food materials at present, although this situation is changing; the technique is fairly application specific, i.e., calibration experiments have to be carried out for each new application; and ultrasound is highly attenuated by materials which contain small air bubbles, which may limit its application to certain foods.

5. CONCLUSIONS

Ultrasound has considerable potential as a tool for characterizing food processing operations. The food industry will benefit substantially from further development and application of ultrasonic techniques. On-line sensors give manufacturers greater control over the properties of the product during manufacturing which leads to improvements in product quality and reduction in costs. The continued development of ultrasound in the food industry depends on the availability of appropriate ultrasonic instrumentation, and workers using a systematic approach to the measurement and interpretation of ultrasonic data.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 5

New methodology using synchrotron radiation to characterize fast events in food processing

A. E. Blaurock

Kraft Foods, Inc., Technology Center
801 Waukegan Road, Glenview IL 60025, USA

1. INTRODUCTION

In a typical food process, a hot mixture is made, which is then cooled rapidly. A main result of cooling is a change in physical state and/or molecular structure. Often, a succession of physical changes occurs as the product cools. Further changes can occur during aging. Chemical reactions may occur as well. This chapter will focus on the more rapid events, particularly changes in physical state, which happen early in the process.

Events occurring at the more rapid cooling rates can be difficult to characterize using common laboratory methods, and there is a pressing need for faster laboratory measurement techniques. The goal of this chapter is to show that X-ray fluxes available today at synchrotron radiation sources make it possible to characterize rapid process events by X-ray diffraction (XRD). Currently, synchrotron X-ray fluxes are up by 3 to 4 orders of magnitude over the best conventional laboratory sources. When used in conjunction with fast electronic detector systems, synchrotron radiation beams can be used to study events occurring on millisecond time scales. Owing to on-going technical developments, the prospect is to be able to characterize events on a microsecond time scale in the near future. A few other potential applications of synchrotron radiation are indicated at the end of the chapter.

1.1. The problem

Many processed foods start with the mixing of ingredients at higher temperature, often with the formation of an emulsion during mixing. Typically, the mix is then cooled rapidly. In the sequence of heating and cooling, chemical changes may occur, such as disulfide bond formation in dairy products, or the major change may be simply a temperature-induced change of physical state, such as starch gelation, protein aggregation or fat crystallization. Whether the changes are physical or chemical, the problem for the food scientist or engineer is to control events in such a way as to produce the optimum product consistently.

The engineer or scientist who seeks to control a food process will do this best by an appropriate understanding of events; such understanding is the price we pay for reliable

control. Problems encountered in developing this understanding are, first, formulating a model system that includes the essential elements of both the ingredients and the process, and second, recording data at sufficiently high rates. This chapter will concentrate on the second of these related problems.

Although food processing rates fall far below the highest cooling rates possible -- biological specimens are frozen at up to 10,000°C/sec for electron microscopy -- our experience has been that unit operations in the Plant tend to outperform the laboratory equipment that we would choose to model process dynamics. For example, margarine is made by forming a hot water-in-oil emulsion. This emulsion is then cooled rapidly in order to cause the oil to crystallize, thereby stabilizing the emulsion. The cooling step from 110° to 40°F is accomplished in roughly 20 seconds, i.e., at an average cooling rate of about 200°F/min. In comparison, the Differential Scanning Calorimeter (DSC) that we use regularly (Perkin-Elmer DSC-7 with Intracooler II) cools at a maximum sustainable rate of <100°F/min over the same temperature range.

As another example, cream cheese is made in a multi-step process that involves two cycles of heating and cooling. First the mix is heated, in order to homogenize and pasteurize it, and then cooled to the culturing temperature. After culturing, the mix is heated once again, and then the curd is separated and cooled in stages, in order to make the final product. Both heating and cooling may be accomplished at average rates ranging from 200° to 400°F/min.

Given these moderately high cooling rates, the problem is to find a methodology capable of following the physical events of interest. The problem is not a trivial one. For example, the DSC is widely used to characterize exo- and endo-thermal process events. Although the maximum cooling rate depends on the temperature range under study, the maximum rate achievable by DSC can be less than that realized in some food processes.

In addition, shear is common in food processing, and shear certainly affects texture of the final product. In order to do DSC while shearing a sample, however, the heat generated by mechanical work must be accounted for. As far as the author is aware, no thermal analyzer has been made to accomplish this apparently difficult task.

1.2. Choice of method

DSC, spectroscopic methods (IR and UV absorption), scattering methods (light scattering, X-ray diffraction) and imaging methods (light and electron microscopies) all are capable of following food processing events. Among these methods, X-ray diffraction (XRD) has the advantages that it is a direct measure of structure over a broad size range (~1Å up to 2 microns); that it is relatively non-destructive and therefore can be used to observe dynamic events; and that it averages over many copies of the structure of interest. Moreover, in principle the experiment can be done while the sample is being sheared. In comparison, light microscopy too is non-destructive, it provides a direct image of the material, and it also may be feasible on a sample undergoing shear; however, the size range is limited to 1/2 micron or greater. Electron microscopy provides a direct image as well, and the resolution is comparable to XRD; however, food samples must be physico-chemically stabilized ("fixed") before

viewing. Hence dynamics must be inferred rather than observed directly. Finally, the microscopy methods require a great deal of effort to average over a large population, e.g., to determine droplet sizes. Light scattering methods allow dynamic experiments to be carried out, even as the sample undergoes shear; however, the resolution is limited, and there are restrictions on the allowable concentration of solids.

In passing, it may be useful to record different kinds of data simultaneously. XRD and calorimetric data, for example, complement one another nicely. The calorimetric device made by Mettler (M-84, designed for use with the light microscope) can be used for combined calorimetry and XRD.

In order to introduce the technique of XRD using synchrotron radiation, this chapter will begin with a discussion of the technologies for generating and detecting X-rays. Then some examples of structural kinetics determined using XRD will be presented, which are drawn from the more mature areas of biological and biomedical studies; these areas are chosen because the materials studied are akin to foods. Finally, one of the first applications of synchrotron radiation to food science -- the kinetics of fat crystallization -- will be summarized.

2. XRD TECHNOLOGY

2.1. Production of X-rays

Historically, elapsed times to record one single XRD exposure have ranged from minutes to weeks. The reason for the long XRD exposure times has been the very weak X-ray beams available. The weak X-ray beams have resulted from the inability to use the bulk of the radiation arising in a conventional X-ray generator. Almost all of the X-rays arising inside a conventional generator go off in the wrong direction and are lost.

Note that X-rays cannot be focused with glass lenses in the way that light is: refraction of X-rays is poor and the absorption by glass is too strong. Mirrors employing grazing incidence are commonly used to reflect X-rays, but the maximum angle off the surface is a scant 1/4° (Guinier, 1963). Hence, not much of the total X-ray production can be gathered by such mirrors. Only about one part in one million, or even ten million, of the X-rays generated inside the X-ray tube are going in the right direction to reach the specimen. In addition, only a fraction of this radiation has the desired wavelength. Thus even though a kilowatts-worth of radiation can be generated easily, much less than a milliwatts worth of radiation is present in the finely collimated, monochromatic beam required for the XRD experiment.

The advent of synchrotron radiation sources is radically changing the situation. In the United States, there are currently synchrotron radiation laboratories at Stanford University (SSRL: Stanford Synchrotron Radiation Laboratory); Cornell University (CHESS: Cornell High Energy Synchrotron Source); Brookhaven National Laboratory (NSLS: National Synchrotron Light Source); the University of California at Berkeley (ALS: Advanced Light Source); and the University of Wisconsin. About one year from now a more technically advanced source will open at Argonne National Laboratory

(APS: Advanced Photon Source). There are also sources in various other countries, including England, France, Germany, Italy, and Japan. These sources generate X-rays with an efficiency similar to the efficiency with which light is generated in a laser.

The phenomenon of synchrotron radiation is not new. From the inception of high-energy accelerators, physicists carrying out experiments at the above laboratories have paid a high price: the continual loss of valuable energy as elementary particles, moving inside a vacuum tube, follow a circular path within "bending magnets". Particle energy is lost in the form of light, ultra-violet rays and, principally, X-rays.

The radiation process is analogous to the emission of radio waves from a radio tower, but with a twist: Because of a relativistic effect, the radiation from the high-energy particles cannot go off in all directions, as for a radio antenna. Instead, the radiation goes off in the near-forward direction. The result is a highly directional X-ray beam. The X-ray beam so generated has been compared to the light coming from the headlamp of a locomotive moving around a bend in the tracks.

Because of the directional emission, a much higher proportion of the X-rays produced in a bending magnet can be used in the XRD experiment. As a result, instead of 10^9 photons, as in an experiment using a conventional generator, the beams now produced at these sources have up to 10^{12} photons per second. Even higher fluxes are expected in the near future as "third-generation" sources start up at various laboratories around the world.

Further spectacular developments are under way. Devices now being reduced to practice at synchrotron sources will deliver X-ray beams with energy fluxes ranging from 10 to 350 watts per square millimeter. These fluxes are comparable to the heat and light radiating from the surfaces of meteoroids entering the earth's atmosphere - 100 to 600 watts per square millimeter. (In comparison, the Sun radiates a mere 60 watts/mm^2 at its surface.)

2.2. Detector technology

Another key development is the technology for recording and storing XRD patterns. As might be expected, development of detector systems has been stimulated by the production of the intense synchrotron beams.

From the time of their discovery 100 years ago, X-rays have been detected both electronically and by photographic film. A relatively recent development are the position-sensitive X-ray detectors (PSD's). An outgrowth of detectors used in high-energy physics, PSD's use fast electronic processing techniques. They register, not only the presence, but also the location of X-ray photons, processing each photon in less than 1 microsecond.

PSD's offer several advantages over previous techniques for recording and processing XRD patterns. They record photons all but simultaneously at the various points in a diffraction pattern, whereas a scintillation counter is stepped sequentially from point to point. Film also records simultaneously at the various points, but PSD's have the advantage of recording photons individually against a low background of noise (cf. the ubiquitous photographic 'fog'). Also, natural background is low since X-ray sources are rare. Finally, PSD's avoid the time-consuming and tedious

photographic development process: instead of physically replacing one piece of photographic film with another, these devices allow the rapid electronic transfer of each succeeding diffraction pattern out of the detector memory and into an electronic storage device. Thus the entire XRD pattern is stored away in 1 millisecond or less. Once the pattern is placed in storage, the detector is ready to start the next sub-second exposure. Other kinds of detectors with similar capabilities are being perfected.

Taken together, the developments in efficient X-ray production, recording of low-noise patterns, and rapid storage of multiple patterns mean that a new class of dynamic experiments becomes possible. Using these twin technologies -- high-intensity synchrotron X-ray beams and fast electronic detector systems -- allows one to do 'time-slice' exposures at a rate that was once inconceivable. After summarizing work in biological kinetics, two examples from the study of fat crystallization will be presented, that demonstrate second and even sub-second time resolution.

3. APPLICATIONS TO BIOLOGICAL KINETICS

As discussed below, there are only a few examples of the use of synchrotron XRD techniques to study food process kinetics. Therefore, the potential of the method will be illustrated first using examples drawn mainly from biological studies, where there is a substantial history of this kind of work. Such studies include: (1) protein catalysis, initiated using stopped-flow or 'caged' metabolite techniques, including reactions that can be induced to take place **within** protein crystals; (2) structures of proteins in solution, such as soya globulin; (3) structural kinetics, e.g. the assembly of microtubules in biological cells, where the process is initiated by a change of temperature; or the assembly of a plant virus; (4) lipid crystallization events which are initiated by a rapid change of temperature or pressure ('temperature-jump' or 'pressure-jump' experiments); and (5) crystallization that is induced by shear, as in the study of industrial polymers.

3.1. Protein catalysis mechanisms

Mechanisms of catalysis within a protein crystal can be characterized if the process is synchronized over all the protein molecules. In order to synchronize events, an inactive, photolabile precursor is allowed to diffuse into the crystal over a period of time and then subjected to a flash of laser light, in order to release the active metabolite all over the crystal simultaneously (Bartunik & Bartunik, 1992). This approach relies on the large amount of water in a crystal, which can occupy well over half the total volume. Other approaches for triggering structural catalysis in a crystal include rapid temperature change (T-jump); release of protons ("caged" protons); and optical "pumping". Using the same methods, catalysis also can be studied in solution. Studies of events that require milliseconds are being carried out. The third-generation synchrotron radiation sources currently being built will facilitate the study of events that occur in microseconds (non-cyclic events) or even nano-seconds (cyclic events).

Another example is one of optical pumping. In this case, cyclic structural changes occurring in a membrane protein have been investigated by XRD (Koch et al., 1991). The protein, called bacteriorhodopsin, uses light energy to pump protons across a cell membrane. The absorbing chromophore is a protein to which a retinal molecule is bound covalently. Two different protein structural changes, occurring in seconds and milliseconds after light is absorbed, have been characterized and compared to optical spectroscopy observations.

3.2. Protein structure/dynamics in solution

The 11S soya globulin has been characterized in a 5% solution, using synchrotron radiation (Miles et al., 1984). The measurements are more rapid than for conventional sources by 100- to 1000-fold. The shorter exposure times forestalled well known structural changes -- aggregation and/or denaturation -- that occur during the longer time required for conventional XRD exposures.

Muscle has been studied extensively by XRD, and synchrotron exposures have greatly improved the time resolution of the structural kinetics of contraction. In ancillary studies, a muscle protein, troponin C, has been studied in solution in order to understand the role of Ca in contraction (Ueki, 1991).

3.3. Assembly of organelles

As an example of characterizing structural kinetics in solution, the growth of virus rods from protein monomers has been characterized using synchrotron radiation (Ueki, 1991).

3.4. Structural kinetics in phospholipid membranes

Rapid crystallization events in phospholipid membranes have been characterized using synchrotron radiation. The events have been initiated by gradual heating and cooling, at rates of the order of 5°C/minute (Quinn, 1992); or by a very rapid drop in pressure, which can have a similar effect to very rapid heating (Caffrey, 1991).

3.5. Crystallization under shear

A study of direct relevance to many food processes is crystallization under shear. Under conditions where a quiescent sample of plastic takes an hour to begin to crystallize, the process begins in seconds under even a low shear (Moitzi and Skalicky, 1993).

4. APPLICATION TO TRIGLYCERIDE CRYSTALLIZATION

Turning at last to foods, the rates at which oils crystallize and undergo polymorphic transitions are important in determining texture and physical stability in a variety of food products (Larsson, 1982). Edible oils consist almost entirely of triglycerides, with small amounts of mono- and di-glycerides and other minor components (Swern, 1979, Chapter 1). Hence attention has been concentrated on crystallization of triglycerides (TG's).

Before continuing this section, it may be useful to the reader to review a few basics. Thus the "diffraction angle", denoted "2θ", is defined as the angle between the line of the incident beam, continued through the sample, and the line of any given diffracted (or "scattered") ray from the sample to the X-ray detector. This is the angle that appears in the following figures.

By determining the diffraction angle, and knowing the X-ray wavelength, it is a simple matter to calculate the "Bragg spacing", or simply "spacing"; spacings are given in Å in the figures. These spacings, which measure packing at the scale of molecules and above, are diagnostic of specific structures, e.g. crystal forms of fat or starch . Finally, it may help to note that, in the reciprocal fashion of diffraction science, **smaller** angles of diffraction correspond to **larger** spacings in the sample, and of course, larger angles of diffraction correspond to **smaller** spacings.

4.1. Classification of lipid crystals

XRD has given useful insights into food structures and dynamics, at the molecular and supramolecular levels. Thus XRD of fats demonstrated sixty years ago that crystal polymorphism is the basis for the intriguing phenomenon of multiple melting points of fats (Clarkson and Malkin, 1934), which are observed even when these compounds are painstakingly purified (Duffy, 1853). Even today, XRD is a primary method for classifying TG crystals (Fig. 1).

Although there have been disagreements in the past regarding the classification scheme (Clarkson and Malkin, 1934; Bailey et al., 1945; Lutton, 1945; Malkin, 1952), there appears now to be general agreement (Chapman, 1962). Three major forms recognized are: alpha; beta prime (with two sub-forms [Simpson and Hagemann, 1982]); and beta (two sub-forms also are indicated [Kellens et al., 1990]). A less common variant is the sub-alpha form (Jackson & Lutton, 1950; Fig. 1); structurally, the sub-alpha form resembles a rather disordered beta prime (Eads et al., 1992). The same crystal forms seen in purified TG's also are seen in commercial oils. In general, the different crystal forms are related by monotropic transitions, i.e. transitions that cannot be reversed directly (Hernqvist, 1990); the reversible transition between alpha and sub-alpha is an exception to this rule.

4.2. Kinetics of lipid crystallization

As noted, lipid crystallization is key to processing foods and food ingredients such as margarine, spreads, shortening, peanut butter and chocolate, and it plays an important role in cream cheese and ice cream making.

The sequence of events occurring when margarine emulsion is cooled -- rapid crystallization to a less stable form, followed by a slower transition to more stable crystals (Fig. 2) -- has been characterized using a conventional laboratory X-ray generator. However, the recording of such a process by XRD with fine time resolution has had to await the development of synchrotron radiation methods.

The first synchrotron radiation experiments on fat crystallization were reported in 1990 by a group based in Belgium and using a synchrotron source in Germany

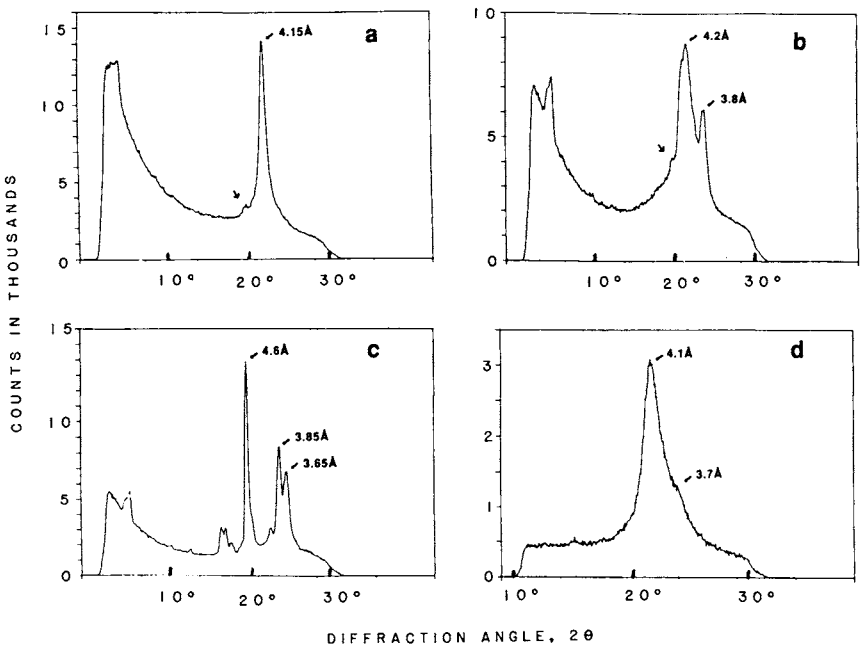

Figure 1. Wide-angle diffraction patterns from major crystal forms of TP. (a) Alpha form. (b) Beta prime form, β'_2 variant (Simpson and Hagemann, 1982). (c) Beta form. (d) Sub-alpha form, with its β'-like combination of a peak and shoulder; the shift to slightly smaller spacings is due to the low temperature (-130°C). Reproduced from Eads et al. (1992) by permission of the American Oil Chemists Society.

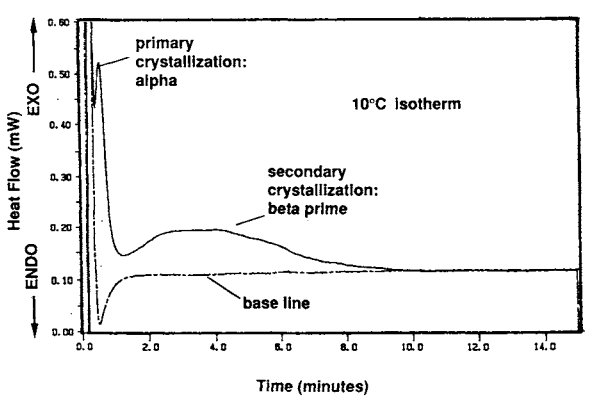

Figure 2. DSC showing crystallization events in a margarine oil. Crystals formed during primary and secondary phases were identified by XRD using a conventional X-ray generator.

(Kellens et al., 1990). Exposure times were 5-10 seconds. Working on a "simple" TG, tripalmitin (TP; three C16:0 chains), this group documented the transition from alpha to beta TP as the alpha form is heated at 5°C/min (Fig. 3). They also suggested, tentatively, that beta prime TP appears very briefly between the alpha and beta forms. A pair of heavy dashes in Figure 3, one at 20 1/2° and one at 23 1/2°, do indeed suggest the brief existence of an intermediate form just as the alpha TP crystals melt at about 50°C.

A year later, the same group reported the formation of beta TP crystals directly when alpha TP crystals were heated at 1.25°C/min. This time, the beta prime intermediate was excluded (Kellens et al., 1991).

Working at NSLS, Malcolm Capel and myself recorded crystallization data from trilaurin (TL), TP and tristearin (TS). We studied very rapid crystallization events: (1) formation of alpha crystals from the melt (see below), and also (2) the transition from alpha to beta during heating (Fig. 4). In the latter case, an intermediate form, identified as beta prime, is seen beyond any shadow of doubt.

In order to form alpha crystals, the molten trilaurin (TL) was cooled rapidly (~40°C/min). The crystals were then heated at a similar rate. The series of 1/2 second XRD patterns recorded during heating was then summarized in a pseudo-color plot; a color version of this plot has appeared on the cover of INFORM (Blaurock, 1993). The black and white version in Figure 4 shows the single X-ray reflection expected for alpha, near the bottom of the figure, and the three major reflections expected for beta crystals, near the top of the figure (refer Fig. 1). Figure 4 also demonstrates the existence of intermediate crystals - which clearly are neither alpha nor beta. The intermediate form is identified as beta prime TL (Blaurock et al., 1992).

The intermediate beta prime crystals in Figure 4 were expected on the basis of DSC curves which showed two exothermic events upon rapidly heating alpha TL (Blaurock et al., 1992). In conformity with the monotropic transition scheme (Hernqvist, 1990), the two events were interpreted as follows: (1) a peak for the formation of beta prime crystals; and (2) a second peak with onset about 10 seconds later, interpreted as the transition from beta prime to beta TL. Hence the brief existence of the the intermediate crystals in Figure 4 was anticipated by DSC observations (see also Lutton, 1946). In the case of TP, a similar pair of DSC exotherms has been published by Kellens et al. (1990; 1991), which are consistent with the beta prime TP intermediate suggested by these authors (Kellens et al., 1990).

At the same time, there is a definite surprise in Figure 4: the beta prime TL peaks are considerably broader than usually observed (cf. Fig. 1). In the reciprocal manner of XRD, **broader** peaks indicate **smaller** structures (Guinier, 1963). In fact, the broad peaks near the middle of Figure 4 indicate crystallites less than 50Å in diameter! The miniscule size of these beta prime crystallites may explain why they are so short lived, i. e. the surface/volume ratio is extraordinarily high, and there is correspondingly a large surface energy. As noted above, DSC heating curves demonstrate that beta prime TL crystals formed in this way are quite unstable while those formed directly from the melt are more stable. Presumably this is so because the latter are larger, as is indicated by the narrower diffraction peaks in Figure 1(b).

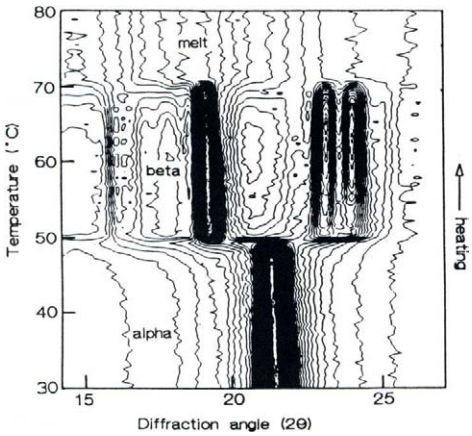

Figure 3. Kinetics of alpha to beta transition in TP. The series was recorded at the rate of one pattern every 5 sec as the sample was heated at 5°C/min. At the bottom, alpha TP is represented by a single peak at an angle of 21 1/2°. The transition to beta TP at about 50°C, with three main peaks, appears abrupt. Reproduced by permission of Elsevier Scientific Publishers; c 1990.

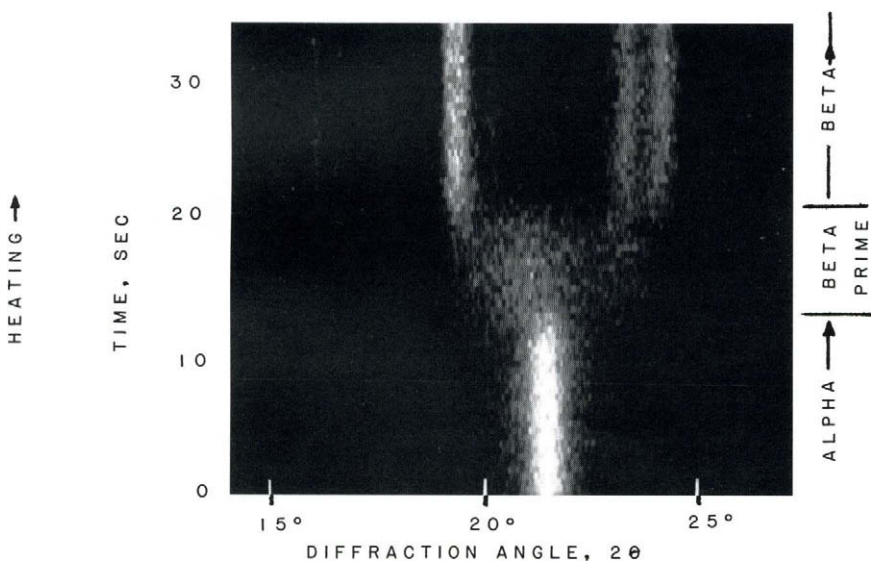

Figure 4. Kinetics of alpha to beta transition in TL. The series was recorded at the rate of one pattern every 1/2 sec as the sample was heated at about 40°C/min. The transition from alpha (bottom) to beta (top) is mediated by an intermediate form that lasts 9 sec. This form is identified as tiny beta prime crystals (see text). Reproduced by permission of the American Oil Chemists Society; c 1993.

The kinetics of the **formation** of alpha crystals also have been recorded (Blaurock et al., 1992). This work is the most severe test of the technique since alpha crystals form more rapidly than the other forms. Indeed, owing to the monotropic relations between forms (Hernqvist, 1990), alpha crystals would not be seen at any time if they did not form more rapidly than the more stable forms.

As described above, diffraction patterns were recorded at the rate of one every 1/2 second in our study. In analyzing short exposures like that shown in Figure 5, we took advantage of the fact that the total intensity in a diffraction peak is defined with good statistical accuracy when the number of counts in the entire peak is of the order of one thousand. If the peak is covered by a large number of recording points (electronic "channels"), say 40, then the number of counts per channel may be small, i.e., just 25 counts on average. This means that the peak shape in Figure 5 is not so precisely defined, but the total intensity is known with a 3% statistical uncertainty (Poisson statistics). As to peak shapes and peak positions, we relied on published data, as well as our own longer XRD exposures (Fig. 1), showing the alpha, beta prime and beta diffraction peaks (Bailey et al., 1945; Hoerr, 1964; Hernqvist and Larsson, 1982).

An example of the formation of alpha TP crystals from the melt is shown in Figure 6. Exposures were taken at the rate of one every 1/2 second, with about 1 millisecond between exposures required to store the pattern before starting the next exposure. The 'S'-shaped curve in Figure 6 is typical of crystallization events (Avrami, 1939).

The series of integrated intensities in Figure 6 have been analyzed in terms of the kinetics theory put forward by Melvin Avrami (1939). At the onset of crystallization, the data are fit well by a curve of the form t^3. A curve of the form t^2 fits the data significantly less well. A curve of the form t^4 fits the data well, but this would require an early start-up of crystallization, for which there is as yet no supporting evidence (Blaurock et al., 1992).

These results can be used to test possible kinetic schemes such as those put forward by Avrami (1939). The data in Figure 6 are consistent with two, alternative kinetic schemes, both of which are simple but unexpected. One interpretation is that (a) nucleation of alpha crystals is a random event with equal probability at all times, as is found for many other crystals (Avrami, 1939), and (b) the accumulation of crystal mass is proportional to the square of the time, rather than the cubic dependence seen for crystals in general. A second possible interpretation is that crystal mass does, in fact, accumulate as the cube of the time, as for other crystals (Avrami, 1939), but that nucleation of alpha crystals is not a random event. Either of these interpretations would account for the t^3 fit. It should be noted that t^4 kinetics - as found for other kinds of crystals (Avrami, 1939) - seem unlikely, but they have not been ruled out decisively (Blaurock et al, 1992).

In closing this section, it may be mentioned that XRD data are commonly divided into two regimes, namely the small- or low-angle region; and the wide-angle region. The two regions are distinguished on the basis of the different technologies typically used to record the XRD pattern. The patterns discussed in this chapter are all wide-angle patterns, but useful, complementary information can be obtained by recording the small-angle patterns as well.

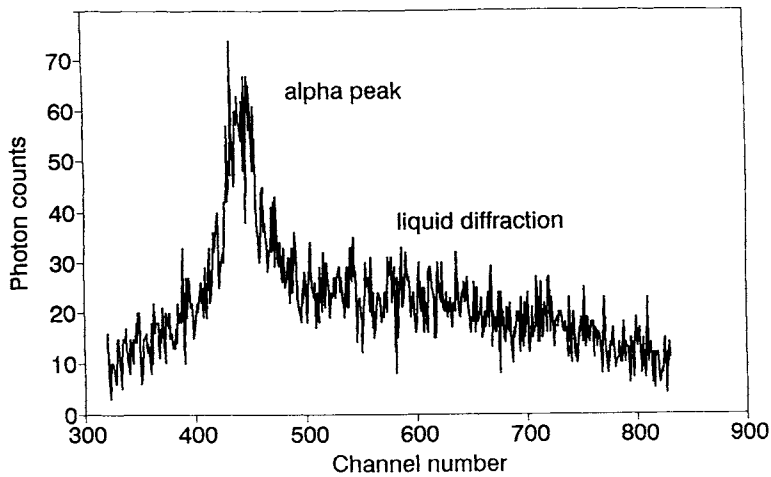

Figure 5. Formation of alpha TL from the melt. Both the narrow alpha peak and a broad liquid-oil peak are present in this 1/2 sec pattern, which was taken midway through the 1/2 min crystallization process.

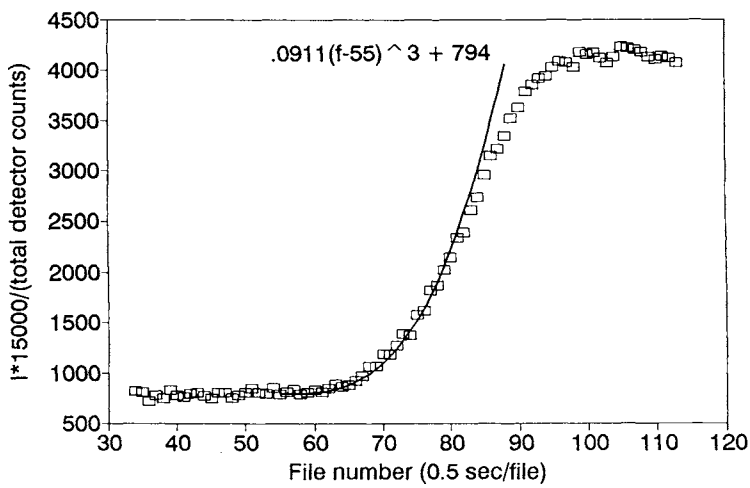

Figure 6. Kinetics of the formation of alpha TL. Open boxes represent intensities integrated over the alpha peak (half height to half height in Fig. 5). The solid line is the t^3 curve best fitting the start-up. The deviation of the data points from the curve at later times is as expected since the crystallization process must end as the liquid TL is used up.

5. CONCLUSIONS

In conclusion, it has been argued in this chapter that synchrotron radiation allows the determination of structural kinetics of food processes. In addition to the application reviewed here, there are other obvious applications. One such broad area is emulsion science. Thus synchrotron radiation experiments already have been reported in the literature, which investigate the structures of monolayers on the surface of a Langmuir trough.

A second broad area is the kinetics of aggregation of macromolecules during processing. The intense synchrotron radiation beams at APS naturally are highly collimated, owing to the high particle beam energy selected (7 GeV), and the X-ray beams therefore will be very well suited for ultra-small angle experiments (Shenoy et al., 1988). Recalling once again the reciprocal relation between diffraction angle and spacings inferred, such beams can be used to probe dimensions as large as 2 microns, and rapid kinetics will be accessible owing to the high beam intensity. Note that, while XRD requires regular structure, the structure need not be strictly crystalline.

A phenomenon that might be called facilitated nucleation is well recognized: new crystals nucleate near the surfaces of existing crystals (summarized in Avrami, 1939). Based on this fundamental understanding, it is clear that the way in which a food formulation is sheared, while nucleation and crystallization are under way, can be expected to have profound consequences for texture of the final product. The study of such a process using synchrotron radiation techniques can be expected to yield valuable insights.

This chapter necessarily is speculative since the potential of synchrotron radiation in the food industry remains largely to be demonstrated. Nonetheless, the application to fat crystallization shows that this potential is real and not "academic". What actually happens in future may depend on the will of various food companies to pursue long-term, fundamental research, a pursuit to which historically there has been limited commitment. Note that industrial work is welcomed and encouraged at the various synchrotron sources. The food and agricultural industries are represented at these sources, but as yet they are minor users (see e. g., Barnes et al., 1991).

For further information on synchrotron radiation experimentation, the reader is referred to other sources such as the "Handbook on Synchrotron Radiation", which is a series of volumes edited by Ernst-Eckhard Koch and published by North Holland Press; volume 1(a) and (b) were published in 1983. There is also a three volume series referred to as the "Hercules course" and published together by Springer-Verlag and Les editions du physique.

ACKNOWLEDGMENTS

I thank Frank Sasevich for masterly DSC work, insightful discussion and assistance in experimental work; and Sue Gaud and Peter Boyle for their support and encouragement of the original experimental work reported here.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 6

New developments in membrane processing

Gun Trägårdh

Lund University, Department of Food Engineering
P.O. Box 124, S-221 00 Lund, Sweden

1. INTRODUCTION

The use of membrane technology in the food industry is increasing. Established membrane processes in this field include reverse osmosis (RO), ultrafiltration (UF), microfiltration (MF) and electrodialysis (ED) and, to some extent, nanofiltration (NF). The application of new membrane processes, such as pervaporation (PV), is under development for the food industry. In 1991 the annual sale of membranes and membrane modules for the food industry was estimated at about 235 million US $ or about 10% of the annual sales, distributed as follows: MF 130, UF 60, RO 25 and ED 20 million US $ [1]. It should be observed that about half of the annual sales is accounted for by devices for blood purification and about 30% for water purification.

Most of the microfilters installed to date are run in the dead-end mode. The main applications are found in the beverage industry, where microfiltration is used to achieve sterile filtration and clarification. The use of crossflow microfiltration is, however, increasing, leading to better performance and a substantially longer membrane lifetime. In this chapter only the crossflow applications will be discussed.

Ultrafiltration often presents a unique separation solution, for example the use of ultrafiltration for fractionation. In other applications, ultrafiltration is an alternative to other separation processes. When used for concentration, ultrafiltration sometimes competes with evaporation, for example.

Reverse osmosis is normally used for the concentration of liquid foods and food process effluents, and, in some cases, for fractionation purposes, e.g., in the

beverage industry for production of beer with reduced alcohol content.

Partial demineralization can be obtained by using membranes in the intermediate range between UF and RO, often called nanofiltration. High-molecular-weight compounds are concentrated simultaneously. A higher degree of demineralization can often be obtained using electrodialysis. In this case, however, the product is not concentrated simultaneously.

Membrane separation technology offers a number of advantages in the food industry. Some of these are gentle treatment of the product at the chosen temperature, (resulting in significant improvements in the product quality compared with other techniques where more severe heat treatment is necessary), unique separation properties, concurrent fractionation and concentration, desalination and purification of solutions, low energy consumption, increased efficiency and simple plant layout. Motives to utilize membrane technology were as follows according to a survey of Japanese companies [2]: improvement of product quality (82.1%), reduction of production costs (32.1%); fortification of products against the products from competitors (28.6%).

Among the disadvantages is fouling, the deposition of material on the membrane surface and/or in its pores, leading to a change in the membrane performance. Fouling causes a reduction in flux and sometimes also changes in the separation properties. Methods of reducing fouling, especially in crossflow microfiltration, will be discussed later in this chapter. The maximum degree of concentration is restricted by viscosity and osmotic pressure, for example. To partly overcome this, plate-and-frame modules with specially designed flow channels have been developed. Reverse osmosis modules and membranes withstanding pressures well above 10 MPa are being developed.

2. FACTORS OF RELEVANCE FOR THE PERFORMANCE OF MEMBRANE PLANTS

2.1. Properties of the raw material

To facilitate the choice of membrane, process and process conditions, the composition and characteristics of the raw materials should be determined as extensively as possible. Some factors are general, some are specific for the treatment of biological material. Characteristics which should be considered are: type and content of micro-organisms, viscosity, total solids (TS) content, fat

content, protein content, salt content (including iron, magnesium, calcium and potassium salts), type and content of surfactants, polysaccharide content, fibre content, oxidized compounds such as free chlorine, hydrogen peroxide, dissolved oxygen, aroma compounds, traces of solvents, pH and pH change with time, etc.

During RO, NF, UF and also to a large extent during MF, micro-organisms are retained by the membrane and are concentrated in the retentate. This is sometimes an advantage, but may be a disadvantage in other processes. High-quality raw materials (low levels of micro-organisms) are essential in the food industry, especially when the retentate is the main product. The presence of surfactants in the process liquids can cause problems such as reduced capacity and increased retention. This is the case, for example, with antifoams which are sometimes used in the food industry. The various effects are dependent on type, concentration and membrane material.

Biological materials often change very quickly. Factors such as storage, transport etc., can affect properties which are important for the quality of the product as well as the final result of the separation process. The properties and handling of the raw materials should, therefore, be documented and controlled as accurately as possible in order to obtain reproducible results. It is also important to investigate and document the variation in the raw material with time (day-to-day variations as well as seasonal ones) and to carry out tests on samples of the material in order to detect variations.

2.2. Pretreatment

Mechanical pretreatment is used to remove fines, fat and fibre, for example. Heat treatment (pasteurization) is a form of pretreatment often used for biological material to kill micro-organisms, inactivate enzymes, prevent a lowering of pH, etc. In some cases it might be necessary to heat-treat the retentate prior to further treatment. Heat treatment of milk before UF (at 55 °C for 15-30 minutes) prevents the precipitation of calcium phosphates on the membrane surface or in the membrane. As the solubility decreases with temperature, the calcium phosphate precipitates during the heat treatment and no longer causes problems during ultrafiltration. Chemical pretreatment, e.g., adjustment of the pH may also be necessary.

2.3. Membranes

Important membrane properties are: selectivity, flux and surface characteristics, and thermal, chemical and mechanical stability. Selectivity is a measure of the

ability of the membrane to fractionate components in the feed stream and thus an important parameter in, e.g., ultrafiltration.

High selectivity is essential to obtain the desired fractionation, as low selectivity leads to multi-stage processes which, in most cases, are uneconomical. A high flux (the volume or mass of permeate, or any component in the permeate, passing through the membrane per unit area per unit time) is desirable in order to minimize the size of the membrane plant and thus the cost. It is, of course, also important that a high flux is retained for long periods of operation.

For ultrafiltration, polysulphone is the most common membrane material due to its high thermal and chemical resistance, an advantage especially for cleaning purposes. This material is, however, quite hydrophobic and encounters, sometimes, fouling problems. More hydrophilic membrane surfaces are being developed. Such polymeric membranes are generally a little less resistant than polysulphone. The development of ceramic membranes with hydrophilic surface properties and excellent thermal and chemical properties, is of great interest for the treatment of liquid food and food waste waters. At present, these membranes have mainly been used for crossflow microfiltration due to the high fluxes obtainable compared with those for polymeric membranes. The number of companies offering ceramic microfiltration membranes is increasing, while development of asymmetric polymeric MF membranes seems to be very limited. Recently, more resistant ceramic ultrafiltration membranes have been developed. For UF, the flux improvements are negligible compared with polymeric membranes, and the matter of fouling prevention will be of major importance.

For reverse osmosis, polyamide is the main membrane material, having reasonably good thermal properties but being extremely sensitive to oxidizing agents such as chlorine. Development of more chlorine-resistant polymeric RO membranes is in progress and some are already in the market [3,4,5]. Recently some work aiming at producing inorganic nanofiltration membranes has been initiated. Such membrane materials could have great potential if the separation properties prove to be good enough.

2.4. Modules

For food processing, and especially in the dairy industry, where large volumes of liquids are treated, the use of spiral wound sanitary ultrafiltration modules has increased rapidly due to the decreased cost compared to the plate-and-frame systems or tubular systems. In New Zealand, the use of spiral wound modules has

increased enormously in the dairy industry and plants with membrane areas of thousands of square metres are being installed. Dow recently introduced a cassette module for ultrafiltration and microfiltration with short transport distances and thus much lower pressure drops than in traditional spiral modules. This module is being tested for water treatment. For highly viscous process streams, a specially designed module was developed by DDS (now Dow).

For reverse osmosis, membranes and modules withstanding higher pressures than those used traditionally have been and are being developed, making it possible to attain higher concentrations and thus to decrease the need for further concentration steps. This is of special interest for the food industry, where heat-sensitive liquids are often processed. The development of steam-sterilizable modules is of interest for the biotechnology industry but also of benefit to the food industry.

2.5. Process conditions

Sometimes, the conditions under which the process is run are more important than the membrane material itself. Start-up mode, trans-membrane pressure, etc. play a significant role in the separation behaviour and fouling tendency, especially in microfiltration. Residence times and running temperature during membrane filtration are of great importance for the bacteriological status of the final product. To minimize the clogging of the membrane, the process liquid should be preheated to the process temperature before being pumped into the membrane equipment. For the same reason, rinsing prior to cleaning should also take place at the process temperature. The permeate side should also be full during normal running, as well as during cleaning and disinfection, as air pockets may result in the risk of microbial growth.

2.6. Process mode

In the application of crossflow microfiltration of liquid foods, very high fluxes are often encountered in the beginning followed by a rapid flux decrease together with a change in separation properties (increased retention). The importance of the processing mode has gained more and more attention. Starting up, circulation velocity, transmembrane pressure etc. are very important. Low transmembrane pressures and high circulation velocities cannot be combined in traditional module designs. For dairy applications of microfiltration using ceramic membranes from SCT, France, a substantial flux improvement was obtained using the Uniform Transmembrane Pressure (UTP) process patented by Alfa-Laval [6]. In this process, a uniform transmembrane pressure is maintained by circulating the

permeate at high speed concurrently with the retentate, inside the module, but outside the module element.

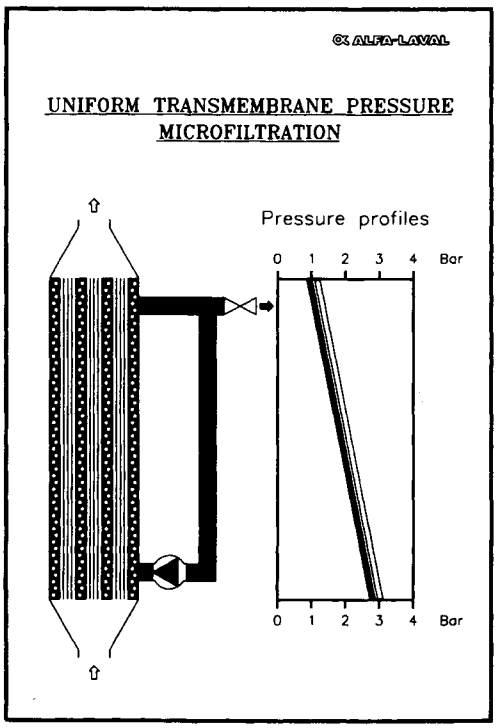

Figure 1. Circulation systems in the MF module with uniform transmembrane pressure. (Reproduced with the permission from Reference [7]).

On the permeate side of the module, the space which is normally empty is filled with plastic grains in the UTP version as indicated in Figure 1 [7]. The high-speed circulation of the permeate causes a pressure drop inside the channels. The pressure drop on the permeate side is adjusted by the permeate pump. This results in a uniform transmembrane pressure over the whole membrane area which in turn results in an optimal and controllable utilization of the membrane area. When the membranes start to clog, the pressure/permeate valve is opened, thereby increasing the transmembrane pressure. Thus, the permeate flux at the outlet is constant. As the transmembrane pressure determines the flux, a higher transmembrane pressure leads to a higher flux. However, clogging of the membranes then takes place faster and the running time is decreased.

For polymeric, hollow fibre microfiltration membranes, the Dutch company X-flow has a patent pending concerning the use of the so-called "BACKSHOCK process", a further development of backflushing, a mode of operation in which the transport direction through the membrane is reversed by applying pressure in the opposite direction so that the feed enters the module through what is normally the permeate outlet. The backflush process is optimized both for the duration of the backflushing pressure and for the backflushing interval. The improvement in the product flow rate achieved with backflushing is mainly a function of the backflushing pressure and the interval between two backflushes. X-flow found that extremely good results could be obtained using very short backflushing times (typically 0.06 second) with a time interval of maximum of 5 seconds, preferably 1 to 3 seconds. Since the effective backflushing time is very short and the backflushing pressure is relatively high (typically 0.1 MPa over the feed pressure), the name BACKSHOCK was introduced. The loss of permeate during backshocking is said to be very low and hardly affects the net permeate flow. With this technique, quite good fluxes were obtained using very low pressures and crossflow velocities [8].

2.7 Membrane cleaning and desinfection

The cleaning and desinfection of membranes as well as the auxiliary equipment is essential, in order to maintain high fluxes, as well as to maintain a good bacteriological status. In the membrane treatment of biological materials, e.g., liquid foods, membrane equipment is usually cleaned at least once per day [9,10].

Much work has been devoted to the investigation of cleaning methods, often based on trial and error. Mechanical cleaning can be effected by introducing a high shear rate at the membrane surface. Some material can easily be removed by rinsing with water at a high rate of circulation and reduced pressure.

Chemical cleaning is essential. The method of cleaning depends, naturally, on the membrane material and its chemical and temperature resistance, as well as the composition of the process liquid and the deposits on the membrane surface or its interior. Acids are used to remove mineral deposits, e.g., 0.5% HNO_3. Protein deposits as well as other types of deposits are often removed with an alkaline cleaner containing 0.5-1% NaOH. Formulated cleaning agents, containing alkalis, phosphates, complex-forming agents and tensides, are often used. For some types of membranes and deposits, cleaning agents containing enzymes are used, e.g., proteases and amylases. Some kinds of surfactants can cause severe flux reduction problems and is thus very important to follow the membrane manufacturer´s

recommendation and not to change the cleaning agent without consulting the manufacturer. The use of cleaning agents containing unsuitable surfactants may result in having to replace the membranes in the whole plant.

Chlorine is often present in alkaline cleaning agents. These agents clean more effectively than the same agent without chlorine and also kill bacteria. Hypochlorite has a certain cleaning effect on its own, and has been successfully used for the cleaning of membranes after the ultrafiltration of potato fruit juice. Some membranes, however, cannot withstand oxidizing agents, and a degree of caution is therefore recommended. The use of chlorine in the food industry is debated and it might not be allowed in the future.

Hypochlorite, hydrogen peroxide, peracetic acid or sodium bisulphite are usually employed for the disinfection of membranes. Some inorganic membranes can be steam sterilized.

3. NEW DEVELOPMENTS IN FOOD APPLICATIONS

3.1. Dairy applications

Ultrafiltration of milk and whey for the enrichment of proteins and the use of reverse osmosis for concentration are established processes in the dairy industry. The introduction of crossflow microfiltration using ceramic membranes at constant transmembrane pressures applying the UTP mode mentioned earlier, has opened new and exciting possibilities, and during the last few years, membrane separation processes for the fractionation of milk proteins have been developed [11].

Examples of interesting products are defatted whey protein concentrates, lactoferrin, lactoperoxidase, β-lactoglobulin, α-lactalbumin and κ-glycomacropeptide, opening up new worldwide markets with potentially high added value. The same approach has been investigated for the fractionation of caseins. Micellar casein-enriched milks obtained through the use of microfiltration have provided opportunities for cheesemakers to improve the efficiency of their equipment. Native phosphocaseinate, also separated by microfiltration, will easily compete with traditional products. β-casein, separated by microfiltration, in addition to expanding the manufacture of cheese varieties through modification of the α_s/βcasein ratio in cheesemilk, is the required substrate for producing numerous peptides thought to have physiological activities [11].

3.1.1. Fat removal from whey

The production of whey protein concentrates (WPC) with a protein content of 35-80% of the total solids using ultrafiltration has been a commercial process for many years [12]. WPC's with high protein contents have a substantial amount of fat which leads to decreased functional properties and shorter storage time. Part of the fat is valuable phospholipids. Residual fat can be separated as proposed by Maubois et al. [13] by exploiting the ability of the phospholipids to aggregate through calcium binding when subjected to a moderate heat treatment (55 °C, 8 min). The resulting precipitate, as well as non-aggregated fat, is removed by microfiltration through a 0.2 μm membrane. The defatted whey leads to higher fluxes in UF compared to untreated whey, but UF membranes with smaller cut-offs than for untreated whey are required to obtain the same whey protein retention [14]. Whey fat aggregation is optimized by using ultrafiltered whey retentate instead of normal whey, adjusting and maintaining MF retentate pH at 7.5, and finally applying the heat treatment described above. This has also led to major improvements in MF performance such as flux rate and protein recovery. Defatted whey is also a very good raw material for the production of lactoferrin and lactoperoxidase through ion-exchange chromatography.

Purified β-lactoglobulin and α-lactalbumin can be prepared from the defatted WPC due to the fact that at low pH and moderate heat treatment (55 °C) α-lactalbumin polymerizes reversibly entrapping most of the other whey proteins except β-lactoglobulin. By using microfiltration (0.2 μm) β-lactoglobulin can be fractionated from the other proteins. Further purification of β-lactoglobulin is carried out by ultrafiltration coupled with electrodialysis or diafiltration [14]. Purification of α-lactalbumin from the MF retentate is accomplished by solubilization at a neutral pH and then ultrafiltration through a 50,000 dalton membrane. Further work is required to improve the purity.

Alfa-Laval [7] has developed a process for the production of defatted, functional WPC containing 85% protein from sweet whey in which the whey is first ultrafiltrated (volume reduction factor 2.5-4) in order to reduce the whey volume and thus the membrane area of the microfiltration plant. In the MF plant, 90% of the feed is removed as permeate. The retentate contains nearly all the fat and denatured proteins from the whey and at least 99.5% of the bacteria and spores. A minimum constant capacity of 30-45 l/m^2h during 20 hours for UF-treated raw whey using 0.1 μm membranes and uniform transmembrane pressure microfiltration is possible, at a volume concentration factor of 4 in the UF plant. The MF retentate is then further concentrated in a second UF plant giving a final protein

content of 85% and a fat content of less than 0.4% in the dried product [7]. When MF is performed on raw whey at a concentration factor (CF) of 10, the overall flux is 90-100 l/m^{2}h, while the MF flux of the UF retentate (CF 4) is about 40 l/m^{2}h. meaning that the overall MF flux is about 4x40=160 l/m^{2}h. This means that the overall gain in the MF flux is due to the removal of the free fatty acids and the phospholipoproteins which are normally the main source of irreversible fouling material in the MF ceramic membranes.

3.1.2. Removal of bacteria from milk

In the Bactocatch process developed by Alfa-Laval [6] for the extension of the shelf-life of pasteurized milk or for the improvement of the quality of cheese milk, the principle of uniform transmembrane pressure is used during microfiltration of skim milk through 1.4 μm ceramic membranes. In this way, the bacterial content of the permeate is reduced to less then 0.5% of the original value, while the retentate contains nearly all of the bacteria as well as spores. The retentate is then mixed with cream and heat treated at 130 °C for four seconds before remixing with the permeate. Finally, the mixture is pasteurized. The bacteriological quality of the milk is improved significantly. Fluxes of about 600 l/m^{2}h during up to six hours are reported [6]. Several commercial plants have been installed worldwide, a few of them in Sweden.

In spite of the decrease in rennetability of MF/HTT cheesemilk compared with low pasteurized milk, several cheese varieties were produced successfully without nitrate addition. From the view point of spore reduction, the MF/HTT technique is reported to be more efficient than one-stage bactofugation [15].

3.1.3. Fractionation of caseins

Ceramic membranes with a pore size of 0.2 μm allow a specific concentration of micellar casein in skim milk. The resulting permeate has a composition close to that of sweet whey, but it does not contain caseinomacropeptide, phospholipoproteins or bacteria. Its content of high molecular weight whey proteins, such as immunoglobulins and bovine serum albumin, may be different from that of a normal whey. The retentate is an enriched solution of native calcium phosphocaseinate. Depending on the concentration, it can be utilized as a casein standardized cheesemilk. If diafiltration is performed during the MF step, purified phosphocaseinate is obtained (up to 90% protein of total solids content) [11]. Microfiltration using 0.2 μm membranes also allows for the separation of β-casein when this component is solubilized from the casein micelles giving process streams suitable for modifying the β-casein/α_s-casein ratio in cheese milks and consequently

the texture and flavour of the cheeses [16]. The main interest in β-casein is related to the presence of peptides with biological activities in its sequence. The recovery of small amounts of valuable components or an alteration in composition of dairy liquids is regarded to be major challenges possible by membrane technology.

3.1.4. Ultrafiltration used in cheesemaking

Intensive work has been, and is being done, on ultrafiltration for the manufacture of fresh, soft, semihard and hard cheeses. Examples are given in Table 1.

Table 1.
Different types of cheese processed by ultrafiltration. Data from [17]

Cheese type	Structure		UF concentration		UF and
	Closed	Open	Partial	Total	evaporation
Quarg	x			x	
Pate Fraiche	x			x	
Cream cheese	x			x	
Cast Feta	x			x	
Domiati	x			x	
Ricotta	x			x	
Cheese Base	x			x	x
Structure Feta		x	x		
Mozzarella		x	x		
Queso Fresco		x	x		
Quartirolo		x	x		
Blue cheese		x	x		
White mould cheese		x	x		
Blue-white cheese		x	x		
Tilsiter type		x	x		
Gouda type		x	x		
Cheddar (APV Sirocurd)		x	x		

There are various ways of using ultrafiltration in cheesemaking [17].

* Protein standardization: The protein content of milk varies during the year.

Ultrafiltration of the milk results in a more homogeneous and constant cheese quality.

* Preconcentration: The capacity of existing equipment can be doubled if milk is preconcentrated by ultrafiltration. This does not increase the yield substantially, however.

* Partial concentration: Between 20 and 40% total solids is mostly used for open-structured cheeses.

* Total concentration: Ultrafiltration is used to obtain a total solids content equal, or almost equal to the concentration in the final cheese.

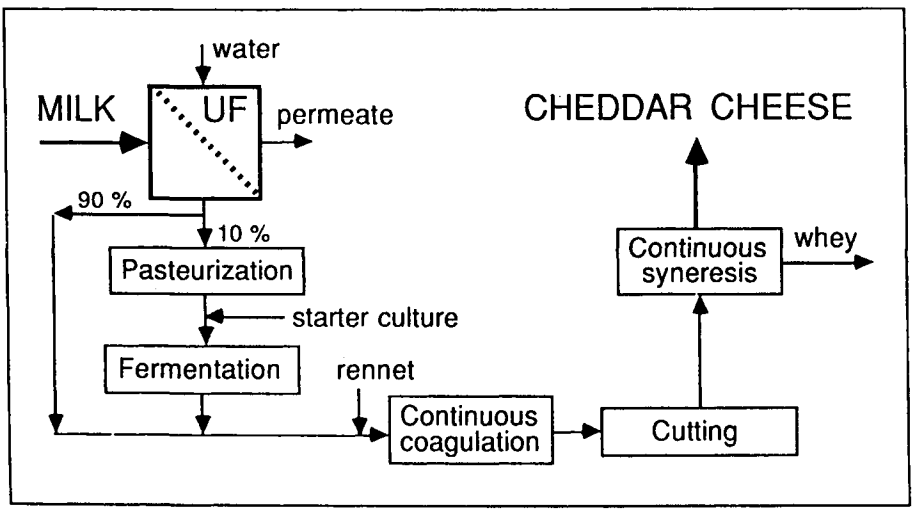

Figure 2. Flow scheme for the APV-SiroCurd process. (Reproduced with permission from Reference [18].

So far, the use of ultrafiltration for the production of semihard and hard cheeses is limited. Cheddar cheese, however, can be successfully made commercially from UF retentates by a process (APV-SiroCurd) developed in Australia [18]. In this process, the standardized and pasteurized milk is ultrafiltrated and diafiltrated giving a retentate having the required solids, lactose and salt balance. Part of the retentate is pasteurized and inoculated, mixed with the bulk retentate and rennet and coagulated [19]. There is a certain whey drainage, which is not the case when

fresh and soft cheeses are produced. A flow scheme for the cheddar cheese process is shown in Figure 2. The first commercial plant was commissioned in Australia in 1986, a second was installed in the USA in late 80'ies.

In many cases, traditional process technology must be modified when UF concentrates are used instead of milk. It is, for instance, very important to carefully adjust the mineral balance in order to obtain the correct rheological properties and taste in the final product. Ultrafiltration at the normal pH of milk often results in too high a calcium content in the final product, giving off-flavours and undesirable rheological properties.

3.1.5. Cheese brines

Another application of microfiltration is the removal of micro-organisms and fines from cheese brines. It has been shown that pathogenic bacteria such as Listeria are able to survive in cheese brine and that Staphylococcus sp. are able to grow even in a brine with a salt content up to 20% [20]. Various methods of brine purification are used, the most common one being pasteurization, addition of NaOCl and kieselguhr treatment. Microfiltration is an interesting technique in this context since with this technique the bacterial content is reduced without changing the chemical composition of the brine. It has been shown that momentary heating of brine to 40-50 °C, immediately before the membrane filtration, causes precipitation of calcium phosphate complexes on the membrane as the solubility of these complexes decreases with increasing temperature [15]. This precipitation will cause heavy fouling of the membrane and will lead to a rapid and serious flux drop, more than cancelling out the improvement in flux due to the temperature. Thus, use of a filtration temperature close to the natural brine temperature is recommended. A temperature of 20 ^{0}C will minimize the need for cooling and change the mineral balance of the brine as little as possible during the filtration process [21].

3.1.6. Nanofiltration of whey

Lately, membranes with selectivity in the intermediate RO/UF cut-off range have been made available. Using this new class of modified, thin-film composite membranes, which has a high retention of low-molecular-weight organic substances and a fairly high salt permeation, it is possible to partially remove Na^{+}, Cl^{-} and other monovalent ions from, for example, whey without a significant loss of lactose. The process, sometimes referred to as nanofiltration and sometimes to loose reverse osmosis or ultra-osmosis, is a very interesting alternative to ion-exchange

and electrodialysis if moderate demineralization is required. One advantage of nanofiltration compared with the other two processes is that nanofiltration is quite a simple process through which partial demineralization and concentration can be obtained simultaneously (and in one step).

So far, very little work on the transport mechanisms in nanofiltration has been published. The salt permeability depends on the nature of the component as well as on the membrane itself. It is much higher for monovalent ions than for divalent ions. Increasing the NaCl content leads to a higher permeability of monovalent ions. At pressures in the range of 1.7-2.5 MPa, negative Cl^- retentions were obtained with sweet whey at volume reduction factors of more than two. The higher the volume reduction factor, the more negative the Cl^- retention [22].

Applications of nanofiltration in whey processing [23]:

* Concentration and partial demineralizing whey UF permeates prior to further processing into lactose and lactose derivatives.

* Converting "salt whey" to normal whey while solving a disposal problem.

* Preconcentration and partial demineralization of sweet whey to make 50% demineralization products or to act in sequence with ion-exchange or electrodialysis to produce 90% demineralized products.

* Partial demineralization and concentration of hydrochloric acid casein whey to convert it to a low-chloride "sweet whey".

* Partial deacidification and concentration of cottage cheese, fresh cheese (fromage frais), quarg and lactic acid casein wheys so as to convert them to "sweet wheys".

* Treating cheese brines solutions for reuse.

* Partial demineralization of lactose mother liquor (delactosed whey).

The partial demineralization of whey (or milk) UF permeates prior to the manufacture of lactose is reported to be employed commercially at three or four plants. The conversion of "salt whey" to sweet whey by NF is reported to be carried out in about 10-15 commercial plants with capacities ranging from 9 m^3 to 22 m^3 "salt whey" per day. Benefits are realized through the recovery of whey solids and

a reduction in disposal costs [23].

3.2. Fruit juices and wine

3.2.1. Clarification

In juice processing, the process stream contains compounds such as pectins, cellulose, hemicellulose, starch and proteins, which cause an undesirable turbidity when the product is stored. It is thus necessary to clarify the juice. Since the late 1970's, ultrafiltration has been applied commercially for the clarification of different types of fruit juices. Most ultrafiltration plants have been installed for apple juice clarification, but commercial systems are also in operation for grape, pear, pineapple, cranberry and citrus juices. MF is now also used for the clarification of juices. Advantages of using membrane technology for the clarification are the following: a simplified and continuous process, shorter treatment times, lower personnel costs, reduced amounts of additives, increased efficiency, better colour, pasteurization unnecessary if the pore size is less than 0.2 μm.

One disadvantage is that the juice may exhibit turbidity when stored (past clouding). This phenomenon is ascribed to certain substances which can pass through the membrane.

Microfiltration is now used, to an increased extent, for the clarification of fruit juices and wines. In 1992, MF had replaced kiselguhr filtration in wine making in some 400 plants, producing 20-30% of the German wine. MF plants are small, typically less than 200 m^2 in area.

3.2.2. Concentration

Fruit juices are concentrated in order to prolong their shelf-life and to minimize the cost of distribution and storage. Before retailing, the concentrated juice is diluted, pasteurized and packaged. Concentration normally takes place by means of vacuum evaporation in one or more stages. During this operation, many of the volatile aroma compounds (typically various organic substances such as esters, aldehydes, alcohols etc., which are present in very low concentrations, i.e., ppm levels) in the juice are lost in the vapour, resulting in reduced product quality. In order to maintain a high quality, aroma compounds must be recovered and added to the juice concentrate. For juices such as apple, pear and some berries, the vapour from the first of the evaporation stages is often taken to a distillation

column where it is concentrated and cooled to a low temperature. The aroma concentrate is stored separately and then added to the diluted juice concentrate before pasteurization. On an industrial scale, such distillation techniques result in a very low yield. Also, the aroma compounds are treated at a relatively high temperature for quite a long time, which has a negative effect on the quality of the final product.

Pervaporation using hydrophobic membranes has showed to be a very interesting method for the recovery of aroma compounds from, for example, apple juice but also from beverages containing ethanol [24]. The possibility of using a low process temperature allows very gentle treatment and thus an improved flavour compared with aroma recovered by distillation. The process seems to have great commercial potential.

Bengtsson et al [24] obtained high enrichment factors, especially for esters and aldehydes, which are most important for e.g., apple flavour. When concentrating a natural aroma condensate from an apple juice concentration plant by pervaporation, a sensory study showed that the pervaporation permeate had a more natural flavour than the concentrate obtained conventionally at the plant (Alfa-Laval, Aroma Recovery Units (PAR)).

The mass transfer in the liquid feed can greatly affect the pervaporation process and it has been shown that the relative permeate composition of five different aroma compounds varies depending on the Reynolds number on the feed side. Operation with optimal feed flow conditions, i.e., a turbulent flow regime, can in some cases, such as for 2-methylbutanal, give a flux that is 10 times higher than the flux at the lowest feed flow (Re = 10). Although it might seem optimal to operate the pervaporation process under turbulent conditions in order to achieve maximum fluxes, other feed flow conditions might be optimal with respect to the sensory perception of the aroma concentrate produced. It is thus possible to control the relative composition, or in other words the flavour, of the aroma concentrate by a simple manipulation of the feed flow [25].

Reverse osmosis is used commercially for the concentration of different types of fruit juices. High aroma retention is reported using polyamide membranes. However, due to the osmotic pressure and/or the viscosity, traditional RO is used only as a preconcentration stage to reach 20-25 °Brix [26].

Separa Systems, a joint venture of the FMC Corporation and the Du Pont

Company, has developed a very interesting process, the "FreshNote System", mainly for the concentration of citrus juices [27]. In the first step, the juice is ultrafiltrated giving a clarified permeate stream and a low-volume retentate stream containing some soluble solids and all of the insoluble solids, pectins, enzymes, and the micro-organisms that would affect the stability of the concentrate. The permeate stream contains soluble compounds, particularly the sugars and the flavour and aroma compounds. The retentate is subjected to mild pasteurization which destroys enough of the micro-organisms to provide the necessary stability for the juice concentrate under typical storage conditions.

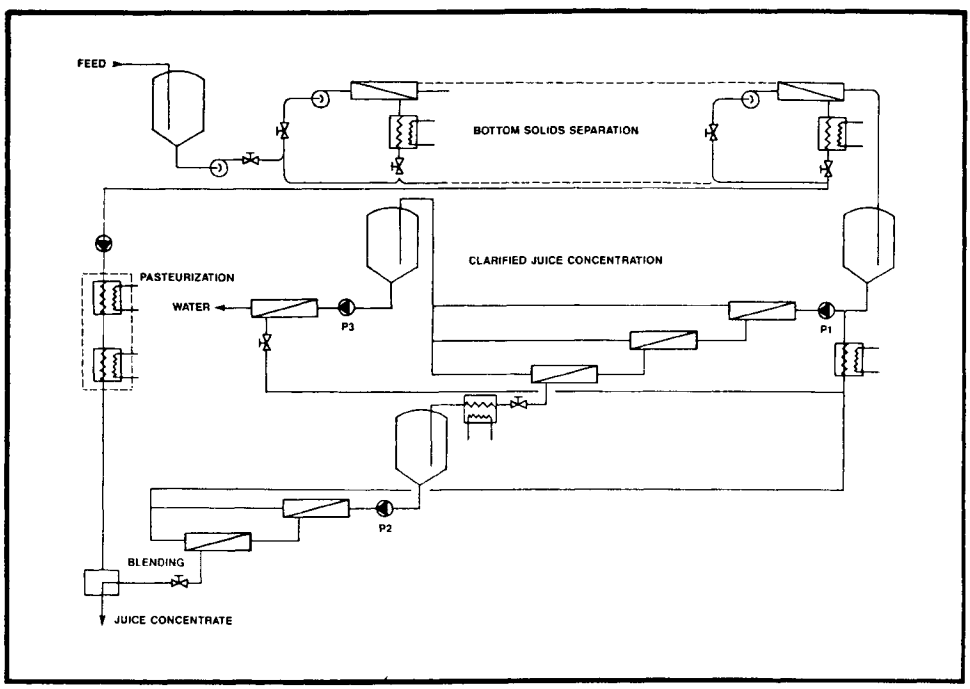

Figure 3. A schematic diagram of "FreshNote" juice concentration process [27].

The clarified permeate is concentrated in a series of reverse osmosis, hollow fibre modules at pressures between 10 and 14 MPa. By using membranes with different degrees of sugar retention and returning any permeate with a non-zero sugar retention to the stage where the sugar retention is the highest, it is possible to achieve very high levels of sugar concentration. 55 °Brix has been demonstrated commercially and concentrations as high as 70 °Brix have been demonstrated on

pilot-plant scale. The permeate from the first stage can be treated in a second reverse osmosis stage to further remove traces of sugar and aromas. The concentrated clarified juice stream is recombined with the pasteurized UF retentate. A generic process schematic is shown in Figure 3.

An integrated process scheme for the wine and juice industry has been suggested by Drioli [28] as shown in Figure 4.

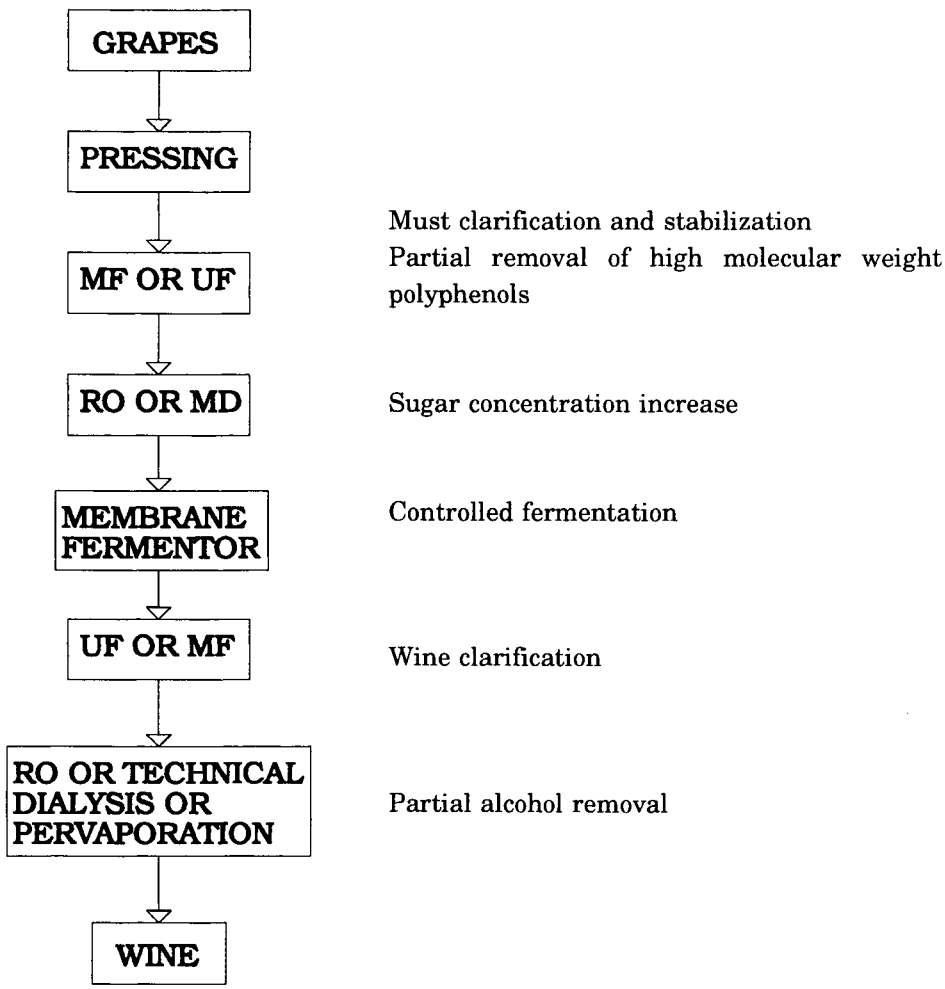

Figure 4. A possible cycle in the wine and juice industry with integrated membrane processes. (Reproduced with the permission from Reference [28]).

3.3. Beer

In the beer industry, several full-scale installations exist for the production of low-alcohol beer by tight RO membranes, followed by water dilution of the retentate. Apart from the cost, the loss of aroma compounds in the permeate is an important factor. Generally, cellulose acetate membranes (ethanol retention about zero) are used in order to remove sufficient amounts of ethanol [29]. Such membranes have a poorer retention of aroma compounds than polyamide membranes, but the ethanol retention of polyamide RO membranes is as high as about 70% and they are thus not suited for the production of low-alcohol products. The aroma loss problem can, to some extent, be overcome by changing the brewing process, i.e., producing a more "aromatic" beer.

In beer production, almost all the yeast and a significant proportion of the micro-organisms are removed in the traditional filtration step, which includes kiselguhr filtration [30]. Also tank bottoms, which contain some beer, can be further processed in order to recover the beer. Much research has been devoted to the application of microfiltration for beer clarification and for beer recovery from tank bottoms. Another potential application is the replacement of the final pasteurization by microfiltration. The thermal load is avoided using crossflow microfiltration, and the beer clarity is also improved, since particles passing the traditional filter can be removed at the same time [30].

Crossflow microfiltration has a significant potential in brewing. The impact would be substantial if many current processes could be combined into a single stage, e.g., rough beer clarification, tank bottom recovery and sterilization. The use of this technology for wort separation provides an exciting opportunity to bring continuous processing into the brewery and to match further developments in continuous fermentation [31]. Much work has been done using ceramic membranes and high crossflow velocities. Fouling problems have occurred, leading to decreased fluxes and changes in separation properties, which result in the risk of losing some colour, bitter flavour components and foam stabilization proteins.

Using the "BACKSHOCK" process described earlier for membranes with a so-called reversed asymmetric structure (the feeds in contact with the larger pores of the membranes, while the flow-determining pores are located at the interface of the membrane and the permeate), very stable fluxes over 200 l/m^2h have been reported when microfiltering beer. In this study, performed with polymeric membranes, very low transmembrane pressures (less than 0.01 MPa) and very low crossflow velocities (less than 0.5 m/s) were used [8].

In a few years, crossflow microfiltration will most probably replace traditional methods to a quite large extent in the beer industry.

3.4. Non-aqueous systems

The use of membrane technology for the separation of solutes from nonaqueous solutions is a new field. As the solvent resistance of membranes is being improved, it is of increasing interest to apply ultrafiltration and microfiltration in the process for refining of edible oils.

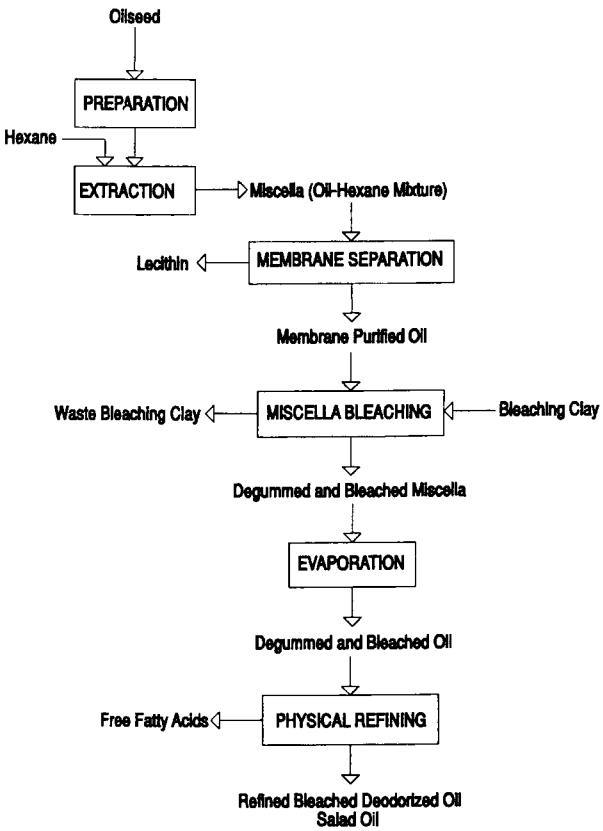

Figure 5. Comparison between a classical process and a UF process for refining of edible oil a) UF process (Reproduced with the permission from Reference [32]).

CLASSICAL PROCESS

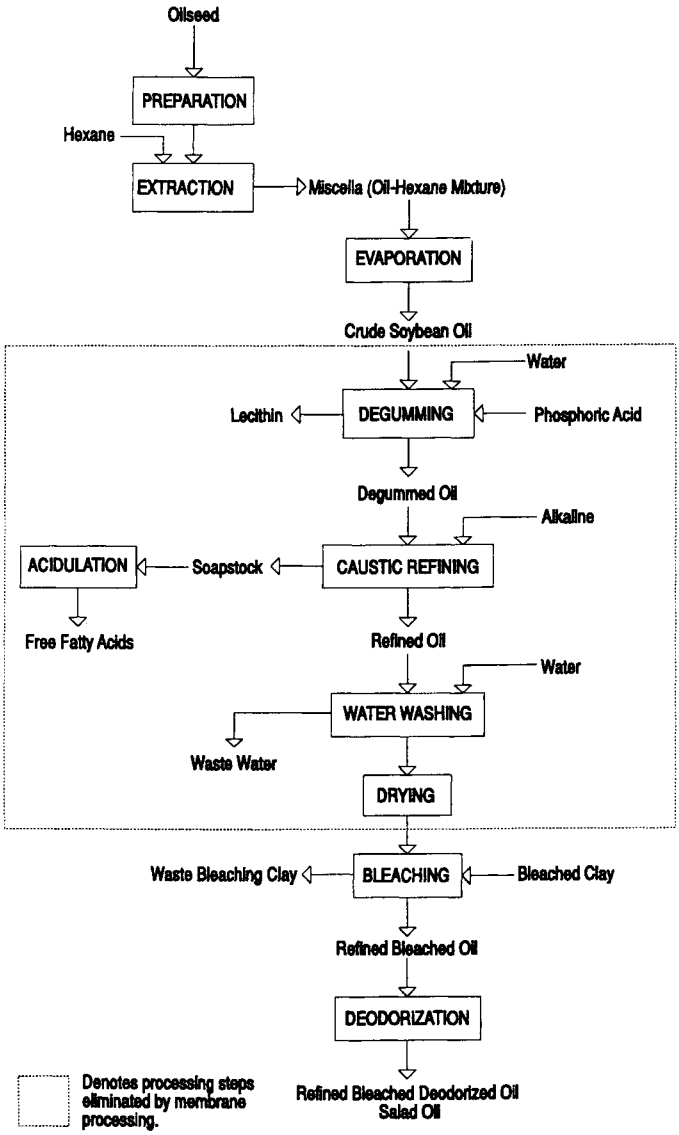

Figure 5. Comparison between a classical process and a UF process for refining of edible oil b) Classical process (Reproduced with the permission from Reference [32]).

In general, vegetable oils for edible use, such as soybean oil or rapeseed oil, are purified and produced by a chemical refining process including hexane extraction and stripping, degumming, deacidification, washing, bleaching and deodorization. Chemical refining has disadvantages. Large amounts of energy are used in heating and cooling oil between process steps and in generating the required vacuum levels during the various process steps: chemical damage occurs due to severe treatment with alkaline solutions, resulting in considerable loss of oil. Also, chemical processing with water and chemicals results in large volumes of highly contaminated waste water. By degumming crude micella by ultrafiltration, and by removing hexane from the permeated micella, a degummed oil can be obtained in one step, replacing the traditional degumming and deacidification steps and perhaps also replacing the bleaching step, as shown in Figure 5 [32]. Potential applications are e.g., the recovery of phospholipids from oil-hexane and oil-IPA micella; removal of catalyst from hydrogenated oils and rejuvenation of used frying oils [33].

3.5. Waste water processing

It is of interest to treat waste streams from the food industry by membrane separation technology, partly for environmental reasons, and partly for the recovery of valuable substances and the reduction of water consumption. Examples of such applications are the treatment of different rinse waters, i.e., diluted products obtained when changing to another product or before cleaning and the treatment of cleaning solutions to recover alkali etc. By recovering proteins from effluents, e.g., potato fruit juice and red meat abattoirs, the product value can be increased. The environmental problems caused by olive oil production are substantial in most of the Mediterranean countries. The waste water contains large amounts of suspended and dissolved compounds, such as sugars, nitrogenous compounds, fats, acids, polyalcohols, polyphenols, pectins, and salts. The polyphenols can cause a reduction in the protein bio-availability when used as animal feed; on the other hand the recovery of polyphenols could give economical benefits if recovered and used as natural antioxidants and pigments. An integrated process for the treatment of effluents from olive oil mills was developed in a project supported by the European Community [34]. The process includes a physical-chemical pre-treatment, UF and RO stages and a final polishing phase. It was found to be possible to recover polyphenolic substances with good antioxidizing properties from the waste water. The dephenolized residue could be used as an animal feed.

4. FUTURE TRENDS

Emerging reverse osmosis applications in the food industry are reported to be in dairy processing, sweetener concentration, in juice and beverage processing, production of low-alcohol beer and wine, and waste stream processing [35]. For ultrafiltration, the prospect of future applications in the food industry is excellent according to Eykamp [36], who states that many applications in broad food areas are based on the ability to change protein and starch/sugar, salt and water ratios. Longer term prospects include refining of oils. Applications will be widespread and increase with technical progress and customers' acceptance. Future applications for microfiltration appear also to be excellent [37]. Many applications in broad food areas are based on the ability of microfiltration to retain micro-organisms without affecting desirable properties. Applications are predicted to be large, but the growth rate slow.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 7

Applications of microporous glass membranes: membrane emulsification

Kazuhiko Kandori

School of Chemistry, Osaka University of Education,
Asahigaoka 4-698-1, Kashiwara-shi, Osaka 582
Japan

1. INTRODUCTION

Emulsification has been used in various industrial applications for processing food, cosmetics, medicine, paints, etc. Numerous studies have been reported on the preparation of both Oil-in-Water(O/W) and Water-in-Oil(W/O) emulsions (1-7). Also, many emulsification equipments such as colloid mill, homogenizer, ultrasonication etc. are being used in various industrial fields (8). However, their emulsification conditions cannot be precisely controlled and the emulsion droplets are polydispersed. According to the DLVO theory (9), the monodispersity of emulsion droplets is necessary for forming a stable dispersion against aggregation. Therefore, a new emulsification technology for producing monodispersed emulsions became attractive. Incidentally, in many applications the monodispersity in droplet size can be regarded as a plus.

Recently, Nakashima and Shimizu developed a new type of porous glass, the pores of which are uniformly controlled in size, synthesized from CaO-Al_2O_3-B_2O_3-SiO_2 type glass, and made from "Shirasu" which is available as volcanic ashes in the southern part of Kyushu, Japan. This was named ***Shirasu-Porous-Glass (SPG)*** (10,11). In 1988, they proposed a method for the preparation of emulsions using SPG and referred to as "***SPG Membrane emulsification***" (12). The monodispersed oil droplets were simply formed by pressing an oil into a water phase through the uniformly controlled pores of SPG membrane. Because of its simplicity and less energy consumption, this technique became attractive. In this chapter, the feature and application of this new membrane emulsification technique will be explained, specifically highlighting the methodology, on the basis of a pioneering work of Nakashima and Shimizu and the author's investigations (13-15). Applications of microporous glass membranes in emulsification to produce monodispersed droplets, production of uniform silica hydrogel particles, preparation of uniform polymer microspheres, preparation of uniform multiple emulsions, and ozonation of liquids are discussed.

2. SPG MEMBRANE

2.1. Preparation of SPG Membrane

In recent years, porous glass became attractive as a material for formation of unique structures. They are expected to find application in various industries (16). In the earlier stage, Na_2O-B_2O_3-SiO_2 type glass, which was made by utilizing phase separation of Na_2O, B_2O_3 and SiO_2, was used as a primary glass for producing porous materials (17). Recently, Nakashima and Shimizu found a new stable phase separation region in CaO-Al_2O_3-B_2O_3-SiO_2 type glass by utilizing "Shirasu" and made a unique new type of porous glass (10).

Figure 1 shows the process for manufacturing SPG membrane using the CaO-Al_2O_3-B_2O_3-SiO_2 type glass as a primary glass.

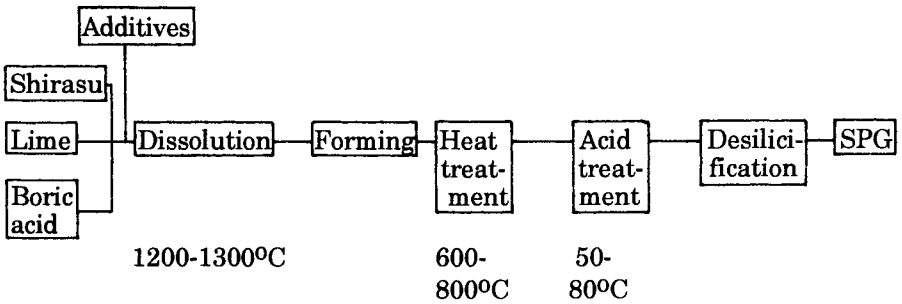


Figure 1. Production procedure for SPG

The basic process of separating the phase by heat treatment and dissolving the separated phase in an acidic solution is identical with that of Corning Process, so-called ***Porous Silica Glass (PSG)***, except for the addition of CaO and Al_2O_3 as major components. "Shirasu", used as a raw material, is volcanic ash and has a composition listed in Table 1.

After primary glass was formed into a tube, plate, powder, beads or fiber depending on the application of SPG membrane, it was subjected to heat treatment at a temperature of 600 to 800°C for several hours. This heat treatment causes the homogeneous primary glass to produce phase separation of SiO_2-Al_2O_3-rich and CaO-B_2O_3-rich glasses. Since the latter phase easily dissolves in acidic solution, the desired porous SPG can be obtained by immersing the phase-separated glass into a hydrochloric acid solution. Table 2 summarizes the chemical composition of the primary glass and SPG along with that of PSG made by the Corning process. It can be seen clearly from this table that the chemical components of SPG differ from PSG because it contains less SiO_2 and more Al_2O_3.

Table 1
Chemical Composition of "Shirasu"[11]

Components	wt. %
SiO_2	72.5
Al_2O_3	13.65
Fe_2O_3	2.14
CaO	1.26
MgO	0.29
Na_2O	3.04
K_2O	2.68
Ig. loss	4.54
Total	100.10

Table 2
Composition of Primary Glass, SPG and PSG[11]

Composition	Primary glass	SPG	PSG
SiO_2	49.27	69.41	94.0-99.5
Al_2O_3	9.47	12.81	0-0.5
B_2O_3	15.70	6.90	0.2-6.0
CaO	17.12	2.12	
Na_2O	4.97	4.59	0.1 to less
K_2O	2.20	3.68	
MgO	0.19	0.03	
Fe_2O_3	1.07	0.45	
Total	99.99	99.99	

The size of the separated phase determines the pore size of SPG. The pore size is closely related to the temperature (T) and time (t) of the heat treatment. Nakashima and Shimizu (11) revealed that the average pore radius (r) can be expressed by Eqn. (1) when T is changed under a constant t

$$\ln r = -E/2RT + C \quad (1)$$

Also r depends on t as is expressed by Eqn. (2) when t is varied under a fixed temperature T

$$r = At^{1/2} - B \quad (2)$$

where E is the apparent activation energy, R is the gas constant, and A, B and C are the constants determined by the composition of primary glass.

2.2. Physical Properties of SPG

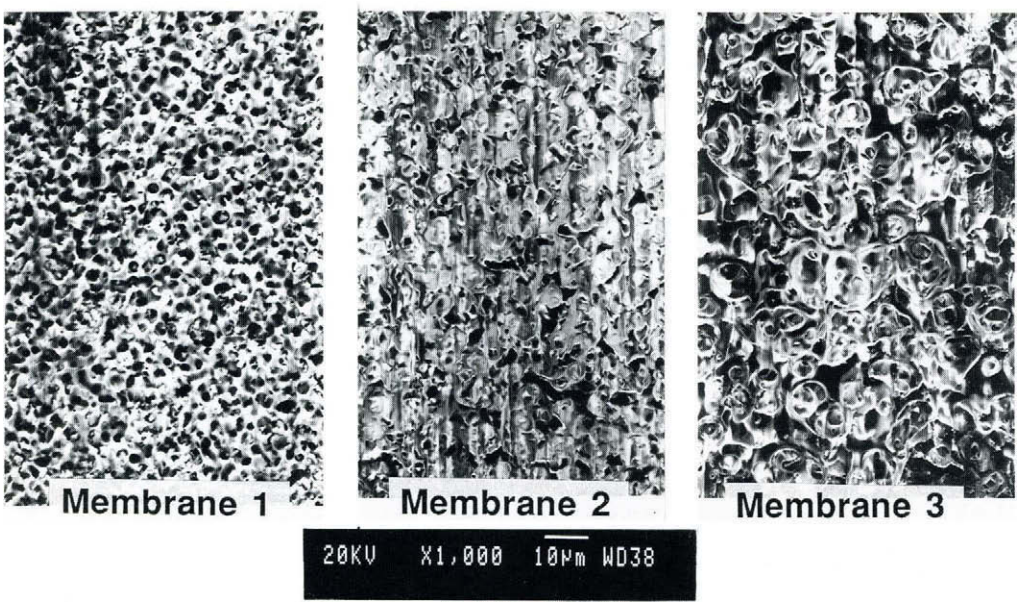
One of the features of SPG is the unique porous microstructure, which is related to mechanical properties such as strength. Figure 2 displays scanning electron micrographs of SPG membranes, and pore size distribution histogram measured by a mercury penetration porosimetry. It is clearly seen in Figure 2 that the outlets of Membrane 1 are uniformly controlled in shape and size. However, the irregularities can be seen for the surfaces of Membranes 2 and 3, while the pore diameter in all three membranes is narrowly distributed. This surface roughness of Membranes 2 and 3 could be due to the dissolution of SPG in a highly acidic solution resulting in enlargement of the pore size. The pore size of SPG can be varied over a wide range from several nanometers to 10 micrometers. This is the distinguishing feature of SPG. Also, the many hydroxyl groups which cover the surface of SPG membrane make it an excellent material for surface modification by reaction with organic silanes such as octadecyl trichlorosilane. Table 3 shows physical properties of a typical SPG membrane. Detailed information on SPG is given elsewhere (11).

Table 3
Physical Properties of SPG

Pore diameter	20 nm to 10 μm
Porosity	50 to 60 %
Pore volume	0.4 to 0.6 cm^3/g
Specific surface area	0.2 to 8.0 m^2/g
True density	2.5 g/cm^3
Thermal expansion coefficient	$60x10^{-7}$ K^{-1}
Heat resisting temperature	650 °C
Compressive strength	2000-3000 kgf/cm^2
Bending strength	710-840 kgf/cm^2

2.3. Availability and price of SPG

The SPG membarne can be formed into tubes, flat sheets, particles or beads of various sizes. Tubular membranes are typically made in 10 mm outer diameter, and are generally available incorporated in filtration module. Flat sheets are cut into disc or square configurations. Several beads and particles are also made for chromatography separations. The standard products and their prices are listed in Table 4.



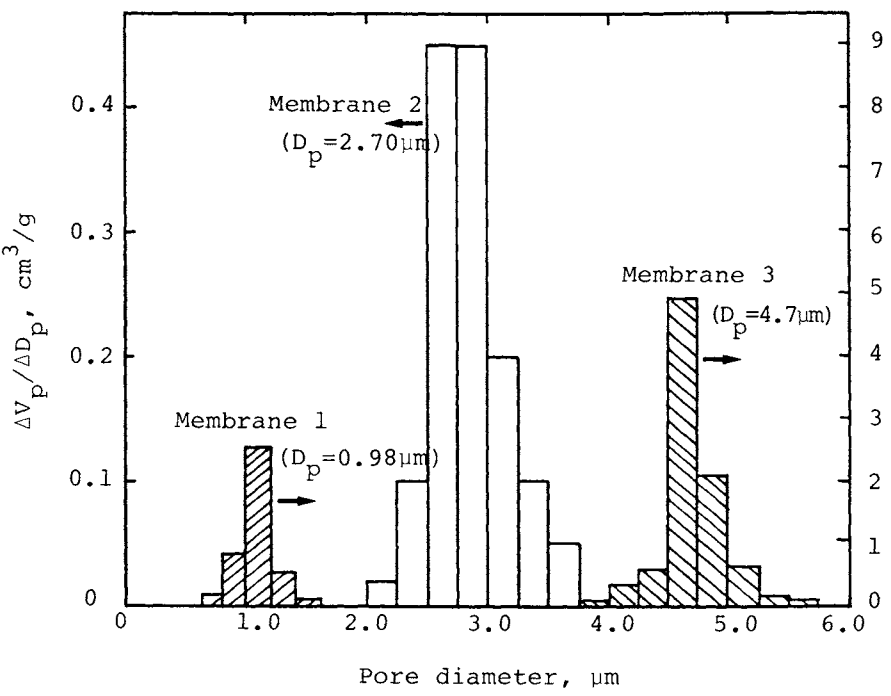
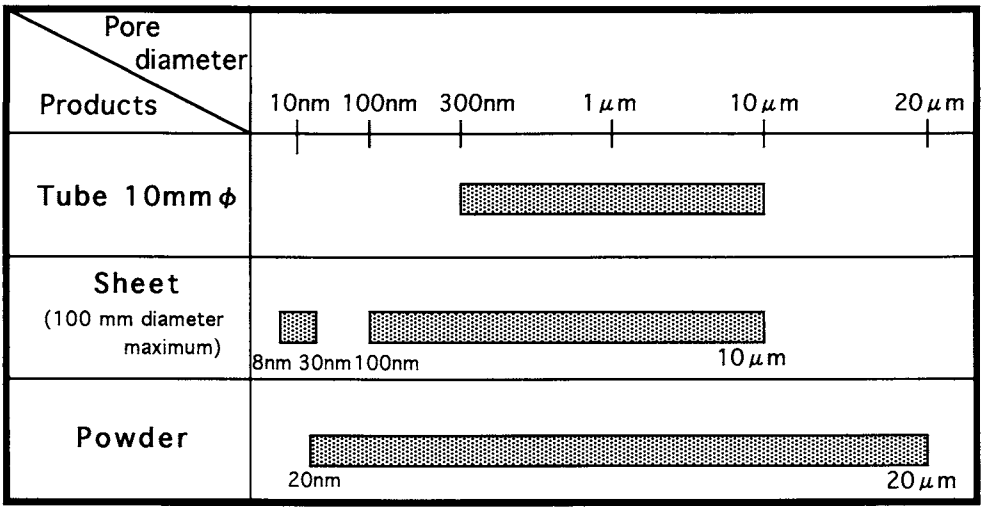


Figure 2. Photomicrographs and pore size distribution of SPG Membranes 1, 2, and 3

Table 4
Nominal pore size range of SPG.



Prices:

Tube; 10 mmφ, 120 mm length *U.S.$ 40-50 (for an order of more than 2)*
Sheet; square 50x50 mm, 0.5 mm thickness *U.S.$ 50*
circle 47 mmφ, 0.5 mm thickness *U.S.$ 70*
Powder; 80-120 mesh *U.S.$ 2/g (for an order of more than 1kg)*

The prices are valid only in Japan (January, 1994)
(Courtesy of Asahi Glass Co. Ltd., Tokyo, Japan)

3. SPG MEMBRANE EMULSIFICATION TECHNIQUE

3.1. Principle of Membrane Emulsification

Figure 3 depicts principle of this technique. Two immiscible phases were separated by the membrane and pressure was applied across the membrane to obtain drops of the dispersed phase into the dispersion medium (continuous phase). The microporous membrane applied for the membrane emulsification must provide uniform pore size distribution as well as tolerable mechanical strength. SPG membrane suites such requirement, because this membrane possesses uniform cylindrical interconnected micropores which are not present in other currently well-known microporous membranes. In addition, because it permits the change of the micropore size from the submicron to micron range (Table 3), this membrane allows one to obtain a monodispersed emulsion of desirable dropsize.

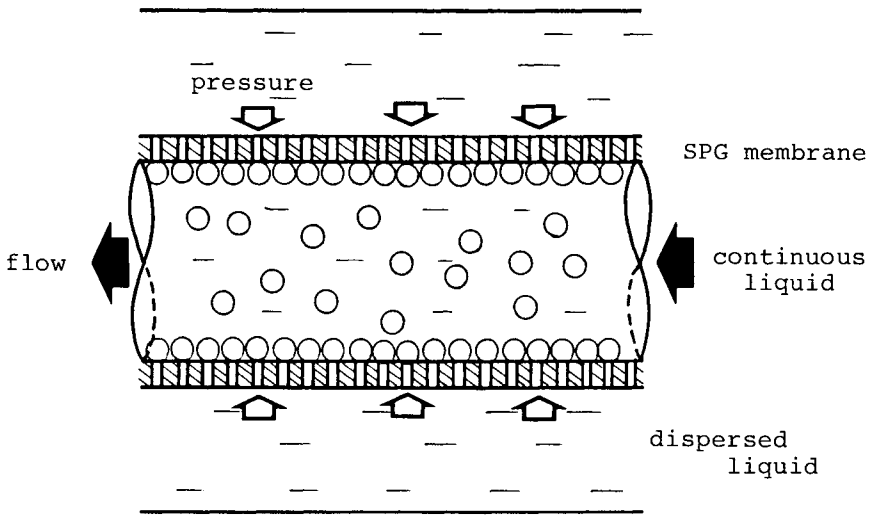


Figure 3. Principle of Membrane Emulsification

A schematic diagram of the continuous membrane emulsification apparatus is shown in Fugire 4(a). The continuous method consists, mainly, of SPG membrane module, a pump for pressure source, dispersion phase (Liquid 1), a storage vessel, an emulsion vessel and a circulation pump for continuous phase (Liquid 2). In the case of a batch method, the bottom of the SPG membrane was sealed by fusion and was immersed in Liquid 2 as shown in Figure 4(b). Liquid 1 was pressured into a Liquid 2 from the inside of the SPG membrane using the same procedure as for the continuous method. The change in water pressure during emulsification can be monitored by a pressure gauge. A commercially available SPG membrane emulsification apparatus is depicted in Figure 5.

3.2. Preparation of Monodispersed Emulsions by Membrane Emulsification Technique

3.2.1. O/W emulsion

Oil-in-water emulsion was successfully produced by Nakashima and Shimizu using a membrane emulsification technique (12). The photomicrographs of a O/W emulsion prepared by the SPG membrane emulsification technique and conventional homogenization technique are shown in Figures 6 and 7, respectively. These emulsions were made using the soybean oil containing 1wt. % of Span 80 and water containing 1wt. % Tween 20 and 1wt. % of NaCl. The interfacial tension of this system was 1.5 dyne/cm. The mean pore diameter (D_p) of SPG membrane

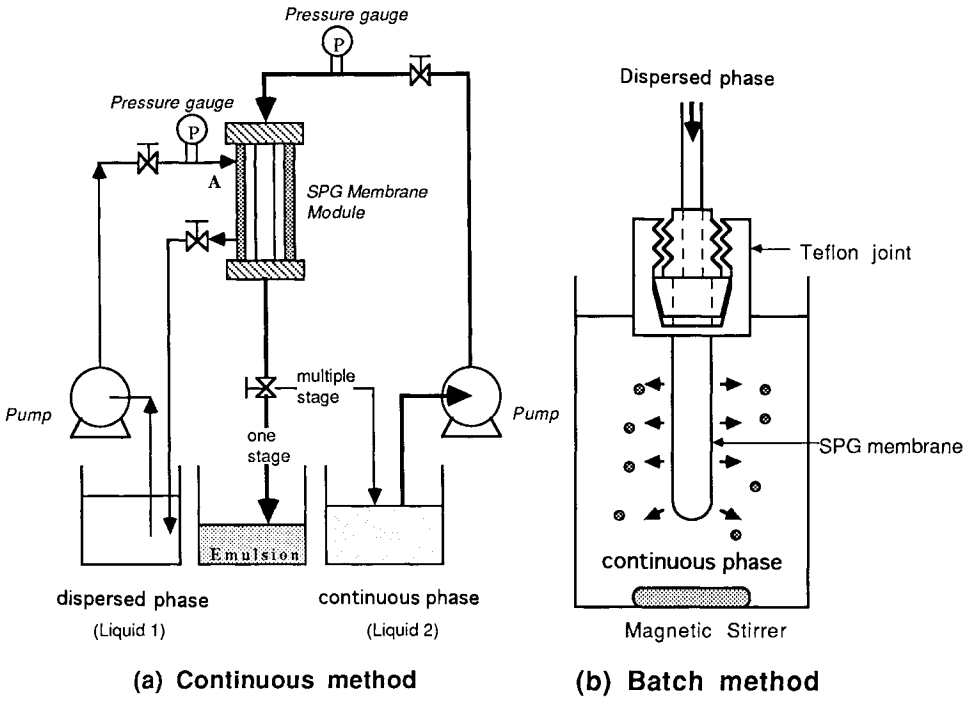


Figure 4. Schematics of an apparatus for the SPG membrane emulsification technique

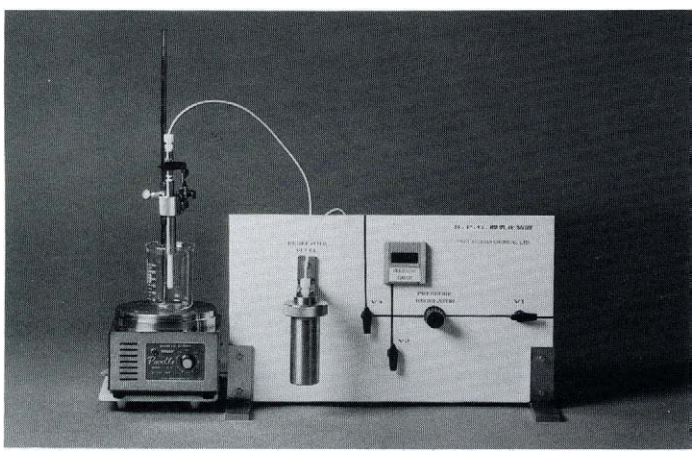


Figure 5. Photograph of commercially available SPG membrane emulsification apparatus (Courtesy of Fuji-Silysia Chemical Ltd.)

used was 0.70 μm. A monodispersed emulsion was obtained using the SPG membrane emulsification technique (Figure 6) while a polydispersed emulsion was obtained using a homogenizer (Figure 7). A comparison of the size distribution for droplets obtained using the SPG membranes to that obtained using the conventional method is shown in Figure 8. In this case, kerosine was used as an oil phase and 0.2% SDS as a water phase. Porous ceramic membrane yielded a very wide size distribution, while the SPG membrane yielded a very narrow size distribution of the oil droplets. The oil droplets were stabilized by the electrical double layer repulsion force and/or a steric repulsion force of adsorbed surfactant layer. In the case of membrane emulsification, the mean diameter of the oil droplet (D_o) is related to the mean pore diameter of SPG membrane (D_p) used by Eqn. (3) (18).

$$D_o = 3.25\, D_p \tag{3}$$

Emulsification conditions	
Oil phase:	Soy bean oil containing 1 wt.% Span 80
Water phase:	Water containing 1 wt.% Tween 20 and 1wt.% NaCl
Membrane:	Hydrophilic SPG; Dp=0.7 μm
Temperature:	288-293 K

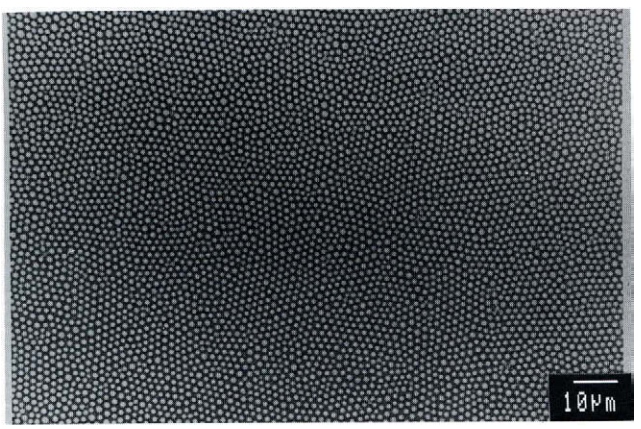


Figure 6. Photomicrograph of O/W emulsion obtained using the SPG emulsification technique (Courtesy of Nakashima and Shimizu[18])

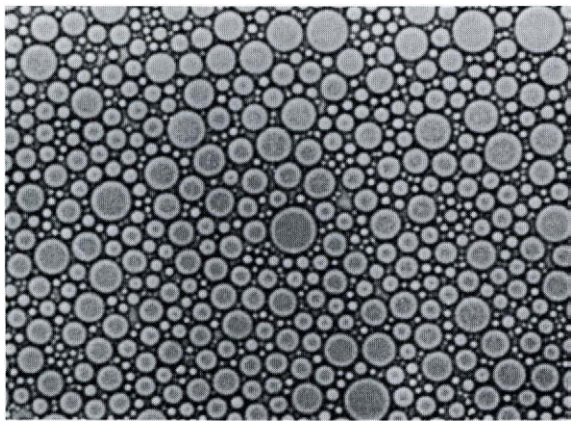


Figure 7. Photomicrograph of an O/W emulsion obtained using the homogenizer (Courtesy of Nakashima and Shimizu[18])

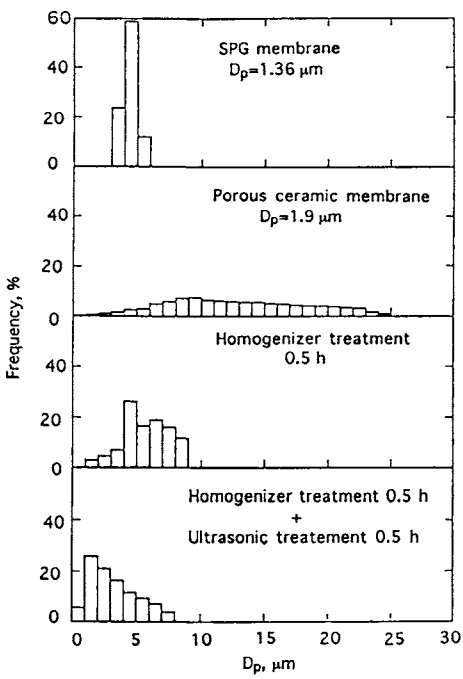


Figure 8. Particle size distribution of oil droplets in O/W emulsions prepared using the SPG membrane emulsification technique and conventional methods. [SDS] = 0.2 wt.% in water. Oil is kerosine. (Courtesy of Nakashima and Shimizu[18])

3.2.2. W/O emulsion

The preparation of a monodispersed W/O emulsion is difficult compared to a O/W emulsion because the water droplets are difficult to stabilize by an electrical double layer repulsion force in an oil phase having a low dielectric constant (9,19). Therefore, it is preferable to choose an emulsifier such as macromolecular type surfactants which form a viscoelastic adsorbed film at the oil/water interface (20-22). The author prepared a monodispersed W/O emulsion by using poly (oxyethylene-oxypropylene) copolymer type surfactants as an emulsifier. The surfactants were of PE-series and manufactured by the Sanyo Kasei Corp., Kyoto, Japan. The molecular formula of PE-series surfactant is $HO(C_2H_4O)_n$-$(C_3H_6O)_m$-$(C_2H_4O)_n$-H and their average numbers of m and n are listed in Table 5. Deionized and distilled water was used after filtering through Millipore filter (220 nm). Toluene was used as an oil phase. Three kinds of SPG membranes, formed into a tube of 3 mm diameter and 0.6 mm film thickness having the different average pore sizes, were used. These are referred to as Membrane 1, 2 and 3 and used without further surface treatment (membrane surface is hydrophilic). The average pore diameters (D_p) of Membranes 1, 2 and 3 as measured by a mercury porosimetry were 0.98, 2.70 and 4.70 μm, respectively. The scanning electron micrographs of the SPG membrane surfaces were already shown in Figure 2. The volume ratio of water to toluene solution of surfactant was fixed at 1:10, and emulsification was performed at room temperature. The PE-series surfactants were dissolved in toluene before use.

Table 5
Properties of PE-series surfactants

	n*	m*	m/2n	cloud point(°C)$
PE-61	2.2	30.1	6.8	24
PE-62	5.0	30.1	3.0	30
PE-64	13.4	30.1	1.1	59

* Molecular structure of PE-series surfactants
$ Data for 1 wt% aqueous solution.

The mean diameters (D_W) and the standard deviations of the water droplets are summarized in Table 6 together with monodispersity ratio (U) and the values for critical pressure of water penetration (P_c). U is the ratio of weight-mean diameter(D_1) to number-mean diameter(D_2), $U=D_1/D_2$, and P_c is the water pressure when the permeation of water through the SPG membrane was started.

As can be seen in Table 6, continuous and batch methods using Membrane 1 gave fairly constant D_W values of ca. 0.66-0.70 μm over the entire range of PE-64 concentration studied. For the Membrane 2 system, D_W values were almost constant ca. 1.59-1.87 μm. Also, the monodispersity ratio was close to 1.0. On the contrary, a large D_W value and broad size distribution were obtained from the

Table 6
Results of W/O emulsions prepared by SPG membrane emulsification technique

Method	PE-64 (wt%)		Membrane 1 D_W (μm)	Membrane 1 U^* (-)	Membrane 1 Pc (kPa)	Membrane 2 D_W (μm)	Membrane 2 U^* (-)	Membrane 2 Pc (kPa)
	10	no SPG	2.23±1.23	1.254	-			
Batch	2	SPG	-	-	87.5	1.59±0.26	1.025	29.4
	5	SPG	0.67±0.11	1.025	90.7	1.87±0.34	1.032	26.5
	7.5	SPG	0.66±0.09	1.020	92.2	1.74±0.26	1.022	29.4
	10	SPG	0.69±0.09	1.017	85.3	1.62±0.22	1.019	28.9
Continuous	2	SPG	0.67±0.10	1.023	10.8	-	-	-
	5	SPG	0.69±0.11	1.022	9.3	-	-	-
	7.5	SPG	0.70±0.09	1.017	22.1	-	-	-
	10	SPG	0.67±0.10	1.022	88.2	-	-	-

* Monodispersity ratio, $U=D_1/D_2$, where D_1 and D_2 are weight-, and number-mean diameter of water droplets, respectively.

batch method without the SPG membrane, indicating that the pores of SPG membrane make the size of water droplets uniform. The D_W values for the Membrane 1 system are smaller than those for the Membrane 2 system as expected. However, the D_W values for both methods are smaller than the D_p values of the SPG Membrane used.

This result is opposite to that obtained for the O/W emulsion in which the size of the oil droplets was larger than that of the pore diameter as designated by Eqn. (3). This fact may be interpreted by the difference in the interfacial tension in these two cases. As we shall see in a next section, the interfacial tension for the W/O emulsion system produced by PE-64 was less than 0.1 dyne/cm, which is much less than O/W emulsion system produced by Tween 20 (Figure 6). The effect of interfacial tension will be described in the next section. Furthermore, it can be seen in Table 6 that the P_c values of batch method using Membranes 1 and 2 are

	Emulsification conditions
Oil phase:	Soybean oil containing 0.5 wt.% Span 80
Water phase:	Water containing 1 wt.% NaCl
Membrane:	Hydrophobic SPG; Dp=2.56 μm
Temperature:	288-293 K

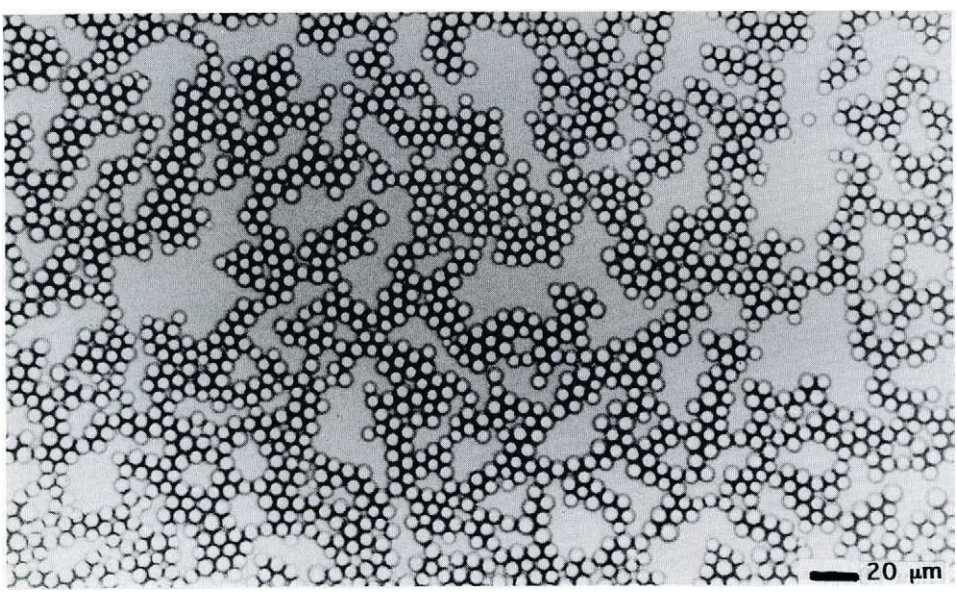


Figure. 9. Photomicrograph of W/O emulsion (Courtesy of Nakashima and Shimizu[18])

fairly constant in the vicinity of 90 and 28 kPa over the range of concentration of PE-64 studied. Taking into consideration the difference in the D_p values between Membranes 1 and 2, this result seems to be reasonable.

Nakashima et al. prepared a W/O emulsion from an edible oil by membrane emulsification technique using hydrophobic SPG membrane. A photomicrograph of this emulsion is shown in Figure 9 (18). It should be mentioned here that the surface of the SPG membrane was treated, to render it hydrophobic, by octadecyltrichlorosilane (ODS) and triethylchlorosilane (TMS). Single emulsifier (Span 80) is added to the continuous oil phase (soybean oil) prior to permeation of water phase. Particle size of the emulsion thus obtained had a quite uniform distribution. The size of the water droplets was bigger compared to the W/O emulsions produced by the author as described before. This would be due to the difference in the surface hydrophobicity of the SPG membranes and the interfacial tension.

3.2.3. Factors Determining the Size of the Droplet

Effects of the interfacial tension on the size of the droplets produced in W/O emulsion by the SPG membrane emulsification technique were verified. The author produced the W/O (water-in-toluene) emulsions with Membranes 1, 2 and 3, at various interfacial tensions using PE-series surfactants as an emulsifier. The D_W, U and P_C values for the resulting W/O emulsions are summarized in Table 7 and will be discussed in a next section.

(a) Interfacial Tension

Figure 10 shows a plot of interfacial tension versus the D_W of water droplets produced with Membrane 3. Here, the D_W values for the PE-64 system are plotted at D_W=0 because of their extremely low interfacial tension. It is interesting to note that the curve can be roughly classified into three regions as A, B and C as follows: (A) γ<1 dyne/cm and D_W<2 μm, (B) 1<γ<5 dyne/cm and D_W ~2 μm and (C) γ>5 dyne/cm and D_W>>2 μm. The dispersions of the monodispersed W/O emulsions formed in A, B and C regions were very stable, metastable (completely separated into two phases after 1 day) and unstable, respectively. The PE-series surfactants adsorb onto the water droplets, thereby lowering the interfacial tension and forming rigid and thin adsorbed layers (20-22). The water droplets are stabilized by steric repulsion of the elastic, adsorbed layers of PE-series surfactants. Therefore, not only the interfacial tension but also the conformation of the PE-series surfactant molecules is resposible for producing stable and monodispersed W/O emulsions by the SPG membrane emulsification technique. It is clear from above results that the interfacial tension is one of the factors for determining the water droplet size. However, the D_W is less than D_p in all cases and the mechanisms of the formation of such fine monodispersed water droplets cannot be ascertained on the basis of these results alone.

Table 7
Results of W/O emulsions prepared by SPG emulsification method

Membrane	Surfactant (wt%)	D_W (μm)	U* (-)	Interfacial tension (dyne/cm)	Pc (kPa)
3	PE-61 (3.2)	2.72±0.67	1.060	7.7	29.9
3	-61 (5.0)	2.25±0.61	1.085	6.9	26.0
3	-61 (7.5)	2.44±0.45	1.035	6.1	26.5
3	-61 (10.0)	2.06±0.44	1.044	5.8	26.0
3	-62 (3.2)	1.92±0.38	1.042	2.0	28.9
3	-62 (5.0)	1.88±0.34	1.035	1.3	32.9
3	-62 (7.5)	1.73±0.31	1.038	0.8	35.8
3	-62 (10.0)	1.67±0.31	1.033	0.3	32.9
3	PE-62/64=9/1 (3.2)	1.90±0.36	1.036	1.2	26.0
3	9/1 (5.0)	1.82±0.36	1.040	0.4	26.5
3	9/1 (7.5)	1.80±0.38	1.045	<0.1	30.4
3	9/1 (10.0)	1.64±0.35	1.048	<0.1	30.4
3	PE-62/64=8/2 (3.2)	1.65±0.32	1.041	0.7	35.3
3	=8/2 (5.0)	1.72±0.27	1.024	<0.1	35.3
3	=8/2 (7.5)	1.60±0.40	1.062	<0.1	30.9
3	=8/2 (10.0)	1.68±0.35	1.048	<0.1	26.5
3	PE-64 (2.0)	2.03±0.39	1.038	<0.1	29.4
3	-64 (5.0)	1.87±0.34	1.032	<0.1	26.5
3	-64 (7.5)	1.74±0.26	1.022	<0.1	29.4
3	-64 (10.0)	1.62±0.22	1.019	<0.1	28.9
1	PE-64 (2.0)	-	-	<0.1	87.5
1	-64 (5.0)	0.67±0.11	1.025	<0.1	90.7
1	-64 (7.5)	0.66±0.09	1.020	<0.1	92.2
1	-64 (10.0)	0.69±0.09	1.017	<0.1	85.3
2	PE-64 (2.0)	1.96±0.41	1.045	<0.1	29.4
2	-64 (5.0)	1.75±0.38	1.050	<0.1	29.4
2	-64 (7.5)	1.59±0.30	1.035	<0.1	29.4
2	-64 (10.0)	1.38±0.37	1.074	<0.1	29.4

* monodispersity ratio, $U=D_1/D_2$, where D_1 and D_2 are weight-, and number-mean diameter of water droplets, respectively.

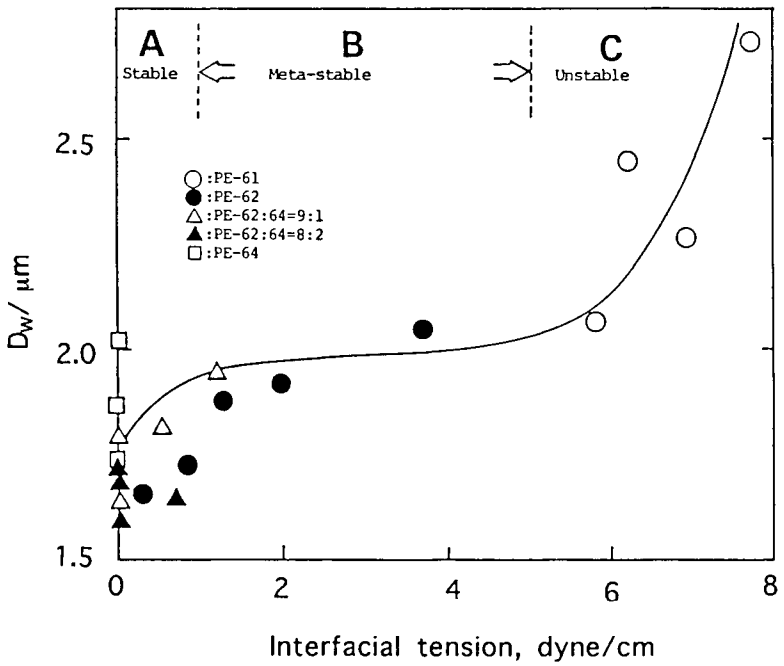


Figure 10. Plots of average diameter of water droplets (D_W) prepared by Membrane 3 as a function of interfacial tension

(b) Pore Size and Structure

The influence of D_p on D_W are compared at different PE-64 concentrations in Figure 11. The D_W increases with increasing D_p, but the extent of increase is reduced above D_p of 2.7 μm (Membrane 2) and no remarkable difference can be observed between Membranes 2 and 3. This result corresponds to the result that Membranes 2 and 3 gave similar P_c (see Table 7). If D_p is a dominant factor to determine D_W, D_W should be directly proportional to D_p. From this paradox on the relationship between D_W and D_p, other factors must be considered.

The drop volume (DV) method, one of the methods used in determining the interfacial tension, is helpful to describe this point because the mechanism of the DV method is very similar to that of the SPG membrane emulsification. In the DV method, the droplets of liquid 1 are formed through the tip of the capillary into a surrounding fluid (liquid 2) as shown in Figure 12. The DV method consists of measuring the volume of a droplet that just detaches vertically itself from the horizontal tip of a sharply cut and polished capillary of accurately known outer diameter. Harkins and Brown (23) showed that the interfacial tension (γ) is related to the droplet volume (V) and the outer capillary diameter (D) by the equation (4);

$$\gamma = \Phi V(\rho-\rho_O)g/\pi D \qquad (4)$$

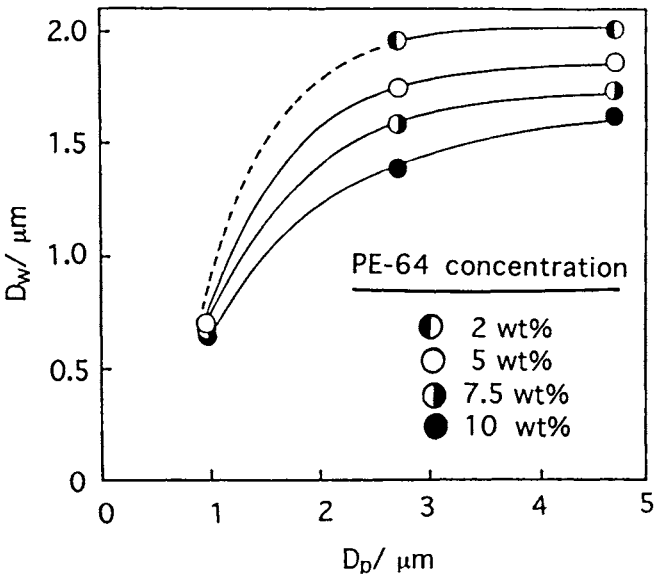


Figure 11. The change of average diameter of water droplets (D_W) prepared by Membranes 1, 2 and 3 as a function of average diameter of Membranes (D_p)

Where g is acceleration due to gravity, Φ is empirically derived correlation factor (1.65) (24), and ρ and ρ_0 are density of droplet liquid and surrounding fluid, respectively. Supposing the theoretical diameter of a droplet as D_W^t, the Eqn. (4) can be rewritten as the Eqn. (5).

$$D_W^t = [6D\gamma/(\rho-\rho_0)g\Phi]^{1/3} \quad (5)$$

The theoretical ratio of D_W was calculated for Membranes 1, 2 and 3 to be 1.0:1.4:1.7 by substituting the corresponding D_p for D in Eqn. (5) and by assuming ρ=1 (water) and ρ_0=0.8625 (toluene) g/cm^3. However, the actual ratio of D_W, prepared with 10 wt. % of PE-64 for example, for Membranes 1, 2 and 3 is 0.69:1.38:1.62 i.e., 1.0:2.0:2.4. This experimental size ratio is much larger than that of the theoretical one stated above. This discrepancy between the theoretical and experimental size ratios indicates that the D_p is not a factor determining the water droplet size. According to this experimental size ratio, the actual size ratio of the effective pore diameters of the SPG membranes on the formation of water droplets can be recalculated from the Eqn. (5) for Membranes 1, 2 and 3 as 1.0:8.0:13.8. Since the D_p of Membrane 1 is 0.98 μm which is close to unity, these values in the actual size ratio of the effective pore diameters of the SPG membranes can be regarded as μm unit. In other words, the effective pore diameters for Membranes 2 and 3 can be estimated to be 8.0 and 13.8 μm, respectively. These calculated effective pore diameters of Membranes 2 and 3, however, are much larger than the D_p of 2.70 and 4.70 μm of respective filter

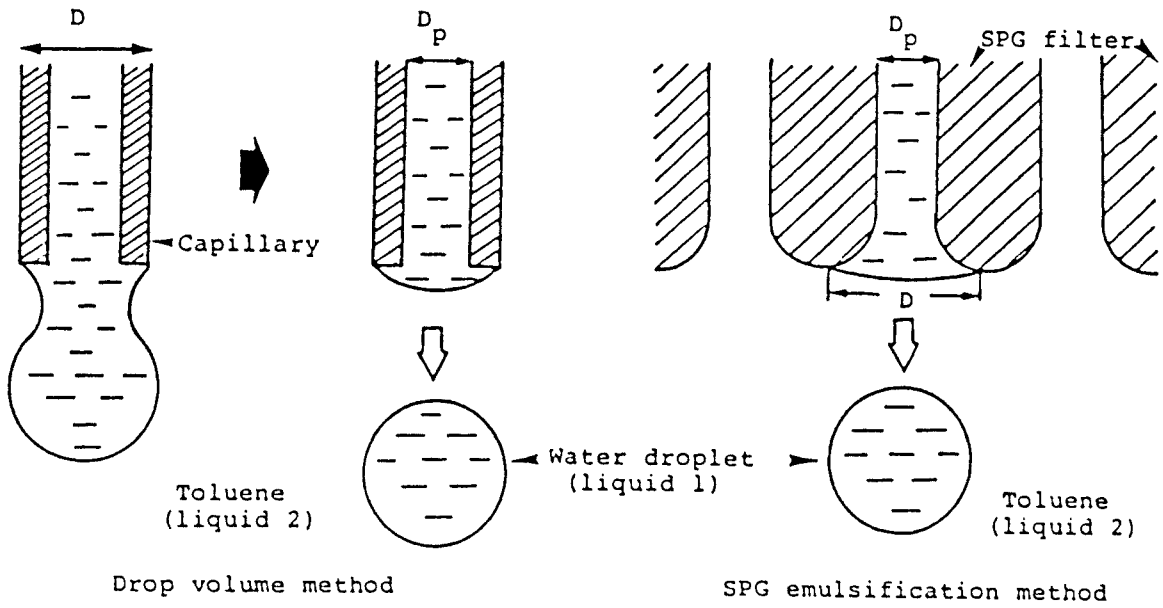


Figure 12. Comparison of schematic diagrams of drop volume and SPG membrane emulsification methods.

measured by a mercury porosimetry.

It can be recognized in Figure 2 that the outlets of Membrane 1 are uniformly controlled in shape and size, but the Membranes 2 and 3 possess irregular surfaces (specifically in the case of Membrane 3) and the outlet diameter of the pores can be estimated to be approximately from 10 to 15 μm which is closer to the theoretical values of the effective pore diameters of 8.0 and 13.8 μm for Membranes 2 and 3, respectively. The consistency in the effective pore diameters estimated by the calculation and by a scanning electron microscope observation confirms that not only the interfacial tension but also the size and shape of the outlets of the pores are the important factors governing the size of the water droplets. The results obtained lead us to an important conclusion that the SPG membrane with the pore outlets uniformly controlled in size and shape is preferable for producing a monodispersed W/O emulsion using the SPG membrane emulsification technique.

3.3. Applications of Membrane Emulsification

3.3.1. Preparation of Uniform Silica Hydrogel Particles

Preparation of many kinds of monodispersed metal oxide particles by the hydrolysis of alkoxide has been widely investigated (25-27). Especially, sol-gel processes with silicon alkoxides, e.g. tetraethoxysilane [TEOS] and tetramethoxysilane [TMOS], have been used extensively to produce silica particles in the submicron range (25-27). Silica has applications in a food industry. Silica gel has not only been used as an adsorbent but also may have applications as *flavor, aroma and nutrient delivery system*. Furthermore, silica gel has been used as a *chill-proofing agent*, because this kind of gel has a selective adsorption capacity to the haze-forming protein of beer. In recent years, SiO_2 nanoparticles were prepared by utilizing W/O microemulsions; specifically, by controlled hydrolysis of TEOS in oil soluble surfactant/ammonium hydroxide solution/cyclohexane or isooctane reversed micellar systems (28-30). However, since the alkoxide compounds react strongly with water, it is difficult to control the hydrolysis conditions during the synthesis.

Since the uniform water droplets were simply formed by pressing water into the oil phase through the pores of the SPG membrane using a copolymer type surfactant as an emulsifier. This method would be applied to precipitate the monodispersed colloidal particles in droplets of water in a W/O emulsion.

Based on this concept, the author prepared a W/O emulsion of sodium silicate solution (5 cm^3)-in-toluene (30 cm^3) at room temperature by a batch method using PE-64 as an emulsifier (15). The pH of the sodium silicate solution (SiO_2/Na_2O of 3.12 ratio) was first adjusted to 2.0 by the addition of a 2.3 mol/dm^3 sulfonic acid solution, to attain slow polymerization of silicic acids at room temperature. To avoid polymerization, the acidic sodium silicate solution was prepared just before its use. The sodium silicate emulsion so prepared was further polymerized in a 100 ml Teflon-sealed and screw-capped vessel by gently mixing with a magnetic stirrer for 7 days at room temperature. In a preliminary experiment, the acidic sodium silicate solution was solidified and converted to a

hydrogel under the same conditions. For the sake of comparison, the control W/O emulsion [water (5 cm^3) dispersed in toluene (30 cm^3)] was prepared under the same conditions.

The mean diameters of freshly prepared sodium silicate droplets (D_{S0}) and that of the silica hydrogel particles formed after the polymerization for 7 days (D_{S7}), the monodispersity ratio (U) and P_C values are tabulated in Table 8 along with those of water droplets in a control W/O emulsion (D_W). The U values of D_{S0} and D_{S7} in the micron range are close to unity, indicating that the sodium silicate emulsions and silica hydrogel particles are of fairly narrow size distribution. Moreover, D_{S0} is comparable to D_W, especially for the Membrane 2, while P_C for sodium silicate emulsions is slightly higher than that for the water droplets in the control W/O emulsion, implying that the mechanism of formation of sodium silicate emulsion is essentially identical to that of the W/O emulsions described in a section 3.2.3. However, D_{S0} of the sodium silicate emulsions does not depend on the concentration of PE-64. Similar result can be seen for D_W, which can be interpreted on the basis of the low interfacial tension (<0.1 dyne/cm) between aqueous solution and toluene phase over the whole concentration region as described before.

The discovery that D_{S7} is smaller than D_{S0} suggests that the silicic acid polymerized slowly and released water molecules in the sodium silicate droplets and that the hydrogel particles shrunk as the polymerization progressed. The gradual shrinkage of hydrogels can be due to the slow gelling rate of acidic silicic acids at pH 2 and room temperature (31-33). The net polymerization reaction yields three dimensional gel networks of polysilicic acid which gradually converts into a rigid silica hydrogel particle releasing water (34).

The silica hydrogel thus prepared can be converted to silica gel particle by removing the liquid medium. The structure of the silica gel is compressed and the porosity reduced to at least some degree. Because of its high specific surface area, the silica gel in monodispersed sphere, granular and/or pellet forms appears to be a catalyst, carrier, an *adsorbent* and a *desiccant*. Some miscellaneous observations in food processing are outlined below. According to Kennedy et al. (35) *polysaccharides* are preferentially adsorbed on silica surfaces coated with polyaromatic molecules. It is surprising that a hydrocarbon surface should have any special affinity for such highly hydrophilic molecules. Antioxidants of the polyhydroxyl aromatic type can be adsorbed on fine silica gel particles. It proved to be as good an antioxidant as carbon black yet the film is clear (36). Recently, Matsuzawa and Nagashima developed a high performance new hydrated silica gel, which gives chill-stable beer at low dosage rates in a short period of contact with beer (37).

Nakashima et al. prepared monodispersed microspheres of silica which are indispensable for HPLC by combining a two step membrane emulsification technique with interfacial reaction used for the preparation of inorganic microspheres. The preparation conditions and scanning electron micrograph of the resulting silica microsphere are displayed in Figure 13.

Table 8
Formation of sodium silicate and W/O emulsions by the SPG membrane emulsification method

		sodium silicate emulsion						control W/O emulsion		
Filter Dp(μm)	PE-64 (wt%)	D_{S0} (μm)	U_{S0}* (-)	D_{S7} (μm)	U_{S7}* (-)	$P_C$$ (kPa)	D_{S7}/D_{S0}	D_W (μm)	U_W* (-)	$P_C$$ (kPa)
0.98	2.0	1.4±0.6	1.20	1.2±0.3	1.07	74.0	0.86	1.2±0.3	1.05	72.0
	5.0	1.2±0.5	1.15	0.9±0.3	1.13	78.9	0.75	0.8±0.3	1.12	71.6
	7.5	1.3±0.6	1.17	0.9±0.4	1.16	78.5	0.69	0.9±0.3	1.07	76.5
	10.0	1.6±0.4	1.07	1.0±0.3	1.07	87.8	0.63	1.0±0.1	1.01	77.5
2.7	2.0	2.0±0.3	1.02	1.3±0.4	1.10	31.9	0.65	2.0±0.4	1.05	29.4
	5.0	1.8±0.3	1.03	1.3±0.3	1.07	24.0	0.72	1.8±0.4	1.05	29.4
	7.5	1.6±0.3	1.06	1.1±0.3	1.06	34.3	0.69	1.6±0.3	1.04	29.4
	10.0	1.7±0.3	1.03	1.1±0.3	1.07	33.3	0.65	1.4±0.4	1.07	29.4

* Monodispersity ratio, $U=D_1/D_2$, where D_1 and D_2 are weight- and number-mean diameters of water droplets, respectively.

$ Critical pressure for the penetration of sodium silicate solution or water through the SPG filter.

Emulsification conditions

	Primary emulsification	Secondary emulsification
Water phase:	4M Na-silicate soln.	$(NH_4)_2CO_3$ soln.
Oil phase:	cyclohexane/n-hexane=4/1 containing 2 wt.% Tween 85	W/O emulsion prepared in the primary process
Membrane:	Hydrophobic SPG; Dp=0.7 μm	Hydrophilic SPG;Dp= 3.16 μm
Temperature:	Room temperature	

W/O emulsion prepared by membrane emulsification → Interfaial reaction → Separation of microsphere of silica

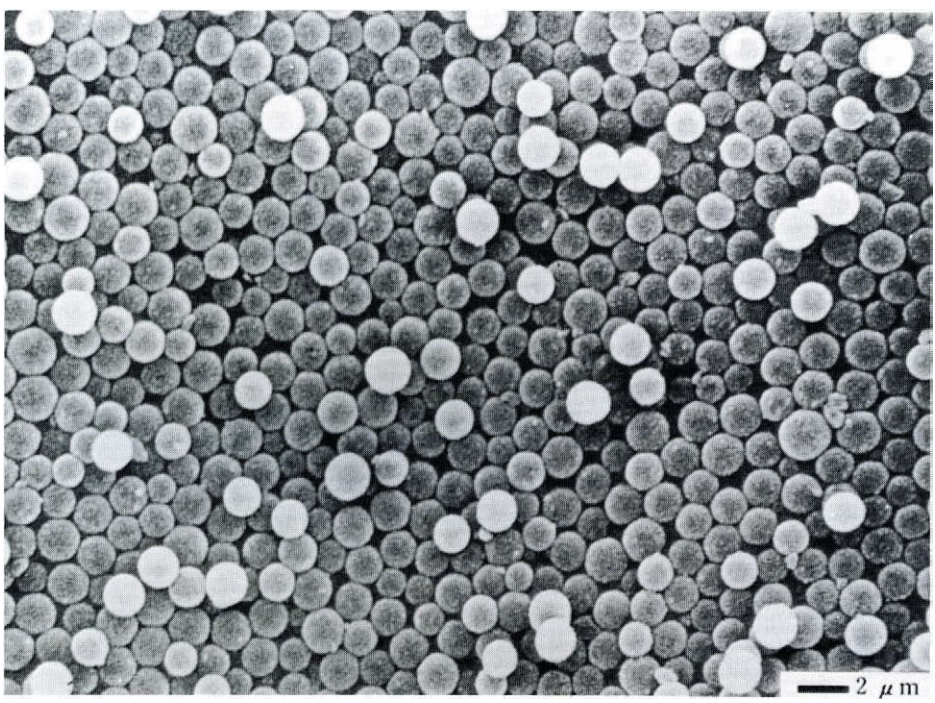


Figure 13. Scanning electron micrograph of silica microsphere(Courtesy of Nakashima and Shimizu[18])

3.3.2. Preparation of Uniform Polymer Microspheres

Spherical and monodispersed polymer latices have attracted a great deal of attention because it is widely used as a model for the study of colloidal stability in

aqueous and nonaqueous media (38-45). Of course, because of its high specific surface area, these polymer microspheres may have the applications similar to silica gel cited in a previous section. Furthermore, from its unique feature of polymer microsphere, the flavor and aroma compounds and nutrients are not only adsorbed on the particle surface but also can be incorporated in the particles by encapsulation. Therefore, their efficiency will be maintained for a longer time.

Figure 14 shows the micrograph of polydivinyl benzene microspheres (PBM). PBM was formed in monodispersed oil droplets dispersed in water phase (O/W emulsion). This method utilized a suspension polymerization and can be applied to other oil-soluble monomers. On the contrary, in the case of water-soluble monomer such as acrylamide, one can prepare water-soluble polymer microspheres as water droplets dispersed in an oil phase by preparing monodispersed W/O emulsion. Such microspheres can also be applied to the preparation of microcapsule delivery system for flavor, aroma and nutrient.

	Preparation conditions
Oil phase:	Divinylbenzene (monomer)
Water phase:	Water containing 0.2 wt.% SDS
Stabilizer:	Polyvinyl alcohol
Catalyst:	Benzoylperoxide
Membrane:	Hydrophilic SPG; Dp=1.36 μm
Temperature:	Room temperature

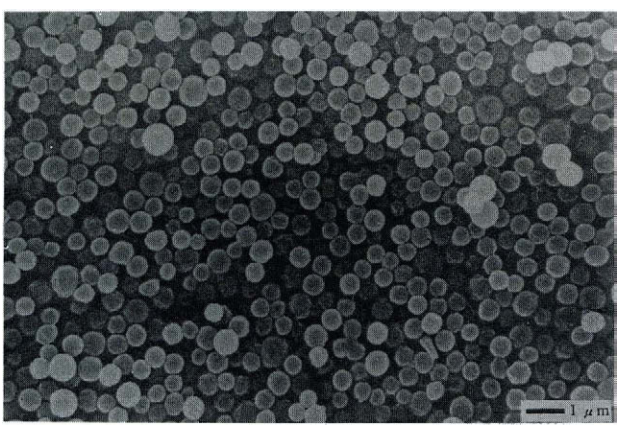


Figure 14. Microphotograph of polydivinylbenzene microspheres (Courtesy of Nakashima and Shimizu[18])

3.3.3. Preparation of Uniform Multiple Emulsions

Multiple emulsion is an emulsion within an emulsion. Water-in-Oil-in-Water (W/O/W) type multiple emulsions are oil-in-water emulsions in which the dispersed oil drops in turn contain dispersed aqueous droplets. These systems were observed as long ago as 1890 (46), but there has been increased interest recently in the use of W/O emulsion in such diverse fields as the food emulsions(47-51), waste water treatment (52), immobilization of enzymes (53) and treatment of drug overdosage (54). It should be noted that the stability of the multiple emulsion depends greatly on the type and concentration of the surfactant. As for the structural characteristics, the single compartment globules of the W/O/W emulsions are morphologically similar to those of the one-lamellar liposome systems, although the size and shape are somewhat different. Multiple emulsion so far has been generally produced by two-step process.

Figure 15 shows a schematic diagram for the preparation of double emulsion by using a double SPG membrane module. The sizes of oil (water) droplet and inner water (oil) phase can be varied by changing D_p of the inner and outer SPG membranes, respectively.

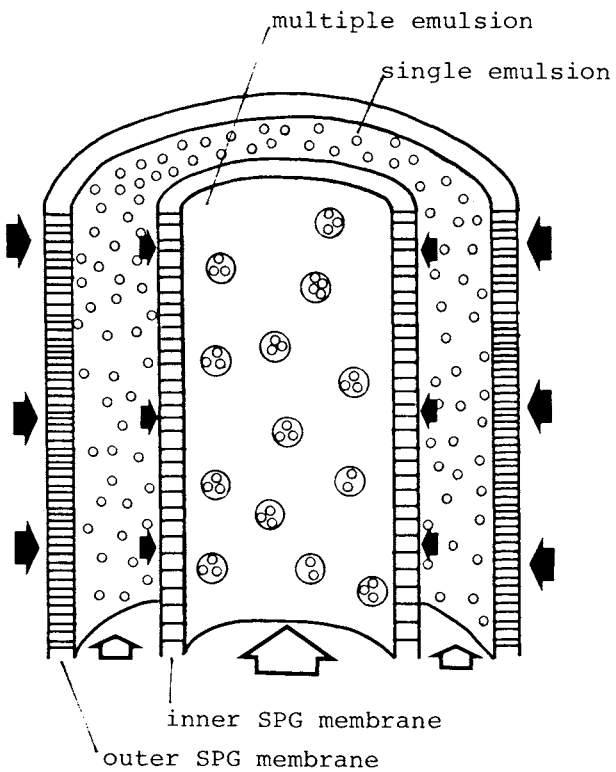


Figure 15. Schematic diagram of the preparation of double emulsion.

Figure 16 exhibits an example of W/O/W multiple emulsion prepared by the double SPG membrane module. First, the W/O emulsion was prepared by dispersing a water phase into a vegetable oil phase using lipophilic nonionic surfactants and then dispersing this W/O emulsion into a water phase containing a hydrophilic nonionic surfactant.

Furthermore, the W/O/W multiple emulsion can be utilized for preparing the monodispersed colloidal inorganic particles via interfacial reactions. Figure 17 displays a typical micrograph of precipitated microspheres. A 1wt. % sodium alginate solution was dispersed in n-hexane solution containing 0.25 wt. % Span-85 and 0.25 wt. % of Tween 85 to prepare a primary emulsion. The W/O emulsion, thus obtained, was dispersed in 15 wt.% $CaCl_2$ solution (secondary emulsification).

Emulsification conditions

	Primary emulsification (W/O emulsion)	Secondary emulsification (W/O/W emulsion)
Water phase:	Water containing 5 wt.% Na_2HPO_4/KH_2PO_4=4/1	Water containing 1wt.% Tween 20 and 0.5wt.% NaCl
Oil phase:	Soybean oil containing 1 wt.% PGCR	W/O emulsion prepared in the primary process
Membrane:	Hydrophobic SPG; Dp=0.36 μm	Hydrophilic SPG; Dp=2.80 μm
Temperature:	Room temperature	

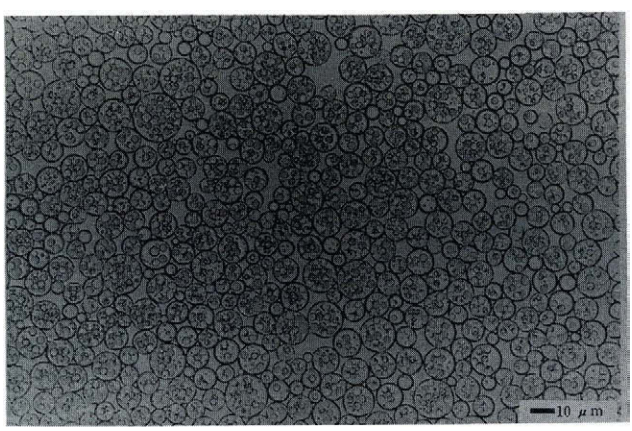
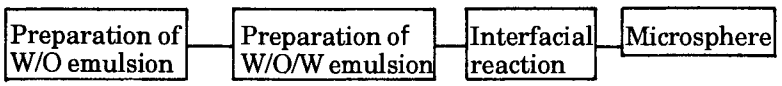


Figure 16. Microphotograph of W/O/W multiple emulsion (Courtesy of Nakashima and Shimizu[18])

Recently, O/W/O emulsion technology has been utilized for producing margarine (55,56). In this case, for example, the oil phase contains lecithin and a sorbitan

Emulsification conditions

	Primary emulsification	Secondary emulsification
Water phase:	1 wt.% Na-alginate solution	15% $CaCl_2$ solution
Oil phase:	n-Hexane containing 0.25 wt. % Span 80 and 0.25 wt.% Tween 85	W/O emulsion prepared in the primary process
Membrane:	Hydrophilic SPG; Dp=0.7 μm	Hydrophilic SPG; Dp=3.16 μm
Temperature:	Room temperature	



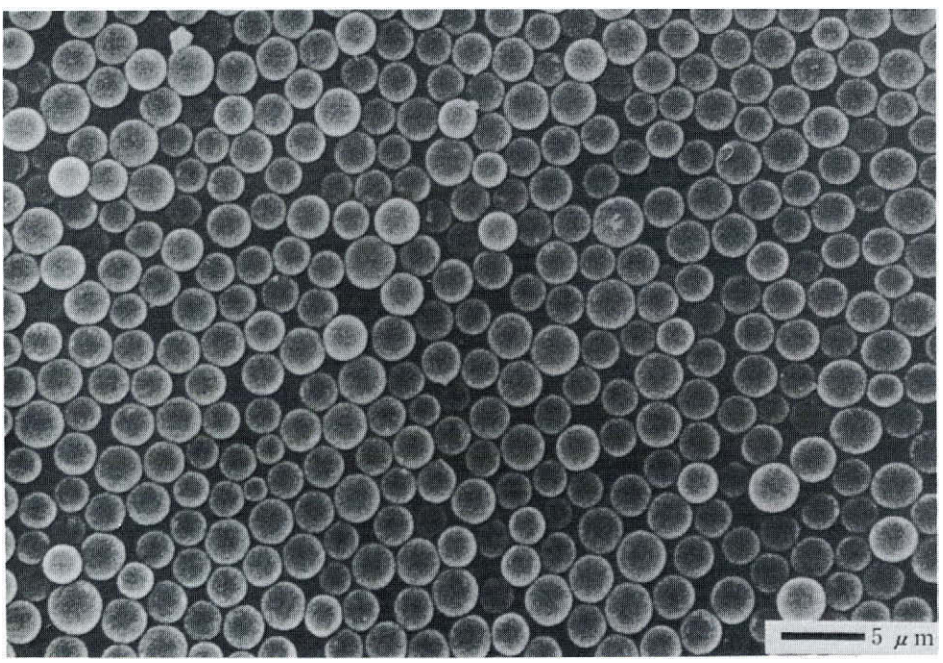


Figure 17. Microphotograph of calcium alginate microspheres prepared by using W/O/W multiple emulsion (Courtesy of Nakashima and Shimizu[18])

fatty acid ester, while the water phase contains skim milk powder, sugar fatty acid ester and sodium hexameta phosphate. This margarine has many characteristics: 1) soft, creamy taste and flavor, 2) water separation from margarine does not occur during whipping, 3) finished cream has resistance to deterioration and mold, 4) cream has an excellent freezing and thawing tolerance, and 5) texture of the cream is maintained well without drying and cracking.

Another O/W/O emulsion (55,56) containing monoglyceride and beta-carotene in oil phase but containing sugar fatty acid ester, whole milk powder, glucose, corn syrup and fresh cream in the water phase produced a new type of cream having additional following characteristics: 1) less oily than ordinary cream, 2) having light mouth feel and good mouth melt properties, 3) tasting similar to a fresh cream, and 4) Freeze-thaw stable like an ordinary butter cream.

3.3.4. Dissolution of Ozone Gas in Liquid (Ozonation of Liquids)

Ozone's well-known properties as an oxidant, bleach and sanitizer make it an excellent water purification and food treatment agent. Ozone is emerging as the more attractive alternative for water and waste water sanitation. The ozonated water is applicable to sterilize vegetable, fish, meat, oyster, raw materials of various foods, and food containers. However, ozonated water having high concentrations of ozone that serves as an effective sanitizer has been difficult to obtain using many conventional techniques.

Asahi Glass Inc. used SPG membranes to obtain a higher concentration of dissolved ozone (55). Producing ozonated water requires that the ozone gas be effectively dissolved and concentrated in water. By virtue of its small pore size and narrow pore size distribution, SPG membranes produce a large number of small and homogeneous bubbles in water.

In practice, water is flowing inside the microporous glass tube and ozone gas (2% in O_2) is pressurized into water from outside of the tube, principle of which is similar to that used in producing emulsions shown in Figure 3. When the ozone gas passes through the pores of the microporous glass, many small ozone bubbles are formed, raising the ozone concentration in water. Asahi Glass Inc. reported that ozone concentration of 10-12 mg/l can be obtained by pressuring ozone gas at 0.8 kg/cm^2 into a 500 liter flowing water (100 l/min) through microporous glass (pore diameter of 3 μm) for 80 min (Figure 18).

With recycling, ozone concentration in water could be reached up to 50 mg/l. The half-life period of ozone water thus produced was ca. 100 min. Many ozone gas bubbles dispersed in water may act as a reservior of ozone gas to attain the long half-life period.

3.3.5.Use of SPG in Processing of Food Emulsions

Since the SPG membrane emulsification method was presented, many applications of this new technique have been developed in the various industrial fields. The *ultra-low fat spread* (margarine) , developed by Morinaga Milk Co. Ltd. in 1993, is the only industrial commodity prepared using the SPG membrane emulsification method (56,57,58). They prepared highly stable monodispersed O/W emulsions by pressing water into the oil phase through the pores of SPG

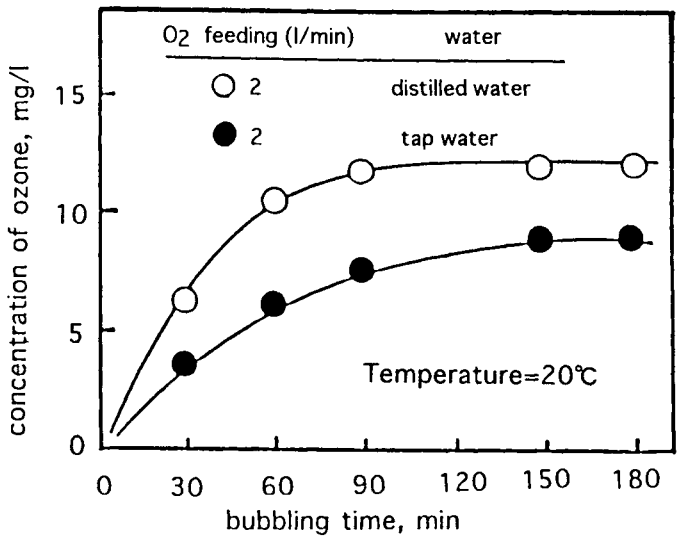


Figure 18. Ozone concentration in water as a function of bubbling time. (Courtesy of Asahi Glass Inc., Tokyo, Japan)

membrane. The content of fat in the ultra-low fat spread, 20%, is considerably low compared to an ordinary low-fat spread which contains a minimum fat content of 40%. The fat content and calorie of the ultra-low fat spread, therefore, could be one-third compared to that of an ordinary low fat spread. The new spread has creamy taste, light mouth feel and good mouth melt properties.

4. CONCLUSION

The SPG membrane emulsification method has a broad potential applicability to form monodispersed particles, droplets and bubbles at low costs. Since the emulsion plays an important role in a variety of industrial fields, this technology is expected to gain importance in the future.

5. ACKNOWLEDGMENTS

The author wishes to express thanks to Dr. Tadao Nakashima and Mr. Masataka Shimizu for permission to use the pictures from their Manual and to Asahi Glass Inc. for providing information about the availability and prices of SPG, the ozone dissolution system and processing of food emulsions. I also thank Professor Tatsuo Ishikawa (School of Chemistry, Osaka University of Education) for his valuable suggestions and comments. Special thanks to Fuji-Silysia Chemical Ltd. for supplying the SPG membranes.

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Chapter 8

Separation processes for biotechnology in the food industry

Gordon W. Niven

Department of Biotechnology and Enzymology,
Institute of Food Research, Reading Laboratory, Earley Gate, Whiteknights Road, Reading RG6 2EF, United Kingdom.

1. INTRODUCTION

Biotechnology may be defined as "the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services" (OECD, 1982). The earliest examples of biological processing include the production of fermented foods and beverages, even though they pre-date any understanding of the nature of the biological agents or the scientific principles involved. The early history of biotechnology is therefore immersed in the history of the food industry. Today, the food industry is the biggest user of bulk enzymes, accounting for 50% of world sales (Harlander, 1989). It can therefore claim to have a substantial interest in commercial biotechnology. The scope and value of enzyme use in the food industry is shown by the data in Table 1.

In recent years, a rapid expansion has taken place in biotechnology, brought about by a substantial increase in knowledge of the biochemistry and physiology of living organisms and their control. In particular, the elucidation of the structure of DNA and the genetic code, and the development of the means of genetic manipulation have given unprecedented potential for the industrial exploitation of living systems.

Biological processes are a particularly attractive means of carrying out industrial syntheses and transformations. As enzyme-catalysed reactions are highly specific, they are less likely than equivalent chemical processes to produce side products. Enzymes or whole cells can often carry out in a single step complex chemical reactions which would require several steps if carried out by conventional means. In addition, such reactions usually occur under mild conditions of temperature, pressure and pH. There exists in nature thousands of enzymes which catalyse the different biochemical reactions involved in the metabolism of all living organisms. This represents a vast pool of potential industrial catalysts. The techniques of genetic manipulation now make it possible for many of these enzymes to be produced on a large scale by well-characterised industrial cell systems. Methods of "protein engineering" are also being developed which enable the structure and function of enzymes to be manipulated by site-specific mutagenesis to improve their suitability for industrial use (Goodenough & Jenkins, 1991; Pickersgill & Goodenough, 1991).

Table 1
The use of enzymes by the food industry (Data from Pilnik & Voragen, 1990)

Industry	Enzymes	Million US $
Sugar & Starch Industry	α-Amylase	110
	β-Amylase	
	Glucoamylase	
	Isomerase	
	Pullulanase	
	Isoamylase	
	Oligoamylases	
	Cycloglucosyl transferase	
	Xylanase	
Dairy Industry	Proteases	80
	Lactase	
	Lipase	
	Lysozyme	
Brewing	α-Amylase	25
	β-Glucanase	
	Bacillus protease	
	Papain	
	Amyloglucosidase	
	Pullulanase	
	Xylanase	
Baking	α-Amylase	17
	Xylanase	
	Fungal protease	
	Bacillus protease	
	Phospholipase	
	Lipoxygenase	
Fruit & Vegetable Processing	Pectinesterase	16
	Polygalacturonase	
	Pectate lyase	
	Arabinanase	
	Hemicellulases	

Various techniques for handling enzymes *in vitro* have been developed which make it possible to apply enzymes to industrial processes in a controlled way. Enzyme immobilization on inert matrices enables the catalysts to be reused in continuous bioreactors and facilitates product recovery (Kennedy et al, 1990). Examples of this which are relevant to the food industry include immobilized β-galactosidase for the hydrolysis of lactose in milk products (Gekas & López-Leiva, 1985) and glucose isomerase for the production of high-fructose corn syrup from corn starch hydrolysate (Carasik & Carroll, 1983). Encapsulation may be used to protect enzymes or to direct their activity. Kirby et al (1987) demonstrated the use of liposome-encapsulated proteases to improve the efficiency of enzymatic accelerated cheese ripening. Encapsulation reduced enzyme losses, ensured even distribution and prevented premature action. Enzymes protected by encapsulation in reverse micelles have been used suspended in non-aqueous solvents (Martinek, 1989). Zaks & Klibanov (1985) demonstrated that enzymes need not only be used in aqueous solution but can be active as direct suspensions in organic solvents. Indeed, certain hydrolytic enzymes such as lipases and proteases can carry out synthetic condensation reactions under these conditions. This principle has been applied to the enzymatic synthesis of food ingredients such as emulsifiers, oligosaccharides and flavours, and to the biotransformation of fats and oils (Vulfson, 1993). Enzymes often have enhanced stability in these systems and they can be used with substrates that have low water solubility. Supercritical fluids have also been used as solvents for enzyme reactions in which substrates or products are sparingly soluble in water (Randolf et al, 1991).

Developments such as these have resulted in a new generation of biotechnological processes which are quite distinct from traditional food fermentations. These operate under highly controlled conditions, carry out highly specific functions and use well characterised enzyme catalysts. Emerging enzyme and genetic technologies have the potential to help meet many of the demands which are made of food manufacturers for products with good storage properties, that are more healthy and nutritious and are more attractive. They also have a role to play in food production and agriculture in the developing world to enhance productivity, reduce crop disease and to control pests. A list of current or impending commercial applications of genetic technology to food production is given by Beck & Ulrich (1993). For further reviews of food biotechnology, the reader is referred to Wasserman et al (1988), Bell & White (1989), Pilnik & Voragen (1990), Whitaker (1990), OECD (1992), among many others.

The latest developments in biotechnology have had their biggest impact in medicine, pharmaceuticals and fine chemicals. It is perhaps surprising, considering the close relationship between the food industry and biotechnology, that their influence has not been greater in food processing. There are several factors which contribute to this. The food industry is by nature very conservative compared to most modern biotechnology industries in which commercial survival is dependent on innovative research and development. There is little history of fundamental research being carried out by the food industry itself. It was noted by de Vogel (1991) that most "high-tech" industries spend 10-20% of their annual turn-over on research and development while such expenditure hardly reached 1% in the food industry. This figure is in agreement with a survey of UK food companies which found that the average expenditure on "innovation" was 0.55% of turnover (CEST, 1993).

Consumers are also conservative with regard to new food ingredients and processes. People are suspicious of new technologies and demand, quite rightly, assurance of the absolute safety of food products. Studies carried out to assess public attitudes to biotechnology have shown that its application to food production is less acceptable than to medicine and health (Frewer, 1992). As profit margins are low in the food industry, manufacturers have to be sure that their investment in new technologies will result in a product which is acceptable to the consumer and to the regulatory authorities. To some extent, the effects of biotechnology on food processing are often not apparent to the consumer. They rarely result in obviously new food products, but most often are used to enhance the properties of products which are already widely accepted (Beck & Ulrich, 1993). In addition, the production of food additives by means of biological agents may be seen as more "natural" than chemical synthesis (Armstrong & Yamazaki, 1986). However, the application of biotechnology in the food sector presents new issues of regulation, particularly for additives which have previously been regarded as safe when encountered in traditional foods, or which have already had approval when produced by conventional chemical synthesis. It is not likely that enzymes currently used in food processing, or well characterised enzymes which are already known to have no toxic effects, will require further safety testing when applied to novel processes. Regulatory authorities such as the Food and Drug Administration in the USA will probably require safety testing of enzymes which are not normally encountered in food products (Kessler et al, 1993).

Another important limitation to the adoption of emerging biotechnologies by the food industry is process economics. The product of a new process must be better and cheaper than that produced by conventional means if a change in production method is to be justified. The costs of research and development, new plant and obtaining regulatory approval must all be met from increased profits. These difficulties are often greater for the food industry where profit margins are small and prices must be kept low. There is little doubt that a great deal of innovative research on novel enzyme technologies for the food industry is being carried out in the laboratory. However, a bottleneck exists in the development of the means to transfer this technology to an industrial scale, particularly for low-cost/high-volume products. The importance of research into the biochemical engineering aspects of bioprocessing to match advances in genetics and enzyme technology has been highlighted in a number of reports (OECD, 1982; Michaels, 1984; OTA, 1984; Lilly, 1992).

Many emerging enzyme technologies require the production of large amounts of enzyme in a higher state of purity than is currently usual in food applications. Process scale enzyme purification is already carried out by the pharmaceutical and fine biochemical industries, but in this sector higher processing costs are offset by high product values. As was demonstrated in an analysis by Nystrom (reported in Dwyer, 1984), there is a strong correlation between the concentration of a product in the starting material and its selling price (Figure 1). The degree of required purification therefore has a significant influence on product cost. For recombinant DNA products, downstream processing can account for 80-90% of total product cost (Dwyer, 1984). It is unlikely that this would be acceptable for a food-related product. The reduction of downstream processing costs will thus be essential if many of the products of modern enzyme technology are to be applied to the food sector. The field of separations is therefore one which can have a significant impact on the economic feasibility of biotechnological processes in the food industry.

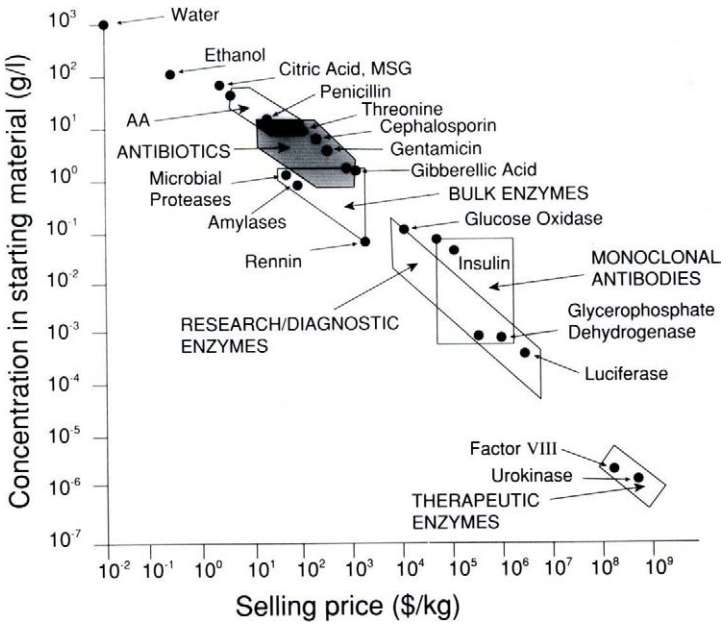

Figure 1. The relationship between the concentration of a biotechnological product in the starting material and its selling price. Reproduced with permission from Dwyer (1984).

Biotechnology undoubtedly offers great potential for novel products and processes in the food industry. The realisation of this potential is dependent on many factors, but the development of suitable downstream processing techniques will play a key role in turning the technical feasibility of emerging biotechnologies into economic viability. In this chapter, some new developments in bioseparations which may help to meet this need will be discussed. It has been the purpose of this introduction to place these developments in the context of a rapidly expanding science where new possibilities, and new issues, must be considered. In such an environment, an innovative approach to research by the food industry will be crucial.

2. THE PREPARATION OF BULK ENZYMES FOR FOOD USE

Although the food industry accounts for a large proportion of industrial enzyme use, the majority of these enzymes carry out a narrow range of reaction types. By far the majority are hydrolases, which catalyse the breakdown of biological polymers such as proteins and

polysaccharides (Table 1). As a consequence of their similar biological functions, they tend to share certain characteristics which make them particularly amenable to technological use on a large scale in food processes. Generally, they are secreted by the cell into the growth medium in order to hydrolyse polymers into smaller components which can then be transported into the cell for further metabolism. In commercial production, this overcomes the need for cell disruption. As these enzymes operate in a dilute environment compared to the cell cytoplasm, they are synthesised in large amounts. In addition, they are also in a relatively high state of purity at the start of downstream processing compared to cytoplasmic enzymes. The importance of this in maintaining low final product costs is illustrated by Figure 1 (Dwyer, 1984). Secreted enzymes also tend to be relatively stable as they have to operate in potentially harsh extracellular environments. The recovery rates from fermentation broths are therefore high and the losses of activity on storage and during commercial use are low.

The current applications of enzymes in the food industry do not require a high degree of enzyme purity. The main objective of downstream processing is therefore to obtain an active enzyme preparation in a form convenient for transport, storage and use. The purification of the specific active enzyme is not usually a consideration. Indeed, a typical industrial enzyme preparation may contain less than 5% enzyme protein (Cowan, 1991). In addition to fermentation residues which are not removed, other components may also be added for bulking and stabilization.

The main processes involved in the preparation of such commercial enzymes are the separation of the biomass from the fermentation medium and the removal of water to obtain the required product concentration. This can be achieved by low-selectivity separation processes such as centrifugation and filtration which are widely used in food processing for a variety of tasks. Figure 2 shows a typical processing stream for the preparation of bulk enzymes based on data from Atkinson & Mavituna (1991).

As enzymes are biological catalysts, a small amount may be used to carry out a biotransformation on a comparatively large amount of substrate. In the majority of current commercial uses in the food industry, enzymes are added in bulk directly to the substrate preparation. Using this method the enzyme cannot be recovered and reused and so its cost must be sufficiently low for it to be consumed during each process batch. Expensive enzymes which are produced in small amounts could not be used in this way in most food-related processes. Techniques such as encapsulation and immobilization can facilitate the reuse of the enzyme preparation over many substrate batches and can enable their application to more sophisticated and controlled processes. These require high-purity enzyme preparations if the maximum enzyme activity is to be achieved by the system. High resolution separation techniques are required to produce enzyme preparations which are free of most other contaminating proteins. The main areas covered by this chapter are therefore those recent developments in bioseparations which may contribute towards making the use of high-purity enzyme preparations more economically viable for food-related applications.

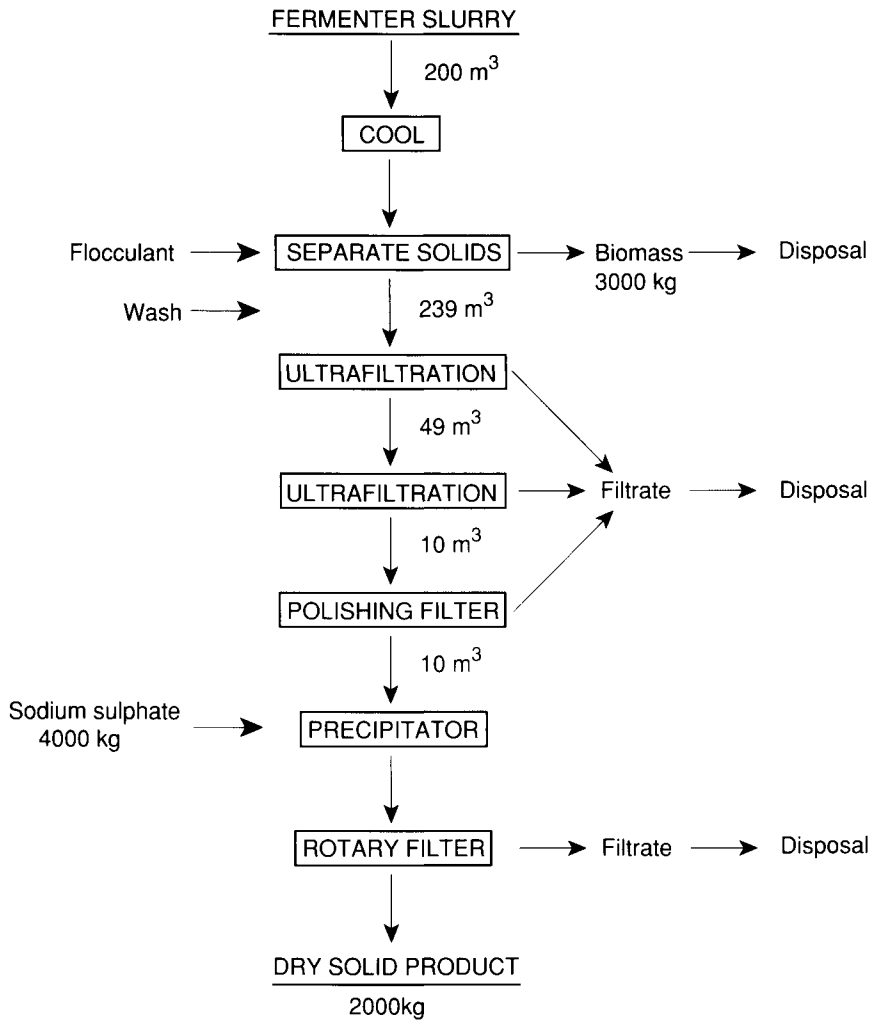

Figure 2. A representative processing stream for the recovery of an enzyme for bulk use. This is based on data from Atkinson & Mavituna (1991) describing extracellular protease recovery.

3. THE ROLE OF GENETIC MANIPULATION IN BIOSEPARATIONS

The ability to manipulate genetic material has revolutionized biotechnology by giving unprecedented access to the activities of biological systems and their control. DNA can be transferred between species so that foreign proteins are expressed in new host cells. The properties of industrial species can therefore be changed in a specific and targeted way which

is no longer limited by the constraints of the cells' natural metabolism and the vagaries of natural recombination. Unique combinations of biological processes can thus be brought together. DNA can be manipulated to create products with new properties, to optimize the synthesis of desired metabolites, or to tailor selected cell functions. The ability to engineer biological systems is not limited to the control of expression and catalytic function of proteins, but can also be used to facilitate protein purification. This makes it possible to consider product recovery as an integrated part of the cloning and expression of recombinant proteins.

There are a number of ways in which recombinant products can be more amenable to purification than native, naturally-expressed proteins. For example, the level of expression of the cloned gene can be increased by using efficient promoters and multi-copy vectors. Recombinant products expressed intracellularly in *Escherichia coli* characteristically accumulate to levels up to 30% of total cell protein, although expression levels of 50% of total cell protein are occasionally reported (Marston, 1986; Kane & Hartley, 1988). If the product is in a high concentration in the crude feedstock it is easier and cheaper to purify than a compound which is a minor component of a complex starting material. As many protein products no longer have to be produced by the native source organism, a wide range of host organisms is available. These may be selected to overcome production problems associated with the use of biomass which is difficult to obtain or is only available in small amounts. Examples relevant to the food industry include the microbial production of animal growth hormones (Schoner et al, 1985; Chen et al, 1992), recombinant chymosin (rennin) for use in cheese manufacture (Emtage et al, 1983; Cullen et al, 1987) and viral proteins for use as animal vaccines (Kleid et al, 1981). The recovery of these products from overproducing bacteria in large-scale culture is much less problematic and more economic than their recovery from animal tissues. A few well-characterized cell systems can be used for the synthesis of proteins from a wide variety of sources. This minimizes problems associated with fermentation and biomass handling as familiar processing plant and techniques may be used for different products. In addition, proteins produced in nature by pathogenic microorganisms can now be cloned into organisms which are more suitable for industrial use.

The recombinant product can take various forms in the host organism and this can influence the downstream purification process. Many are synthesised in a soluble form in the cell cytoplasm. The crude cell extract which contains these products will also contain all the normal cell components in a complex mixture. As stated above, the purification problems can be minimised if the desired product is the major component in the mixture. In some cases, particularly when proteins are cloned into foreign hosts, or are expressed in high levels, the recombinant product forms an intracellular insoluble aggregate (inclusion bodies) (Kane & Hartley, 1988). This is due to the specific association of partially folded recombinant peptide chains produced during the folding process (Mitraki & King 1989). Inclusion bodies differ from other insoluble cell components in size and density and can therefore be isolated relatively easily by differential centrifugation (Taylor et al 1986). However, the insoluble protein is not biologically active and must be refolded into the correct 3-dimensional conformation. This is achieved by first denaturing the protein in the presence high concentrations of agents such as guanidine HCl or urea. On removal of the denaturant by, for example, dialysis or size exclusion chromatography, the protein may refold into the active confirmation.

Some genetic manipulation procedures and expression systems, which are currently used on a commercial scale for the production of recombinant proteins, result in the extracellular liberation of the product. The recovery of the product from the growth medium is easier than from cell extracts as it is a less complex mixture which is likely to contain few other proteins in high concentrations. Certain microorganisms have well-developed systems for the transport of proteins across the cell membrane into the external medium. In particular, those species which are capable of hydrolysing extracellular substrates are often capable of secreting large amounts of enzymes such amylases, lipases, cellulases or proteases. Cullen et al (1987) reported a strain of the filamentous fungus *Aspergillus niger* which could liberate glucoamylase to a concentration of 5 g/l.

Secreted proteins are synthesised as precursors which include an additional series of amino acids on the N-terminal end. This "signal sequence" identifies the protein as a product for export and directs its passage through the cell membrane (Randall et al, 1987). The incorporation of signal sequence code into recombinant protein genes often results in the liberation of the desired product into the growth medium. This strategy has been widely investigated for the production of bovine chymosin by microorganisms. Cullen et al (1987) obtained high levels of chymosin secretion from *Aspergillus niger* by coupling the structural gene of the enzyme to the transcriptional, translational and secretory control regions of the fungal glucoamylase gene. High secretion levels have also been achieved in the bacterium *Proteus mirabilis* using the secretion and expression control regions of streptococcal exotoxin (Klessen et al, 1989). Another example of a food-related target for this type of technology is the plant protein thaumatin which is 100 000 times sweeter than sucrose and has potential as a natural sweetener. Illingworth et al (1988) expressed thaumatin in *Bacillus subtilis* and obtained extracellular liberation using the α-amylase signal peptide. Hahm & Batt (1990) demonstrated that recombinant thaumatin is secreted by *Aspergillus oryzae* using the native plant signal.

A variation on the theme of extracellular liberation of recombinant proteins to facilitate production and downstream recovery is the expression of the desired product in the milk of transgenic animals. This is achieved by fusing a structural gene to the regulatory regions of a milk protein and inserting the construct into a transgenic host. This approach was developed using the expression of human tissue plasminogen activator in the milk of lactating mice by means of the promoter and regulatory sequences of murine whey acid protein (Gordon et al, 1987). Similar methods have since been investigated for the expression of foreign proteins in the milk of various other animals, including rabbits, sheep and goats. Some examples of this technology are described in Table 2. The majority of applications of this new technology which are currently under investigation concern the production of high-value therapeutic proteins. This may yet be extended to the production of enzymes and proteins for food use when the technology is more mature. Combining dairy animal husbandry with the production of biotechnological proteins for use by the food industry is certainly an attractive idea.

The advances in genetic technology described above demonstrate that expression systems for recombinant proteins can be selected which give the greatest advantage for subsequent product isolation. Genetic manipulation also enables the actual physio-chemical

character of the product to be changed in order to facilitate its purification. Just as signal sequences can be incorporated into the product to control secretion from the cell, other sequences of amino acids can be added which confer particular properties which can be used as a "handle" during purification. Sassenfeld & Brewer (1984) and Smith et al (1984) introduced the code for polyarginine into the gene for urogastrone and cloned the construct in *E. coli*. The expressed protein was therefore urogastrone with a C-terminal polyarginine fusion. The great majority of cell proteins are acidic and are therefore negatively charged at physiological pH. The incorporation of the polyarginine tail made the resulting fusion unusually basic and thus very amenable to purification by ion-exchange chromatography. The tail was then removed by cleavage using a carboxypeptidase. By this method, Sassenfeld & Brewer obtained an urogastrone preparation of purity greater than 95% using a 2-step process. Similar techniques have since been applied to a variety of recombinant proteins. For example, polyaspartic acid fusions have been used to make β-galactosidase (Parker et al, 1990) and glucoamylase (Suominen et al, 1992) more amenable to purification by polyethyleneimine precipitation. Persson et al (1988) added code for 4 cysteine residues to the galactokinase gene and code for 11 phenylalanine residues to the β-galactosidase gene. The recombinant proteins were then expressed in *E. coli*. In the former case, the fusion sequence increased the binding of galactokinase to thiol groups during covalent chromatography, the protein being recovered after elution with reducing agents. Polyphenylalanine increased the hydrophobicity of β-galactosidase to improve purification of the enzyme by hydrophobic interaction chromatography.

Table 2
The expression of human proteins in the milk of transgenic animals

Protein	Host organism	Concentration ($\mu g\ ml^{-1}$)	Reference
t-PA[1]	Mouse	0.4	Gordon et al (1987)
Factor IX	Sheep	0.025	Clark et al (1989)
α1-antitrypsin	Mouse	7000	Archibald et al (1990)
Interleukin-2	Rabbit	0.43	Buhler et al (1990)
Urokinase	Mouse	2000	Meade et al (1990)
t-PA[1]	Goat	3	Ebert et al (1991)
α1-antitrypsin	Sheep	35000	Wright et al (1991)
CFTR[2]	Mouse	Not determined	DiTullio et al (1992)

[1] Tissue plasminogen activator
[2] Cystic fibrosis transmembrane conductance regulator

Fusion proteins have also been constructed which enable purification of the recombinant product by immobilized metal affinity chromatography (IMAC) (Smith et al, 1988; Hochuli et al, 1988; Ljungquist et al, 1989). IMAC is a separations method which utilises the interactions between metal ions and certain amino acid residues in proteins, most notably histidine, but also arginine, tryptophan and cysteine (Porath et al, 1975). An IMAC column contains an immobilized chelating agent which can be loaded with a metal ion. A recombinant protein carrying an added amino acid sequence which is rich in these residues binds strongly to the IMAC column. One of the advantages of this technique is that, unlike ion-exchange chromatography, IMAC is compatible with high concentrations of guanidine HCl and urea. This method can therefore be applied to the purification of denatured proteins which are expressed as inclusion bodies. In addition, histidine is a relatively rare amino acid in proteins and therefore the introduction of histidine sequences significantly increases the adsorption of the recombinant product to an IMAC column compared to natural proteins.

Hochuli et al (1988) modified the gene for mouse dihydrofolate reductase (DHFR) such that the protein expressed in *E. coli* carried polyhistidine fusions. The effects of the number of histidine residues on the purification of the recombinant protein by Ni^{2+}-IMAC were investigated. They demonstrated that both purity and yield of 90% could be obtained in a single step by this method, although a $(His)_6$ fusion was required to facilitate adsorption to the column in the presence of guanidine HCl. In the absence of guanidine HCl, increasing the number of histidine residues resulted in a decrease in the recovery of the protein from the column. These data are shown in Table 3 to illustrate the principle of chelating peptide fusions for the purification of recombinant proteins by IMAC.

The highly specific nature of biological interactions is an important aspect of the use of enzymes as industrial catalysts. These specific interactions are also used to purify proteins by means of affinity chromatography. A ligand which binds to the protein of interest can be immobilized in a chromatography column to remove the protein from crude material by specific adsorption (see section 5, Biospecific Separations). Fusion proteins which incorporate well-characterized affinity binding regions enable the highly specific isolation of the recombinant product by affinity chromatography. Germino et al (1983) purified a recombinant regulatory protein for which there was no convenient assay system, by constructing a fusion with β-galactosidase. This enabled purification of the recombinant product by affinity chromatography using a β-galactosidase-specific ligand, and detection of the product using β-galactosidase activity. This approach has subsequently been adapted for the purification of various target proteins. Staphylococcal protein A is another commonly studied protein fusion system for affinity purification of recombinant products (Nilsson et al, 1985; Rondahl et al, 1992). This enables secretion from *E. coli* cells and purification by specific binding to immunoglobulins.

A commercial kit is now available for the expression of recombinant fusion proteins and their affinity purification (Biometra GmbH, Göttingen, Germany). The cloning vector fuses the amino acid sequence Ser-Ala-Trp-Arg-His-Pro-Glu-Phe-Gly-Gly to the C-terminal of the protein which enables purification using a streptavidin affinity column. This technique was developed by Schmidt & Skerra (1993) who used polymerase chain reaction to generate random nucleotide sequences based around streptavidin binding domains. Libraries of

sequences were expressed and screened for binding activity, the above being selected as optimal for affinity purification and for the detection of products by blotting or ELISA. Using this method, a recombinant antibody fragment was expressed in *E. coli* and isolated from a cell lysate with a purification of 650 using a streptavidin-agarose affinity column and elution with 1 mM iminobiotin.

Table 3
The affinity of polyhistidine-dihydrofolate reductase (DHFR) fusion proteins for Ni^{2+}-IMAC adsorbent in the presence of 50 mM phosphate buffer and 6 M guanidine HCl. Data from Hochuli et al (1988).

	Phosphate buffer		Guanidine HCl	
Fusion protein	Retained (%)	Eluted (%)	Retained (%)	Eluted (%)
Native DHFR	<5	-	-	-
$(His)_2$-DHFR	30	10	<5	-
$(His)_3$-DHFR	90	75	<10	-
$(His)_4$-DHFR	>90	30	10	10
$(His)_5$-DHFR	>90	20	50	50
$(His)_6$-DHFR	>90	10	>90	90
DHFR-$(His)_2$	>90	90	<5	-
DHFR-$(His)_3$	>90	80	<10	-
DHFR-$(His)_4$	>90	50	10	10
DHFR-$(His)_5$	>90	40	50	50
DHFR-$(His)_6$	>90	30	>90	90

It is apparent that genetic manipulation can give the technologist increased control of the production of biological products, both upstream and downstream. However, this approach to biotechnology in the food industry has certain disadvantages which restrict its wide-spread application. Firstly, to make use of genetic technology, it is necessary that the gene coding for the protein of interest has been identified and isolated. This requires a significant investment of time, money and expertise on fundamental scientific research. Few companies in the food sector, other than large multi-nationals, have the resources or the specialist facilities required to carry out this type of work. To a certain extent, increased collaboration between food companies and public research centres or universities will help to overcome such problems. Secondly, the acceptability of food-related uses of genetic manipulation is of concern to the general public and to the regulatory authorities. The FDA are currently taking the view that recombinant proteins which are "substantially similar" to those which are known to be safely consumed in food, including those with minor variations

in structure and function, will not require further safety testing (Kessler et al, 1993). However, they would not encourage the use of known toxins and allergens, even if found naturally in certain foods. For those proteins which are not normally consumed in the diet, Kessler et al state that "the degree of testing of these new proteins should be commensurate with any safety concern raised by the objective characteristics of the protein". Proteins which have no history of food use may therefore require extensive testing, depending on their known functions and properties. Some recombinant protein products are now ready for commercial use by the food industry. Indeed, bovine chymosin cloned and expressed in microorganisms has already received regulatory approval in some countries, including the UK and the USA (Law & Mulholland, 1991). In addition, a recombinant version of bovine somatotropin, a hormone for increasing milk production, has recently been approved for use in the USA (Fox, 1993) The number of recombinant products used by the food industry is thus set to increase. The use of genetic manipulation to facilitate downstream recovery is therefore a realistic proposition.

4. COLUMN CHROMATOGRAPHY

Chromatographic separations involve the partition of a solute between a mobile and a stationary phase. Usually, the stationary phase consists of a particulate matrix packed into a tubular column through which the mobile liquid phase passes. The feedstock is introduced as a pulse into the column and individual components are separated by their differential distribution between the phases. For example, in size exclusion chromatography the stationary phase consists of the liquid held inside porous particles. The pores have a distribution of sizes which restrict the access of some species to the stationary phase and so separation is achieved on the basis of the relative sizes of the molecules in the mixture. In adsorptive chromatography, the retention of the solutes in the column is dependent on the strength of their interactions with the solid phase. There are various types of adsorptive chromatography which differ according to the chemical basis of the interaction between the solute and the stationary phase. Separations can therefore be achieved by means of different protein characteristics (see Table 4). To elute adsorbed proteins from the column, the characteristics of the mobile liquid phase are changed such that the interactions between the proteins and the solid phase are disrupted. Very high resolution separations can be achieved using a gradual change by means of an elution gradient. High resolution, combined with the availability of separations based on different protein characteristics, makes column chromatography an extremely powerful technique. By using several methods in sequence it is possible to purify to homogeneity a single enzyme from the thousands present in a cell extract. For this reason, column chromatography represents the state-of-the-art of protein purification.

In a survey of research papers (Bonnerjea et al, 1986), it was shown that ion-exchange chromatography was the most commonly used method of protein purification. On a process scale also, for the isolation of proteins for the pharmaceutical and fine biochemical industries, ion-exchange is used very effectively. However, there are limits to the scale on which such chromatographic techniques can be operated. The flow of liquid through the column is dependent on a pressure drop between the outlet and the inlet. As excessive pressure can

damage the separation matrix, this limits the length of column which may be used if a reasonable flow rate is to be attained. Consequently, most process scale columns achieve large bed volumes by using low bed height and large column diameter. The largest process scale chromatography columns available (Amicon) are 200 cm diameter and 80 cm in height and therefore have volumes of up to 2500 l. Using Whatman DE52 anion-exchange medium (Levison et al, 1990), such a column would be capable of binding in the region of 200 kg of protein.

Table 4
Methods of column chromatography in protein separation

Chromatography application	Basis of separation	Means of elution
Size exclusion	Size	Isocratic
Ion-exchange	Surface charge	Increasing ionic strength
Chromatofocusing	Surface charge	Change of pH toward pI
Hydrophobic interaction	Surface hydrophobicity	Decrease in salt concentration
Affinity	Biological interactions	Increasing concentration of free ligand, or change in pH or salt concentration

For most current uses of enzymes by the food industry, a high degree of purity is not required. The costs of column chromatography are therefore not justified. For those emerging food enzyme technologies which demand a higher degree of enzyme purity, process cost remains a major limitation to the use of column chromatography. In addition to the cost of the chromatography unit process, there are also the costs of associated processes to be considered. For example, particulate materials such as whole cells and cell debris can block the chromatography column and must therefore be removed. It may also be necessary to concentrate the protein or to change the solvent composition to achieve the conditions required for the adsorption of the target compound. Chromatography can rarely be carried out without preliminary processing of the feedstock by, for example, ultrafiltration. High resolution separation techniques are most effective early in the purification stream (Bonnerjea et al, 1986). However, the presence of high concentrations of contaminating proteins in the feedstock at this stage reduces considerably the capacity of the matrix to bind the target protein. Consequently, larger and more expensive plant is required if chromatography is used in this way. If the target protein is in a relatively high state of purity at the start of chromatography then smaller columns may be used. In this case several preliminary low-resolution separation steps such as ultrafiltration and precipitation are often required to remove the majority of contaminants.

As chromatography is such a widely used method, a great deal of research is carried out by manufacturers of chromatography equipment and materials in the development of new

products. If advances in chromatography are to be of benefit to the production of bulk enzymes, the reduction of material and process costs is an essential goal. There are a number of areas of development which could contribute to this. In a study of α-galactosidase purification, Porter & Ladisch (1992) demonstrated that the cost of the stationary phase can be a major component of total process costs, particularly at larger scales of operation. Total costs can therefore be reduced by increasing the operational life of the chromatography matrix, increasing the binding capacity and reducing the materials cost. Process time is also an important factor as this affects through-put, labour costs and overheads. Higher flow rates may be achieved using chromatography media which tolerate higher pressures, or have better flow characteristics. A reduction in the total number of unit processes which are required to prepare the feedstock for the chromatography step would also significantly reduce costs. The achievement of all of these aims in a single product is perhaps an impossible goal as novel chromatography media with higher binding capacities and flow rates than current products are likely to be more expensive. However, several new developments in chromatography do achieve some of the aims outlined above.

Adsorption to ion-exchange matrices is facilitated by the attachment of charged groups to a largely inert base material such as cross-linked cellulose or dextran. Conventionally, the ionic groups are located on short linear molecules and are thus held away from the matrix surface so that they are more accessible to the solute. Müller (1990) suggested an alternative strategy to maximise protein binding. Rather than a rigid array of charged groups, he synthesised adsorbents with the charges grafted laterally on vinyl polymer chains. The high flexibility of the charged chains of these "tentacle" ion-exchangers increased the contact between adsorbent and protein ionic groups, while decreasing the distortion of the protein required for maximum ion pairing. The brush-like layers of charged polymer also reduced contact between the protein and the matrix base material, thus reducing non-specific adsorption. It was demonstrated that the tentacle ion-exchangers had protein binding capacities of 70-140 mg/ml, compared with 35-55 mg/ml for comparable conventional materials. In addition, the resolution of protein separations was much improved.

Chromatographic stationary phase particles are usually porous in order to provide the maximum surface area, and therefore binding capacity. The transport of solute to the outside of the particle is by convective flow, while transport into the interior is by a much slower process of diffusion. If the solvent flow rate through the column is too great and there is insufficient time for diffusion into the particle, this results in a loss of resolution and binding capacity. A novel chromatography particle structure has been developed by PerSeptive Biosystems with the aim of overcoming this problem (Afeyan et al, 1990). Larger pores allow penetration of solute into the interior of the particle by rapid convective flow and give access to smaller "diffusive pores" which provide a large surface area for adsorption (Figure 3). It is claimed that perfusion chromatography using this type of medium reduces the need for a compromise between flow rate, binding capacity and resolution. The manufacturer's literature states a flow rate of 1800 cm/h for a perfusion ion-exchange medium. This compares with approximately 20-200 cm/h for conventional chromatography materials. Lehman et al (1993) applied perfusion chromatography media to the large-scale purification of recombinant tick anticoagulant peptide from yeast culture. In a low pressure capture step, 24.3 g of product was recovered from 400 l of diafiltered fermentation broth. In a subsequent

high resolution step, 16.7 g of product was purified in a series of rapid cycles using a 800 ml high pressure column. Compared to previous methods using conventional chromatography, the process time was reduced by half, recovery was increased from 32% to 47% and the need for low temperature facilities was eliminated.

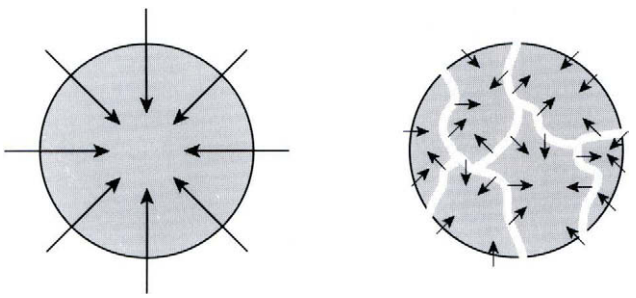

Figure 3. The transport of solute into the porous structure of chromatography particles. Transport into the interior of conventional particles is entirely by diffusion. In perfusion particles, large pores allow initial rapid access of solute by convective flow followed by diffusion further into the structure.

Conventional chromatography systems operate under axial flow, in which the mobile phase moves along the axis of the column. It is possible that some of the limitations of chromatography can be overcome by using an alternative column geometry. In radial flow chromatography, the movement of mobile phase occurs across the radius of the column from the outer surface to an inner compartment (Figure 4). In any chromatography column, it is the area over which the liquid phase is applied which defines the flow rate at a given pressure. In axial flow this is a function of πr^2. With radial flow, the liquid phase is applied over the whole outer surface area of the column, which is a function of $2\pi r$ x length. As a result, faster flow rates can be applied under radial flow than under axial flow for a given column volume. Smaller columns and shorter cycle times can therefore be used to achieve the required throughput. In addition, process scale-up can be carried out by increasing the column length, which is directly proportional to the bed volume and the flow rate.

Huang et al (1988) demonstrated the purification of porcine trypsin by radial flow affinity chromatography. A radial flow cartridge (800 ml) was constructed from spiral-wound sheets of modified cellulose material formed around a central core. Using p-aminobenzamidine as affinity ligand, crude material containing 30 g protein in 4 l was processed at a rate of 17.7 l/h. This resulted in a purification factor of 6.3 and 57% recovery. They also demonstrated a linear scale-up of flow rate and binding capacity with cartridge size,

up to 3200 ml. Saxena & Dunn (1989) described a design of radial flow column which could be packed with conventional chromatography materials. They operated a 60 l production scale column at a flow rate of 200 l/h and estimated that a 160 l axial flow column would be required to match this throughput. In a comparative study of the separation of egg proteins by 100 ml axial and radial flow ion-exchange columns, Lane et al (1990) demonstrated that the radial flow system was capable of operating 6-times faster. However, the axial flow column provided superior resolution, possibly because it had a larger effective bed depth. If the radius of a radial flow column is equal to the length of an axial flow column of the same volume, then the sample application area is twice as great under radial flow.

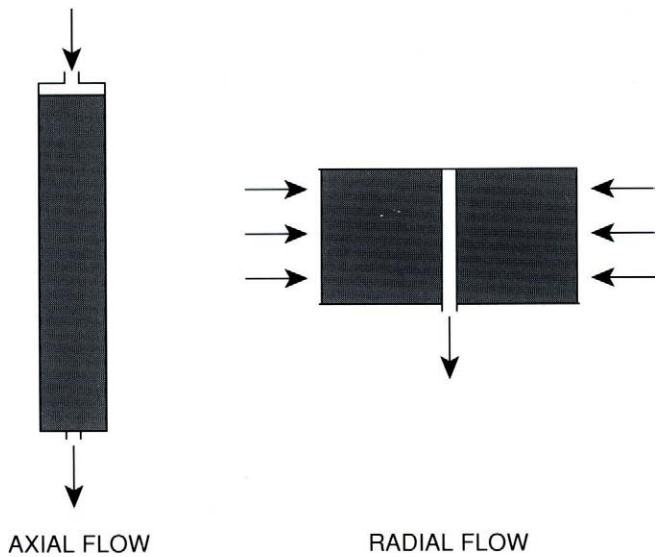

Figure 4. Conventional chromatography columns operate in axial flow in which the mobile phase moves along the axis of the column. In radial flow, the mobile phase is applied to the surface of the column and moves along its radius to the centre.

Another new approach to protein separations is expanded bed chromatography which was recently developed by Pharmacia (Patent No. WO9218237, 1992). This utilizes 200 µm agarose beads with quartz cores to increase their density. The flow of liquid into the *bottom* of the column causes the bed to expand under hydrodynamic pressure. On the introduction of the feedstock, the space between the beads in the expanded bed allows debris and insoluble material to pass through the column while proteins in solution bind to the ion-exchange medium. To recover the adsorbed material, the flow is reversed and proteins are eluted from the bottom of the unexpanded bed, as in conventional chromatography. The advantage of this system is that feedstocks may be applied directly from a fermenter stream, without prior

clarification by centrifugation or filtration (Hjorth et al, 1993). Pharmacia have claimed 40% cost savings using this method for the purification of a bacterial protein from 8000 l of periplasmic lysate (reported by McCormick, 1993).

5. BIOSELECTIVE SEPARATIONS

Highly specific interactions between complex molecules are at the heart of the activity and regulation of biological systems. This specificity results from the complementary 3-dimensional structures and surface properties of interacting species. The binding of enzymes with substrates, cofactors, inhibitors and allosteric effectors are all governed by structural complementarity, as are the interactions of antibodies and antigens, hormones and receptors, etc. Such specificity can also be used to achieve highly selective bioseparations. The most common form of this is affinity chromatography in which one species (the affinity ligand) is immobilized on a chromatography matrix in order to selectively bind a target molecule. Figure 5 shows a schematic representation of an affinity separation. The feedstock containing the target compound is applied to a column containing the affinity adsorbent. The target protein binds to the ligand while other solutes are washed through. The conditions are then changed such that the interaction between the ligand and the bound protein is disrupted and the desorbed protein is recovered in the eluent. The column can be returned to the conditions required for binding in a final regeneration step.

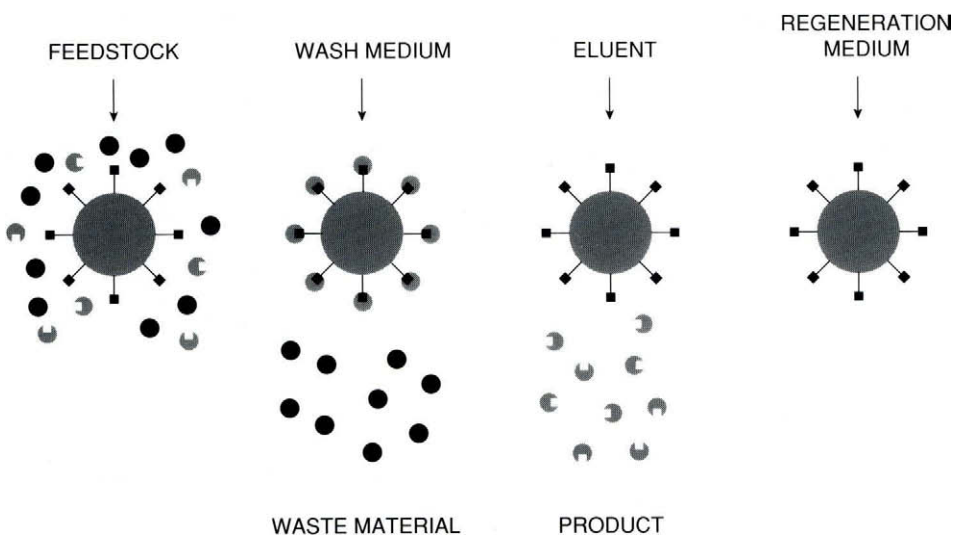

Figure 5. A schematic diagram showing a typical affinity separation. The affinity ligand is represented by the black squares attached to the round support particles. The complementary structure of the target protein is represented by the square cavities carried by some feedstock components. Only these compounds can bind to the immobilized ligand while unwanted materials are washed through. Bound product can then be recovered in an elution step.

Because of the high degree of selectivity which is possible, affinity systems are among the most powerful separation methods available. Some are currently used on a preparative scale for the commercial purification of pharmaceutical and research products. However, there are economic and technical restrictions on the application of these methods to the purification of large-volume, low-cost products such as many of those required for food use. Many ligands are themselves biological compounds and are therefore expensive to synthesise or to purify from biomass. The specificity of the protein-ligand interaction can also be a disadvantage as method development may involve a great deal of research and development which is likely to be relevant only to a single product. Affinity chromatography will also suffer from those limitations of chromatographic methods which were discussed in the previous section, but using more expensive media with a shorter active life.

The use of group-specific ligands can help to overcome some of these problems. These ligands do not have specificity for a single enzyme, but for a group of enzymes which share areas of structural similarity. For example, cofactors such as NAD and AMP may be used to purify the many enzymes which have binding sites for these molecules (Mosbach et al, 1972; Lee, 1983). The introduction of anthraquinone dyes such as Cibacron Blue as group-specific ligands was an important development in affinity chromatography. These originated as textile dyes and are therefore available in bulk at low cost. Haeckel et al (1968) observed by chance that Cibacron blue bound to pyruvate kinase during gel filtration. It has since been used as an affinity ligand for the purification of a wide range of enzymes (Clonis, 1987). The specificity is therefore not absolute but this is off-set by the wide range of potential applications, and the commercial availability of bulk volumes of pre-prepared separation media. Dye-ligand chromatography is the most widely used form of affinity chromatography on a large scale (Jones, 1991). Scawen & Atkinson (1987) listed 228 examples of dye-ligand chromatography published between 1980 and 1987. Of these over 40% described the purification of oxidoreductase enzymes such as alcohol dehydrogenase and malate dehydrogenase, and over 50% used Cibacron Blue as affinity ligand.

The techniques of molecular modelling have been used to establish that Cibacron Blue binds to the NAD binding site of horse liver alcohol dehydrogenase (Biellmann et al, 1979). This has enabled the synthesis of dye analogues with improved binding (Lowe et al, 1992). The ligand can thus be "tailor made" to fit the target enzyme. Group-specific ligands can thus be used as a starting point for the development of a series of ligands of higher selectivity. The rational design of affinity agents, either *de novo* or as analogues of established ligands, will certainly be aided by knowledge of the structure of binding domains in enzymes. Various techniques are now available for the determination of 3-dimensional molecular structures and these are being applied to the modelling of molecular interactions in food research (Kumosinski et al, 1991). As many of the enzymes involved in food technology share common functions (Table 1), they may make good subjects for the rational design of low cost ligands which can be synthesised on a large scale.

An alternative means of achieving selectivity in bioseparations is molecular imprinting. This involves the synthesis of polymer matrices which incorporate an intrinsic affinity for the species to be adsorbed. This is achieved by carrying out the polymerisation reaction in the presence of the target molecule which acts as a template around which the polymer is formed.

Interactions between monomer functional groups and the template molecules cause the resulting polymer to form in a conformation which is complementary to the template structure. Subsequent removal of the template molecule leaves cavities in the polymer matrix which are capable of specifically adsorbing the target species from solution (Wulff, 1993). This procedure is illustrated in Figure 6. The attraction of molecular imprinting is that separation matrices can be synthesised which incorporate the desired affinity in a stable form, and that specific adsorbents can be obtained for molecules for which no complementary ligands exist. This method has been investigated for the resolution of optical isomers of sugars (Wulff & Schauhoff, 1991) and amino acids (Ekberg & Mosbach, 1989).

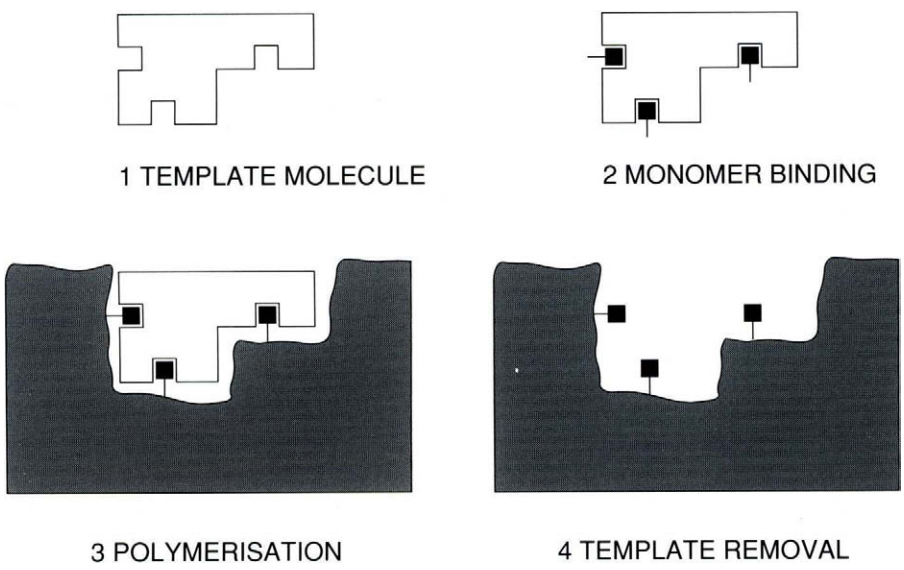

Figure 6. A schematic representation of the process of molecular imprinting. A polymerisation reaction is carried out in the presence of a template molecule which is capable of binding monomer groups. Subsequent removal of the template leaves cavities which are complementary to the template structure and therefore capable of specifically binding the template from solution.

So far, most studies have used small molecules which can be used as templates during polymerisation in organic solvents. However, Glad et al (1985) investigated the application of molecular imprinting to the synthesis of adsorbents for proteins. Imprinted polysiloxanes were formed from boronate-silane derivatives using the glycoprotein transferrin as template. This polymerisation can take place in aqueous solution and the boronate groups interact with glycoproteins. The imprinted polymers were formed as a layer on the surface of silica particles which could then be used in chromatographic experiments after removal of the template molecule. Using 0.35 ml columns, the retention of transferrin was shown to be

significantly higher with transferrin-imprinted boronate silane (1.32 ml) than with BSA-imprinted (0.71 ml), or non-imprinted (0.73) materials. The increased affinity for transferrin was dependent on the presence of boronate groups, and no increased affinity for BSA was observed with BSA-imprinted material. The method was therefore only suitable for the synthesis of glycoprotein adsorbents.

Another means of creating selective adsorbents is to raise antibodies to the target compound. The immune system has the capacity to generate antibodies carrying binding domains capable of recognising a huge array of antigens with a high degree of specificity and strength of interaction. In addition, catalytic antibodies, which are capable of facilitating specific chemical reactions, have been produced using transition-state analogues of the desired reaction as antigens (Jacobs, 1991; Benkovic, 1992). New technologies for the generation and manipulation of antibody-antigen interactions are therefore being developed.

For the purification of low value products by bioselective separations to be commercially viable, advances in ligand design and synthesis must be combined with the development of suitable techniques for the application of these systems on a large scale. The use of affinity interactions is not restricted to column chromatography. The attachment of affinity ligands to various supports makes it possible to increase the selectivity of a low-resolution separation technique by the introduction of an affinity element. For example, in affinity filtration the ligand is attached to a large support compound which can be retained by a filtration membrane. In a two stage process, feedstock is mixed with the adsorbent and unbound contaminants are removed by filtration while the ligand-enzyme complex remains in the retentate. The enzyme is then desorbed and is recovered in the permeate after separation from the affinity support by a second filtration process. Affinity filtration was demonstrated by Mattiasson & Ramstorp (1984) who used whole heat-killed yeast cells as an affinity adsorbent for the purification of concanavalin A from Jack beans. A hollow-fibre membrane with a molecular cut-off of 10^6 was used to retain the yeast cells and concanavalin A was recovered after elution with glucose. In a 5 hour process, 3.4 g were purified to homogeneity with a recovery of 70%. The same group also purified alcohol dehydrogenase from yeast by affinity filtration using Cibacron Blue as ligand bound to insoluble starch particles (Mattiasson & Ling, 1986). The affinity adsorbent need not be insoluble as long as the enzyme-adsorbent complex is significantly larger than the free enzyme and a suitable filtration membrane is available to separate the adsorbent and the proteins. Luong et al (1988) synthesised a water soluble affinity polymer for trypsin adsorption by copolymerization of N-acryloyl-m-aminobenzamidine (a trypsin inhibitor) with acrylamide. This was capable of separating trypsin and chymotrypsin using a membrane with a 10^5 molecular weight cut-off.

Pungor et al (1987) used the principle of affinity filtration to develop a continuous separation process. A conventional chromatography support for the affinity separation of β-galactosidase was recycled between two filtration units, one of which was subject to a continuous flow of feed stock, the other to eluent. A 35-fold purification of β-galactosidase with 70% recovery was maintained continuously for 6 hours on a laboratory scale.

The precipitation of proteins by the reduction of solubility in the presence of salts such

as ammonium sulphate, or organic solvents such as ethanol, are well established separation methods. These are not highly selective but they can be carried out on a large scale with crude extracts and can therefore be useful as a first step in a purification procedure. Bioselective precipitation can be achieved by using affinity ligands which induce precipitation of the target protein, or by attaching affinity ligands to polymeric carriers which can themselves be precipitated. This technique was first described by Larsson & Mosbach (1979) who synthesised a bifunctional NAD compound (Bis-NAD) for the purification of lactate dehydrogenase (LDH). Each ligand molecule consisted of two NAD moieties joined by a spacer. As the LDH had four NAD binding sites, and each ligand molecule was capable of binding two LDH molecules, the result of their interaction was the formation of insoluble cross-linked aggregates. The use of bis-NAD to precipitate various dehydrogenase enzymes was investigated by Flygare et al (1983). In this study, lactate dehydrogenase was purified 40-fold with a recovery of 91%. However, this method was not effective for all of the dehydrogenases tested. It was concluded that the number and spacial arrangement of the binding sites was an important factor in determining precipitation.

From the above studies, it is apparent that affinity precipitation using multifunctional ligands has certain limitations. It is only effective for multimeric enzymes and ligand design may have to be optimised for the spacial arrangement of binding domains in each individual target enzyme, even when using group-specific interactions. This is not the case with affinity precipitation using ligands attached to polymeric carriers. Schneider et al (1981) synthesised an acrylamide-based polymer incorporating a benzamidine trypsin affinity ligand. pH-mediated precipitation of this polymer was used to separate trypsin from pancreatic extract. The polymer was mixed with crude pancreatic extract and precipitated by adjusting the pH to 4.0. The precipitate was recovered by centrifugation and resuspended in water at pH 2.0 to elute the bound protein. In four repeated uses of the polymer, 162 g of trypsin was isolated with 90% purity from 1.4 kg of crude protein. Chitosan is a chitin-derived polymer which is insoluble at pH values greater than 6.5. An affinity ligand may be attached to chitosan which can then form a complex with the target protein in free solution. At increased pH, the complex precipitates and the target protein can thus be removed from the mixture. This technique was demonstrated by Senstad & Mattiasson (1989a) by the 5.5-fold purification of trypsin using soybean trypsin inhibitor as ligand. Lectins have also been purified using this technique due to their affinity interaction with chitin itself (Senstad & Mattiasson, 1989b).

The above purification methods seek to combine the selectivity of molecular recognition with the ease of operation of well established process-scale separation methods. Developments such as these could be important for the future establishment of high-specificity separations of products for food-related uses. Much of the equipment involved such as filtration plant is already widely used on a process scale in the food sector. Continued developments in ligand design and synthesis which reduce costs will further contribute to the commercial feasibility of affinity separations in this area.

In batch affinity adsorption processes, the amount of target compound which can be purified in one run is proportional to the amount of available affinity ligand. Particulate polymeric materials are often used as ligand carrier matrices to give a high binding surface

area. An alternative approach was devised by Niven & Scurlock (1993) with the aim of developing a continuous process and reducing the amount of affinity ligand required. The principle of this method was that a high binding area can be achieved using a rapidly recycling affinity matrix rather than a stationary particulate one.

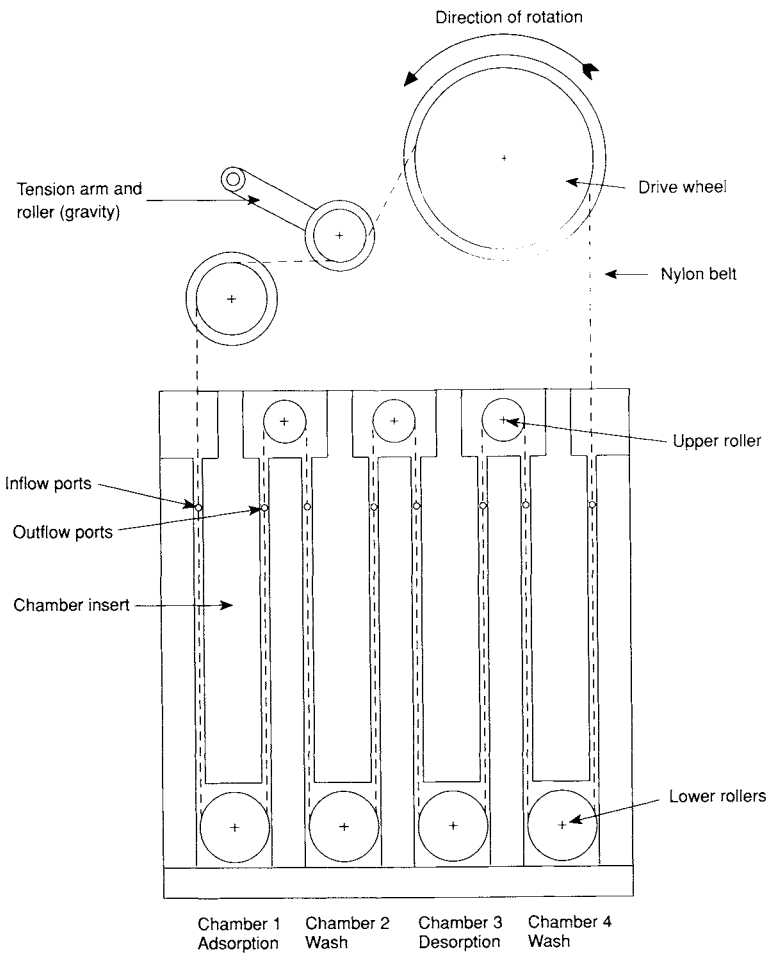

Figure 7. Prototype apparatus used to investigate continuous separations by affinity adsorption (Niven & Scurlock, 1993; European Patent Application No. 94300573.6). The affinity ligand is attached to a mobile belt which is cycled between four chambers in which the processes of adsorption, washing, elution and regeneration take place. Key components of this apparatus were a gift of Mr Peter Wolstenholme of Biometra Ltd, Maidstone, UK.

The ligand was bound to a continuous nylon belt which passed through a four-chambered tank (Figure 7). Each chamber carried a flow of liquid media corresponding to one of the four parts of the affinity separation process illustrated in Figure 5. Selective and continuous isolation of the target compound resulted from repeated transfer of the adsorbent surface between the feedstock and the eluent streams via wash and regeneration stages. Although the planar binding surface carries only a small amount of ligand, adsorption and desorption times are not limited by particle mixing and diffusion into the matrix. Short liquid/solid phase contact times, and hence rapid ligand recycling, are therefore possible. The technical feasibility of this method was demonstrated by the isolation of trypsin from an extract of bovine pancreas using soybean trypsin inhibitor as ligand. During an 8 hour continuous run on a laboratory scale, it was estimated that 12 mg of trypsin were recovered using 0.1 mg of ligand (of similar molecular weight) with a cycle time of 114 sec. The cost benefits of using a small amount of ligand could be important for the application of this method to the recovery of high-volume/low-cost products. In addition, continuous operation introduces the possibility of monitoring and control of the process in real-time. This allows more efficient operation and the production of a constant stream of product of predicable and controllable quality.

6. PARTITIONING IN AQUEOUS TWO-PHASE SYSTEMS

The separation of organic compounds by means of their differing distribution between two immiscible organic solvent phases is a routine method of chemical purification in the laboratory and on an industrial scale. This technique is not generally suitable for the purification of biological compounds such as proteins because of their low solubility and tendency to denature in the presence of organic solvents. However, it has long been known that phase separation can also occur with mixtures of certain polymers in aqueous solution (Dobry & Boyer-Kawenoki, 1947). The application of these systems to the purification of biological materials was pioneered by Per-Åke Albertsson (1956; 1958; 1986), although supported phase systems had previously been used for protein fractionation (Porter, 1955).

The most commonly studied aqueous two-phase systems (ATPS) are composed of polyethylene glycol (upper phase) and dextran or potassium phosphate (lower phase). Many other components such as methyl cellulose, ficoll and polyvinyl alcohol have also been used. At equilibrium, solutes in these systems are distributed between the two phases in accordance with their partition coefficient (K) such that

K = (top phase concentration)/(bottom phase concentration).

The partition of a solute between the phases is a surface dependent phenomenon involving various interactions, including ionic, hydrophobic and hydrogen bonding. The size, hydrophobicity and charge of a protein therefore influence its partition coefficient. The properties of the phase-forming components and the composition of the system also influence solute partition. It is therefore possible to manipulate the partition coefficients of a mixture of solutes in order to optimise their separation. For example, the inclusion of salts in the system can have a significant effect. Unequal distribution of the ions between the phases

creates an electrical potential which influences protein partition according to its net charge. A comprehensive work by Albertsson (1986) describes the theory and application of ATPS in detail.

ATPS are attractive for the large-scale purification of proteins for a number of reasons. The low surface tension and mild operating conditions cause little protein denaturation. As the partition coefficient is independent of total system volume, the method is amenable to scale-up. The processes of phase mixing, separation and recovery require mixers and low-speed centrifuges which are already widely operated on a large scale in the food industry. The phase-forming compounds are non-toxic and are available in large amounts. Unlike chromatographic separations, this method is tolerant of particulate material in the feedstock and so there is little requirement for preliminary clarification processes. Indeed, partitioning in ATPS can also be used as a method of cell debris removal (Datar & Rosen, 1986). A large number of scientific papers have been published since the 1970's which describe the partitioning of proteins in ATPS and the application of this technique to protein purification. Table 5 shows some examples cited by Kroner et al (1984).

Despite the apparent advantages of this method for large-scale use, and the large body of literature on the subject which exists, extraction in ATPS is not yet widely used by industry. At least in part, this is because solute partitioning between two phases offers little scope for the extremely high degree of selectivity required to separate one protein from thousands. While large recoveries can be achieved because of the mild conditions and the ability to obtain extreme partition coefficients, purification factors are most often in single figures. However, some proteins can be purified to a high degree using ATPS, particularly those which have extreme properties or which form a large proportion of total cell protein. For example, thaumatin, which has a pI greater than 10, was purified 20-fold from an extract of *E. coli* with recovery greater than 90% (Cascone et al, 1991). The fluorescent pigment phycoerythrin was purified from the cyanobacterium *Synechococcus* DC-2 in which it forms over 50% of total cell protein (Niven et al, 1990).

The resolution of proteins by ATPS can be increased by the use of countercurrent extraction. This is effectively an automated method of carrying out multiple extractions on a single feedstock sample. After the introduction of the crude material, mixing and equilibration, the two phases are separated and re-extracted with fresh polymer/salt solutions. Repeated extractions result in the distribution of a protein between the extract fractions, dependent on its partition coefficient. Proteins with differing partition coefficients can thus be resolved. Various apparatus have been designed for this purpose in which phase mixing, separation and transfer are carried out automatically (Albertsson, 1986).

As with many other methods for the separation of biological compounds, the specificity can be increased by the use of affinity ligands. This was demonstrated in the context of ATPS by Shanbhag & Johansson (1974) who chemically modified PEG by attachment of palmitic acid as an affinity ligand for albumin. Flanagan & Barondes (1975) investigated the partition of concanavalin A in a polyethylene glycol/dextran system. Because of the affinity of concanavalin A for dextran, it partitioned into the dextran phase. On inclusion of D-mannose, an inhibitor of the affinity interaction, the partition coefficient of

concanavalin A substantially increased. Since then, many other affinity interactions have been incorporated into ATPS, including the use of dye ligands such as Cibacron Blue. Kopperschläger & Johansson (1982) attached Cibacron Blue to PEG and achieved a 58-fold purification of phosphofructokinase from baker's yeast. Other chemical modifications have been made to phase-forming polymers to enhance separation such as the attachment of charged groups, thus introducing ion-exchange effects (Cheng et al, 1990).

Process economics is an important consideration in the large-scale industrial use of ATPS as a protein purification method. Some reports have suggested that the cost of the phase-forming components and waste treatment makes this method unfeasible (Schoutens & Kerkhof, 1987). Alternatively, the high biomass loading which is possible with ATPS means that lower capacity plant is required and that throughput is high compared to other methods. This results in start-up and operating cost savings (Kula et al, 1982). In a theoretical cost analysis, Kroner et al (1984) suggested that extraction in ATPS was cheaper than centrifugation or filtration. It is possible that, by the use of low-cost polymers and phase component recycling, the costs may be reduced sufficiently to make large-scale processes viable. For example, the starch-based polymer Reppal may be used in place of dextran at 10% of the cost (Mattiasson & Kaul, 1986). It is also necessary to consider effluent disposal and so, while phosphate salts may have a low initial cost, their effect on the environment makes recovery essential.

The technical feasibility of ATPS extraction on a large scale has been demonstrated in several studies. Hustedt et al (1988) described computer-controlled plant for continuous cross-current extraction of enzymes. This was operated at approximately 100 l/h with biomass loadings of 20-35% (w/w). Fumarase from *Brevibacterium ammoniagenes* was purified 22-fold with 75% recovery. Other enzymes, including penicillin acylase and aspartase, were processed with similar results. The same group also investigated the possibility of improving the economic feasibility of such purifications by recycling process chemicals (Papamichael et al, 1992). In studies using the separation of fumarase from baker's yeast, they estimated that chemical recycling reduced materials costs but increased labour, capital and utilities costs. The net effect on total operating costs was a saving of 14%. A similar saving was estimated by using continuous processing compared to batch processing.

In technical terms, it is apparent that extraction in ATPS is a feasible and attractive technology for large scale separations. For application in the food sector in particular, the high capacity and the use of non-toxic components and familiar equipment are advantageous. However, the economic feasibility is largely dependent on the particular process under consideration. The lack of wide-spread commercial use of this method suggests that the case for its industrial use is still not proven. The purification by ATPS of enzymes for food applications is likely to require continuous operation and phase component recycling. This increases the sophistication of the plant which is required to carry out these processes and therefore the initial capital investment required. Before this method can achieve its technical potential in the food industry, the economic case will have to be proved beyond reasonable doubt. It is possible that this will only happen after ATPS is better established in other sectors. The continued development of new processes and phase forming systems may yet contribute to the achievement of this potential.

Table 5
Purification of enzymes by extraction in ATPS Data from Kroner et al, (1984)

Enzyme	Organism	Biomass %	Partition	Yield %	Purification
α-glucosidase	*Saccharomyces cerevisiae*	30	2.5	95	3.2
Glucose-6-phosphate dehydrogenase	"	30	4.1	91	1.8
Alcohol dehydrogenase	"	30	8.2	96	2.5
Hexokinase	"	30		92	1.6
Glucose isomerase	*Streptomyces* sp.	20	3.0	86	2.5
Pullulanase	*Klebsiella pneumoniae*	25	3.0	91	2.0
Phosphorylase	"	16	1.4	85	1.0
Isoleucyl-tRNA synthase	*Escherichia coli*	20	3.6	93	2.3
Fumarase	"	25	3.2	93	3.4
Aspartase		25	5.7	96	6.6
Penicillin acylase	"	20	2.5	90	8.2
B-Galactosidase	"	12	62	87	9.3
Leucine dehydrogenase	*Bacillus spaericus*	20	9.5	98	2.4
Leucine dehydrogenase	*Bacillus cereus*	20	15	98	1.3
Lactate dehydrogenase	*Lactobacillus* sp.	20	4.8	95	1.5
L-2-hydroxy isocaproate dehydrogenase	*Lactobacillus confusus*	20	6.5	93	17
L-2-hydroxy isocaproate dehydrogenase	*Lactobacillus casei*	20	11	95	4.9
Fumarase	*Brevibacterium ammoniagenes*	20	3.3	83	7.5
Formaldehyde dehydrogenase	*Candida boidinii*	33	4.9	90	2.0
Isopropanol dehydrogenase	"	20	19	98	2.6
Glucose-6-phosphate dehydrogenase	*Leuconostoc* sp.	35	6.2	94	1.3

7. PROCESS DESIGN AND OPTIMISATION

There are a great number of factors which can influence the performance of separation processes. These include the properties of the target compound, the numbers and properties of the contaminants, the nature of the separation process and the environment in which it is to be operated. The exact effects of these parameters on the outcome of the process is often difficult to predict. This is particularly so for the isolation of biological components of biomass as the starting materials are complex mixtures which can vary in character depending on the conditions under which the organism was maintained and harvested. Biological processes can rarely be defined absolutely, nor controlled with precision. Consequently, the optimisation of purification procedures is largely empirical. The knowledge and experience of the technologist, combined with trial and error, defines the optimum methods and conditions for purification in the laboratory. These are then scaled-up via pilot trials to production level. Frequently, the isolation of the target will involve a series of steps which are optimised individually. The types of unit process which are selected will be determined by factors such as the equipment available and economic constraints in addition to separation performance.

A more rational approach to the design of separation strategies and the optimisation of performance may be aided by a better understanding of the scientific and engineering issues involved. Mathematical models have been constructed which describe various separation techniques including liquid chromatography (Bellot & Condoret, 1991) and extraction in aqueous two-phase systems (Baskir & Hatton, 1989). However, those researchers with an academic interest in the theory of separations do not tend to work with complex multi-component systems of the type relevant to many industrial biotechnological processes (Wang, 1990). A rational approach is therefore limited by the lack of an in-depth understanding of the nature of complex biological feedstocks and their behaviour during separation processes. "Expert systems" seek to combine databases of knowledge with mathematical models to obtain rapid answers to complex problems. A computer algorithm contains a set of rules which define a decision making process and the database provides the information from which the decision is made. The aim is to select the best solution to a problem out of many alternatives in a manner which simulates the rationalization of a human expert. The nature of expert systems and their potential in process control in areas such as the food industry are discussed by Shinskey (1989), Whitney (1989), Aarts et al (1990) and Konstantinov & Yoshida (1992). The possibility that this technique may be applied to the design of large-scale separation process was considered by Asenjo (1990). He concluded that there was insufficient information available for the construction of an expert system for high-resolution separation operations, but that a hybrid approach combining algorithmic and heuristic methods may be feasible. He is seeking to obtain the information required for a separations database by the characterisation of biological products and the components of fermentation streams (Asenjo & Leser, 1993).

It may be that biological materials are too complex and variable for the exact values of process parameters to be determined using mathematical models. Although the processes may be reduced to a series of relatively simple equations, the outcome may be subject to a significant dependency on starting conditions. Small errors in the initial estimations or

measurements thus accumulate and are amplified through the series of calculations. Through the processes of culture inoculation, cell growth, harvesting and multiple-step purification, the actual results may therefore diverge significantly from those predicted. However, mathematical models can place theoretical constraints on the operation of individual methods which will help to define their scope and limitations. This will ease process design and scale-up and will also provide insight into the function of separation apparatus which will contribute to improvements in equipment design and operation.

It is the aim of a process engineer to design a purification scheme which will achieve the desired product specification within the limits imposed by the available resources. Often, it will be necessary that downstream processing costs are kept to a minimum, particularly if the product has a low market value. A certain amount of the product is usually lost during each process operation, either by impairment of biological activity or by loss of material to waste. The use of as few process steps as possible is therefore required for maximum recovery to be achieved. To minimise operational costs, it is also advantageous that each unit process is of short duration, thus expending minimum man-hours and energy. If the material is more concentrated, then smaller volume plant is required and costs per unit product are reduced. It is perhaps obvious that each unit process should also operate as efficiently as possible. In addition, it is becoming increasingly apparent that the optimisation of individual operations is only one aspect of efficient process design and operation. In a series of separations, the performance of each unit will be dependent on the characteristics of the material produced by its predecessor. Overall process efficiency is therefore also dependent on the integration of unit operations into a coherent process stream (Fish & Lilly, 1984).

Various developments in separation technology have made a contribution to process integration. Advances in genetic manipulation have made it possible to consider product isolation at the earliest stages of process development. A purification strategy can thus be built-in to the product, as was discussed in section 3 of this chapter. Other means of integrating production and purification processes have also been devised. Immobilized biocatalysts are an example of this as they allow catalyst reuse while overcoming the need to remove them from the product further downstream.

Bioreactor configurations have been developed which combine a separation element with product biosynthesis such that the product is rapidly removed from the reactor as it is liberated by the cell. This is particularly advantageous for fermentations which are limited by end-product inhibition, such as the production of organic acids and alcohols. Rapid separation of the product from the fermentation medium overcomes inhibition and thus increases productivity. Some recent examples of these techniques from the scientific literature are listed in Table 6. For reviews of integrated production and recovery systems, see Daugulis (1988) and Groot et al (1992).

Combined production/purification strategies have also been developed for secreted enzymes. Aqueous two-phase systems, in which the organism and the extracellular protein have different partition coefficients, have been described for the production/extraction of enzymes. Examples include the production of alkaline protease by *Bacillus licheniformis* (Lee & Chang, 1990), and the production of α-amylase by *Bacillus amyloliquefaciens* (Park &

Wang, 1991). Technology now exists for the commercial production of antibodies on a continuous basis using animal cell cultures in hollow-fibre membrane reactors (Knight, 1988; Griffith, 1992). The cells are retained by the membrane while the antibodies pass through in a continuous flow, thus combining production and separation. Grandics et al (1991) described an automated process in which the antibodies were recovered from the membrane permeate in an integrated affinity chromatography purification loop.

Table 6
Production of organic acids & alcohols by extractive fermentation

Product	Organism	Extraction method	Reference
Ethanol	*Kluyveromyces fragilis*	membrane distillation	Udroit et al (1989)
	Saccharomyces bayanus	liquid membrane	Christen et al (1990)
	Saccharomyces cerevisiae	solvent extraction	Kang et al (1990)
	"	filtration/solvent extraction	Chang et al (1992)
Butanol	*Clostridium acetobutylicum*	membrane assisted solvent extraction	Jeon & Lee (1989)
Propionic & acetic acid	*Propionibacterium acidipropionici*	electrodialysis	Weier et al (1992)
Propionic acid	"	solvent extraction	Lewis & Yang (1992)
Acetonobutylic acid	*Clostridium acetobutylicum*	ultrafiltration/ distillation	Minier et al (1990)
Lactic acid	*Lactobacillus delbreuckii*	solvent extraction	Yabannavar & Wang (1991)
	"	solvent extraction	Seevaratnam et al (1991)
	"	ion-exchange	Srivastava et al (1992)

Continuous operations enhance the possibilities of integrated processing as the characteristics of the product are relatively constant with time. In addition, they facilitate real-time monitoring and control of product parameters so that the process may be fine-tuned during the run. Continuous production methods are now well established through the use of immobilized cell and enzyme systems. Continuous centrifugation and filtration plant are also

in common use for low-resolution separations. These may now be complemented by the development of continuous high-resolution separation techniques. Examples include liquid-liquid extractions (Papamichael et al, 1992) and continuous recycling affinity separations (Hughes & Charm, 1979; Burns & Graves, 1985; Pungor et al, 1987; Niven & Scurlock, 1993). Continuous elution in column chromatography can also be simulated by using a series of synchronised chromatography columns, such that one column is always eluting the product while the others are at various stages in the chromatography process (Nicoud, 1992).

The monitoring and control of biotechnological processes may be greatly aided by the development of biosensors. These utilise a biological component such as cells or enzymes which cause a biochemical reaction in the presence of a particular compound in the medium. This is combined with a transducer which produces a measurable electrical signal in response to the biochemical event. It is thus possible to exploit the specificity of biological interactions to produce detectors which measure the presence of selected compounds in a complex mixture. Various systems have been developed for monitoring compounds such as lactate, glucose and ethanol, some of which are available commercially. Readers are referred to Brooks et al (1991) for a general review, and to Wagner & Schmid (1990) for a review of their application in food analysis. The ability to assay rapidly biological chemicals without the need for lengthy separation and analytical procedures has great potential for the automation of continuous processes. The control of these systems can therefore be based on real-time measurements of the product species, rather than on non-specific parameters such as UV adsorption or pH.

8. SUMMARY

During the initial boom in modern biotechnology, the focus was, quite naturally, on the opportunities presented for novel products and processes. Separations for catalyst isolation, product recovery and effluent treatment were regarded as a minor consideration while this potential was being explored. Now that biotechnology is maturing as an industrial and commercial proposition, the requirements for bioseparations processes are of increasing importance. Although separation science was considered mature when biotechnology was in its infancy, there are now new issues to be investigated and new challenges to be met.

Advances in biotechnology offer great potential to the food industry for the development of novel products and processes. In many cases this will involve the purification on a commercial basis of biological compounds such as enzymes from biomass and bioreactor effluents. Several factors, although individually not exclusive to food industry separations, combine to make this a unique challenge:

- requirement for low cost final product
- high volume operation
- complex, poorly defined starting materials
- variation of starting material characteristics
- rigorous legislation to ensure safety of products and processes
- requirement for R&D specific to the needs of this sector.

Further developments in process engineering and separation technology will be necessary to meet this challenge. Recent advances which may contribute to this include:

- genetic manipulation of organisms for the overproduction and secretion of products and to facilitate purification.
- development of high-resolution separation processes which are capable of operation using large volumes of crude materials.
- increased accessibility of affinity separations through the synthesis of low-cost, stable ligands and methods of large-scale application.
- lower process costs by continuous operations and process integration.
- improved understanding of the nature of biological materials and the operation of separation processes.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 9

Advances in high pressure food processing technology in Japan

Rikimaru Hayashi

Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-01, Japan

1. INTRODUCTION

High pressure treatment to kill bacteria was first described in 1895 by Royer (1). Hite and coworkers (2) of the University of West Virginia reported the effects of high pressure preservation in milk in 1899. Bridgman (3, 4) observed coagulation of "egg albumen" by high pressure treatment in 1914. Since these reports, observations related to effects of high pressure on living organisms and living materials have been gradually made without successful application. A major breakthrough was made in Japan where the application of high pressure on food processing was successfully demonstrated in 1986 (5).

The most common process for food sterilization today is thermal treatment. Although thermal treatment effectively controls microorganisms and bacteria, such processing can alter food's natural taste and flavor and destroy vitamins. Hence, non-heating process for sterilization of food attracted attention of food manufactures. Therefore, introduction of high pressure-processing technology to food industries was received with much enthusiasm. Since then, pressure processing of food has become popular among food industries in Japan.

The intent of this article is not to cover the topic exhaustively, but to summarize recent research and developmental activities in Japan on high pressure technology for processing foods where the most notable progress was made. For further details one may refer to the Proceedings of the Symposia held from 1988 - 1993 (5-10) and references 11-16.

2. PRINCIPLE AND METHOD

The basis for applying high pressure to food is to compress the water surrounding the food (Fig. 1). A decrease in volume of water with increasing pressure is very minimal compared to gases. The volume decrease for water is approximately 4% at 100 MPa, 7% at 200 MPa, 11.5% at 400 MPa and 15% at 600 MPa at 22 °C (4). At above 1,000 MPa and room temperature, however, water changes to a solid (type VI ice) whose compressibility is very small. Usually, irreversible effects on biological

materials are observed at pressure of >100 MPa. Therefore, the pressure of 100 to 1,000 MPa could be useful in food treatment. For reversible effects, the pressure up to 200 MPa may be used. Microbial death at higher pressures is considered to be due to changes in permeability of cell membranes.

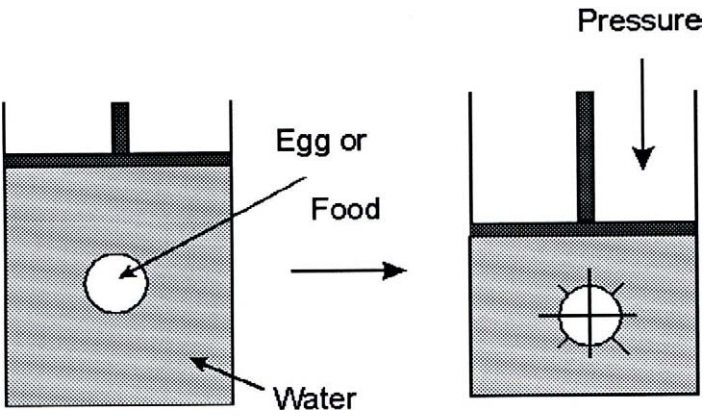

Fig. 1. A schematic view of the application of hydrostatic pressure to an egg or the food enveloped in a sealed plastic bag.

When a protein solution is compressed as described above, protein is denatured reversibly or irreversibly depending upon the nature of the protein and the applied pressure. This is because non-covalent bondings (hydrogen bonds, ionic bonds, and hydrophobic bonds) are destroyed or formed, corresponding to the decrease in volume of the system (see ref. 17 for a review). Covalent bonds do not undergo a change during the pressurization used here. Protein, nucleic acid and starches, whose tertiary structures are composed of non-covalent bonds, change their structures at high pressure and lead to denaturation, coagulation or gelatinization.

Pressure effects are, thus, similar to heat effects on biological materials and foods. In other words, a high pressure is as useful as a high temperature (Table 1). The unique advantage of the high pressure-treatment is that the covalent bonds are kept intact as the liquid water was compressed. Effects such as the Maillard reaction and formation of cooked flavors do not occur during the pressure-treatment. Thus, it is possible to retain natural flavor and taste by application of high pressure treatment to foods.

In a typical experiment, compression of an egg in water to several thousand atmospheric pressure induces coagulation of egg proteins without causing chemical changes, although high temperature sometimes destroys covalent bonds. The egg shell was not crushed under the high pressure and the egg white and yolk were coagulated completely at 620 and 400 MPa, respectively. The coagulated egg proteins increased the proteolytic susceptibility, retained natural taste, color and flavor without lowering

vitamin content. High pressure treatment generally denatures proteins; thus, the high pressure is useful to inactivate enzymes, to gelatinize starches, to sterilize microorganisms and to kill insects and parasites without accompanying destruction of nutrients and without changing flavor and taste.

Table 1
Possible uses of high pressure in cooking, processing, and preservation of foods as compared to high temperature processing.

Phenomenon	Temperature	Pressure
Denaturation of protein	O	O
Coagulation of protein	O	O
Gelatinization of starch	O	O
Chemical changes*	O	X
Enzyme inactivation	O	O
Sterilization of microorganisms	O	O
Killing of insects and parasites	O	O
Stimulation of seed germination	O	O

O, possible; X, not possible
*e.g. Maillard reaction, off-flavor, and vitamin destruction.

3. FOOD PROCESSING BY HIGH PRESSURE TREATMENT

3.1. Processing of food proteins

Soy protein suspended in water and packed in plastic tubes is completely coagulated at 400 MPa. Beef pressurized at 400 MPa and room temperature for 10 min is like a raw ham and the taste of the pressurized beef is intact. When the surface of the pressurized beef is slightly baked, it tastes like a rare steak. Boiled shrimps turn red and curve with the coagulation of the meat, but shrimps pressurized at 400 MPa for 10 min show no apparent change in color or shape but have coagulated meat as boiled shrimps. Oysters also show no change in size and shape, but keep the raw taste and flavor, after pressurization at 300 - 400 MPa and room temperature for 10 min. The pressurized oysters may be microbiologically safe.

In general, pressure-coagulated food proteins, *i.e.*, egg, soy protein, beef, pork and fish meat, are more glossy, transparent, dense, smooth and soft compared to boiled ones. These unique textural properties obtained by the pressurization offer ways to create new food materials (5-10).

Pressure treatment for processed foods including meat, fish and plant are the recent developments. McFarlane (18) in Australia used high pressure to treat meat where the pressure was applied as an electric shock to make meat tender. Meat tenderization is now possible by pressure control of intracellular proteases in meat (8).

High pressure effects on proteins and the fine structure of meat and fish have been studied extensively (see Table 2).

Table 2

R & D on High Pressure (HP*) Food Science and Technology in Japan, 1987-1993 - A summary of Conferences (6-10)

Chemistry and chemical reaction: Difference in liquid and solid compression, difference and similarity of T and P effects, organic synthesis under HP, HP effects on food-related reactions (hydrolysis of protein and starch, Maillard reaction, formation of lysinoalanine), solubility of ethylbenzene, NMR probe.

Enzyme and enzymatic reaction: Peptide hydrolysis and synthesis by proteases, enzyme digestion (soy protein, whey protein concentrates), activation of crude and purified polyphenoloxidases, inactivation of oxidases, HP effects on enzymes (actomyosin ATPase, lysosomal enzymes, muscle proteases, proteases, chymotrypsin, lipase, lysozyme, amylase).

Water and ice: Behavior of water under HP, thermal increase by rapid compression, near IR of water and ice, HP effect on salt solution, rapid thawing, pressure-shift freezing, sterilization of ice-nucleation bacteria, properties of ice I & III for food preservation.

Protein: HP effect on hydrophobic interaction, compressibility and structure, denaturation mechanism of Taka-amylase, chemical modification by HP (carboxymethylation, ferrocenation), HP effect on conectin, HP effect on thermal denaturation, deuterium exchange and fluorescence labeling, HP-induced change in myosin and subfragment, and cytoskeletal protein in muscle, properties of soy protein.

Starches: HP effect on starches, relationship between heat and pressure gelatinization, cooking properties of HP rice, rice wine (*sake*) brewing.

Lipids: Properties of lipids, fish lipid, rice lipid, sardine lipid, and milk fat, heating effect on HP milk fat.

Cellular structure: Cell membrane, subcellular structure of yeast, lysosomal granules, sarcoplasmic reticulum, yeast cytoskeleton, milk fat globules.

Biochemical phenomenon: HP extraction of pectin, solubilization of cellular components.

Microbial physiology: Yeast cell, growth of yeast and *E. coli* under HP, HP-tolerance of heat-shocked yeast, HP-tolerant yeast, HP effect on oxidative stress of yeast, drug sensitivity of *E. coli* grown under HP, HP effect on deep sea bacteria.

Viruses: Herpes virus.

Parasite: Muscular larvae of *Trichinella spiralis*.

Gel and sol: Gel-sol transformation of ovalbumin, emulsion properties of proteins, gel properties of fish *surimi*, gluconolactone gel of soy protein, gel formation of concentrated milk, gel of alginate, properties of HP-extracted pectin, gel properties

of low-methoxy pectin, gel-glass transition, thermal effect on HP-gel of actomyosin and whey protein.

Sterilization: Kinetics, mechanism at low temperature, *Bacillus* spores at high temperature, estimation of sterilization efficiency, effect of antimicrobial substance.

Food sterilization: Meats, fish meats, egg white, milk, milk products, tomato juice, jams, tea drinks, fresh vegetables, mandarin juice, plums, fruits, pickles, coffee, sausage, and sea urchin eggs, pickles in Japan, paste of water melon, soy sauce, seasoning sauce (*tsuyu, tare*), oyster, soybean cake (*tofu*), raw ham, raw sausage, lightly roasted beef.

Processing of meat: Ultrastructure and myofibrillar protein, products of beef muscle, properties of myosin B, gel formation of myosin, properties of HP meat, change in myosin B in high salt concentration, SH content and gel formation of myosin B, rheological properties of HP meat, effect of curing agent on HP porcine minced meats, processing system of meat products.

Processing of fish and fish meats: Texture of Alaska pollack, properties of HP fish meat (*surimi*), water-soluble fish meat, sea urchin, ultrastructure of carp muscle, new meat products with various texture, HP-induced texture of fish sarcoplasmic protein denatured by heating, pH shift or organic solvents.

Processing of agricultural products: Development of jams, fruit sauce and fruit dessert, processing of plum products, orange juice, rice wine (*sake*), rice cake (*moti*), and rice crackers, enzyme inactivation of oranges, food processing by ice nucleation-bacteria, properties of pickles, control of bitter taste in grapefruit juice.

Processing of other foods: Texture of pressurized egg white, cheese processing, whey protein concentrates, HP fermentation of cocoa beans.

Preservation: Pickles, soybean paste (*miso, shiromiso*), rice wine (*sake, namazake*), sea weeds, preservation under HP and sub-zero temperature, quality keeping of HP jams.

Cooking: Egg, meat, fish, oyster, shrimp, Japanese radish, kinetics of hardness of HP radish.

Combination of pressure and temperature: Theory and possibility, sterilization by HP and high or low temperature cooking.

Related techniques: Packaging materials, HP vessel for microscopy, HP indicator, HP fixation for electron microscope, electron microscopy of animal tissue cell, rapid thawing of frozen fishes, equipping of ultrasonic-wave generator.

Others: Economy, future and development of HP.

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*HP also means high pressure-treated or pressurized.

3.2. Processing of starch-containing foods

Starches of potato, corn and wheat are gelatinized by the pressure treatment at several hundred MPa and slightly elevated temperature. Pressurization of starches produces unique properties which are different from those formed by heat-gelatinization. This opens some interesting preparation possibilities. Although heat

treatment destroys starch granules and dissolves starches to give transparent solution, pressure-treated starches keep the granular structure intact. Nevertheless, pressurized starches are digested very well by amylolytic enzymes such as α-amylase (7, 9). Thus, pressure treatment opens a new way to process starches with minimum use of heat. However, further extensive study is clearly needed for understanding the effects of high pressure on starches and other food components.

4. STERILIZATION OF FOOD

Bacteria, yeasts and molds in foods, such as meat, fish and agricultural products, are sterilized by high pressure treatment at 400 - 600 MPa. The pressurization of mandarin or orange juices at 300 - 400 MPa for 10 min is enough to sterilize vegetative microorganic cells, although spores of *Bacillus sp.* are not killed. This retains good taste and flavor of the juice and allows to store it at room temperature for 5 months. When pressure was applied at 45 °C, the results were considerably better than that at the room temperature (6-10).

Pressurized juice should be preserved under chilled conditions to keep its fresh flavor and taste. Low temperature also helps to reduce the development of precipitates, since low temperature storage keeps pectin esterase activity low; thus, pectin esterase cannot participate in the formation of a precipitate. The low temperature storage is important to other pressure-processed foods; for example, pressure-processed jams being kept cold exhibit fresh taste and flavor for long periods (6-10).

Pressure-sterilization is also useful to retain the taste and flavor of traditional pickles where heat-sterilization deteriorates them. Pressure-sterilization is also useful in fermentation of foods to keep the taste and flavor intact (5-10).

The prominent use of the high pressure sterilization is in partially prepared foods or oven-ready foods. Pressure treatment preserves flavor, taste and natural nutrients, but bacterial spores are not killed (see below). Hence, those foods require chilled transportation.

5. COMBINATION USE OF PRESSURE (P) AND TEMPERATURE (T)

Under ambient pressure, protein denatures at high temperature (heat denaturation) and also at low temperature (cold denaturation). In contrast to temperature-dependent denaturation, pressure-dependent denaturation of proteins exhibits two opposite effects depending upon proteins: an increased pressure-denaturation at high or low temperature (enhancement effect of T and P) and a decreased pressure-denaturation at high or low temperature (competitive effect of T and P). This has been systematically studied by altering T and P factors independently (see ref. 17 for a detailed explanation).

Although bacterial spores are not killed by high pressure treatment at room temperature, they are killed at elevated temperatures (45 - 60 °C) at 600 MPa. Vegetative cells of some bacteria and yeasts can be sterilized at low temperatures (*e.g.*, -20 °C) under pressurization (8, 10).

The enhanced effects of high or low temperature can be applied not only to inactivate bacterial spores but also to inactivate enzymes and improve food texture, especially that of starches. An advantage of using both pressure and temperature together lies in its ability to lead to efficient and economical industrial applications; although, the wrong combination of T and P may lead to inefficient inactivation of bacteria or yeasts.

Pre-treatment by T followed by P treatments or *vice versa* is an important consideration for developing high quality foods with good taste, flavor, color and texture. Following scheme summarizes how to introduce P in addition to T:

$$T,\ P,\ T + P,\ P \rightarrow T, \text{ or } T \rightarrow P$$

The use of pressure lower than 200 MPa at 0 to -20 °C is of interest because under such conditions the equilibrium temperature between water and ice is shifted to lower temperature; thus, it may be useful in the following applications:

1. Non-freezing food preservation
2. Rapid thawing of frozen food
3. Rapid freezing of food and biological materials (pressure-shift freezing method)

Two factors should be considered for the combinational use of T and P: 1) the effect of pressure on the increased chemical reaction at high temperature, and 2) the temperature rise of the pressure medium accompanying adiabatic compression. Since the rate of some chemical reactions (*e.g.*, decomposition of nutrients) are accelerated by pressure, adverse effects may take place in food. We have started a study to examine the pressure effects on food-related reactions (7, 8). During the compression of water, adiabatic temperature increase at high temperature is larger than that at low temperature (7). Hence, an accurate control of temperature inside the pressure vessel is required.

6. RESEARCH AND DEVELOPMENT IN EQUIPMENTS AND PACKAGING

6.1. High pressure equipment and the system

In order to meet the requirements for the food industry, a high pressure equipment used in ceramic industry (CIP) has been improved by incorporating additional sensors for pressure and temperature sensing, so as to attain fast pressurizing and depressurizing speeds, and to ensure the safety of the food products.

Test equipments for foods have been developed by several equipment industries and are on the market (5-7). A typical equipment has 500 ml capacity, is made of stainless steel and works at a maximum pressure of 700 MPa. It takes only 90 seconds to attain the maximum pressure. Temperature of the inside water, used as the pressure transducing medium, is regulated by an electric heater outside the pressure vessel. Thus, in this machine, the hydrostatic pressure is directly applied to foods placed in the pressure vessel with high speed under the regulated temperature without any harmful contaminants. A total of more than 100 food companies and government institutions in Japan are equipped with the high pressure test machines in recent years and are performing research and development of new food products based on the high pressure processing.

Industrial equipments for high pressure processing of foods are operational in several food industries: a batchwise system of 10 to 50 liter-capacity and a semi-continuous system of 1 - 4 ton/hour-treatment. The former is used for processing and sterilization of packed foods and the latter for the treatment of liquid foods (6-9). These machines are small as an industrial machine, but a pressure vessel of 50 liters is similar to a heating vessel of 200 liters in capacity. The cycle time for operating the pressure machine is short, generally being 15 min for food sterilization or food processing, while a large pan takes about 1 hour for heating and cooling in conventional processing.

An industrial system for the high pressure processing of foods is similar to the conventional heat processing: raw materials are pre-treated, filled in plastic bags, sealed in vacuum and pressurized. Final products are obtained after drying the bags. Liquid food may be placed directly in the pressure vessel in a semi-continuous way.

It should also be pointed out that the use of pressure processing saves energy and is more sanitary compared to the use of high temperature processing (5-10).

6.2. Packaging

Selection of packaging materials are as important as tastes of food for high presssure food processing. Plastic films are generally acceptable for high pressure processing, while they are not usually suitable for high temperature processing. On the other hand, metal cans and glass wares are not suitable for the pressure treatment. Ochiai and Nakagawa pointed the importance of the head-space in plastic cups and suggested the use of plastic paper as a package material because of its heat-sealability and hygienic safety (7). Packaging materials which prevent oxygen permeability and light exposure should be developed especially for keeping fresh color and flavor of foods.

7. PRESSURE-PROCESSED FOODS IN JAPANESE MARKET

On April 23, 1990, Meijiya Food Company introduced three kinds of jam (strawberries, kiwifruit and apples) made using high pressure treatment without

application of heat. The products are vivid and natural in color and taste. These jams were the first pressure-processed foods in human history. Now, the company produces fruit sauces and desserts using pressure processing (8).

Two juice products (non-bitter grape-fruit juice by Pokka Co. and mandarin juice by Wakayama Co.) are in the market. In the grapefruit juice, pressure treatment was used to inhibit the development of bitter taste, and in the mandarin juice, the pressure treatment inactivates molds and yeasts after squeezing. This primary sterilization is followed by blending and the secondary heat sterilization to meet the Food Law requirements (8). Fruits with fresh flavor and taste added to sherbet and other pressure-processed foods are becoming popular among younger generations.

Recently, Japanese unrefined rice wine (*nigori-sake)* appeared in the market. The Industrial Research Center of Kumamoto Prefecture separated the precipitate and supernatant parts of the wine and sterilized the former by high pressure treatment. The latter part, from which microorganisms were removed by a membrane filtration technique, was remixed with the precipitate under aseptic conditions (10). The new preservable *sake* with white color and fresh flavor, instead of brown color and heated smell, has been brought about at a reasonable cost by the minimum use of an expensive high pressure machine for the treatment of a small amount of the precipitate.

Development of other products including hams, fish-pastes, traditional marine products and tea products continues in small- or middle-sized food companies in many parts of Japan. For traditional preservable foods containing high salt or sugar contents, pressure treatment is an effective alternative to lower their contents.

8. RESEARCH ACTIVITIES

In order to further develop the high pressure technology, studies on high pressure biochemistry and food science are required. Since 1988, the author has organized five annual symposia on the uses of high pressure processing in food. At present, number of researchers working in this area is growing in universities, government institutions and industries (5-10).

In July, 1989, The Ministry of Agriculture, Forestry, and Fisheries in Japan founded the Association of High Pressure Application in Food Industry. The Association included 10 food companies and 7 equipment companies and continued until March, 1993. The First Joint Meeting of Japan and the European Community (EC) on High Pressure Biotechnology was held at La Grande Motte in France in September, 1992, and the First Taiwan Symposium on the High Pressure Technology for Food Science was held at Taichung in Taiwan in April, 1993. The First International Congress on High Pressure Bioscience and Biotechnology will be held at Kyoto, Japan, in November, 1995.

9. CONCLUDING REMARKS

High pressure is useful for the purpose of cooking, processing, sterilizing, and preserving food, as is the high temperature. The advantage of the high pressure lies in the fact that it avoids the destruction of the covalent bonds and retains natural flavor, tastes, color, and nutrients. Thus, the high pressure technology is of great interest to the food industry.

A newest food magazine distributed in Japan (*Shokuhin to Kaihatsu*, December 1994 issue) reported that at least seven food companies now sell high pressure-processed foods. Ice-nucleation bacteria sterilized by high pressure treatment and traditional rice pastes including plant leaf or special bean (*Yomogimochi*) have been added to the list. Basic research on further understanding of the effects of high pressure on biological systems is indispensable for further development of high pressure foods. As more and more high pressure foods with superior quality are brought into the market, a new and growing consumer demand will be created.

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A.G. Gaonkar (Editor)

Chapter 10

Recent progress in aseptic processing of food

Thomas E. Szemplenski

Aseptic Resources, Inc., Overland Park, Kansas, U.S.A.

1. INTRODUCTION

Aseptic processing and packaging of food was invented by C. Olin Ball in the 1930's as the Dole Aseptic Canning system [1]. For many years the system was improved upon by Dr. William McKinley Martin and others, but the Dole Canning System still remained the only equipment for aseptically containing food and dairy products.

Aseptic processing and packaging continues to be one of the most dynamic areas of food processing. New methods of processing and packaging are continually being researched, invented and commercialized. Even during the stages of publication of this book other improvements will be made, thus making it difficult to introduce a comprehensive collection of the latest technology.

The focus of much of this research centers around the aseptic processing of low acid foods containing particulates. Until recently, only low acid homogeneous foods were aseptically packaged, but continued research with products containing particulates has paid off and the result of this research is affording tremendous opportunities for growth. Acid foods containing particulates of significant size have been aseptically processed since the early seventies, thanks mainly to the development of the Scholle bag-in-box filler that allowed the filling of particulates [2]. Products such as fruit for yogurt and ice cream toppings, sliced fruit in syrup, diced peppers and even diced tomatoes have all been aseptically filled for years with the Scholle and other similar bag-in-box fillers. The sterile products going into these packages are all for institutional use or for the reprocessed market. The processing equipment development and design changes that allow significant particulate processing for acid foods are now starting to be applied to low acid foods. It appears that the largest market for aseptically processed low acid foods is not in the larger bag-in-box containers, but in the retail sized and institutional pouch sized containers. In this regard, additional developmental work is needed to aseptically fill particulates into these containers. In pursuit of this goal several new developments are under way. Some of the more important ones will be considered in this chapter.

2. NEW DEVELOPMENTS

2.1. Ohmic heating

The Ohmic heater was developed by EA Technology at Capenhurst, Cheshire in the United Kingdom [3]. It is direct electrical resistance heating of food products by the passage of current through the continuous flow of product. The passage of current

generates heat which sterilizes the food. The depth of heat penetration is unlimited.

As mentioned above, it is one of the food industry's goals to continuously sterilize food products containing larger and larger particulates. Present aseptic processing systems most often utilize scraped surface heat exchangers for heating and cooling food products containing discrete particulate matter. In this regard, most of the damage that is done to the particulates in aseptic processing systems is caused by heating with scraped surface heat exchangers. Due to the inherent design of the Ohmic heater, larger particulates are able to be processed with improved identity. The Ohmic heater is already being used to sterilize low-acid particulates up to 25mm [4].

Particulate identity is not the main advantage of the Ohmic heater. The main benefit the Ohmic heater offers is in the method of heating. The Ohmic heater heats and sterilizes the food quickly and uniformly as the electrical energy is transformed into thermal energy volumetrically throughout the bulk of the food product. This is unlike scraped surface heating which is accomplished almost entirely by conduction. The degree of penetration with Ohmic is unlimited and the extent of heating is controlled by special uniformity of electrical conductivity throughout the product and its residence time in the heater.

A typical in-line heater and power supply will consist of three separate modules: the heater assembly, the power supply, and the control panel. In its simplest configuration, there will be four electrode housings and three interconnecting tubes. The electrode housings are machined from a solid block of polytetrafluoroethylene (PTFE) and are encased in stainless steel. Each contains the working electrodes which are connected to each phase of the secondary side of the transformer.

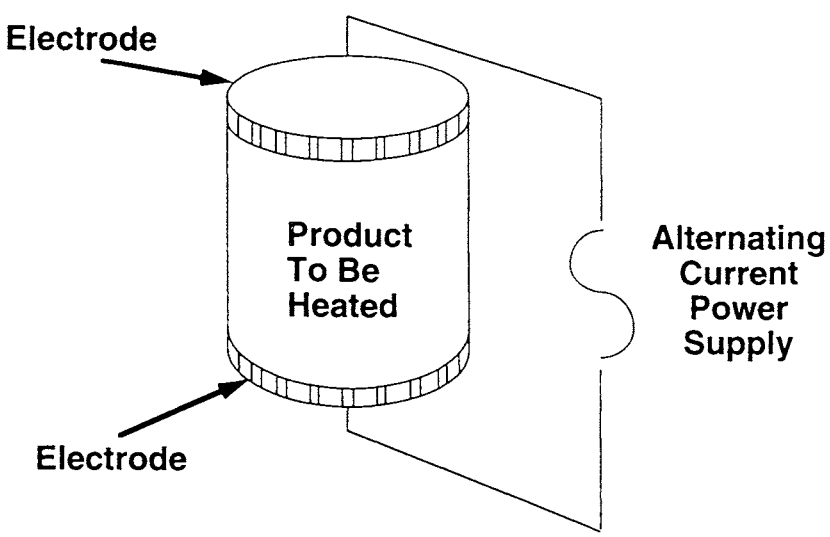

Figure 1. Principle of Ohmic heating (Courtesy of APV)

The interconnecting tube sections of the heater are of stainless steel with suitable electrically insulated plastic lining. The suitable lining material is capable of withstanding temperatures in excess of 145°C. The tube sections are bolted onto the electrode housing by means of flanges. Product sealing between tubes and electrode housing is by the use of flat rubber seals. With some heat sensitive food products, the interconnecting tubes will be jacketed and cooled to counteract the effect of longer residence times near the inner surface of the tubes. The complete Ohmic assembly is mounted in a vertical or near vertical position to ensure complete filling.

At the onset, the energy costs to use the Ohmic heater might seem to be more expensive than conventional tubular or scraped surface heat exchangers as electricity is thought to be a very costly energy source; however, the conversion efficiency is greater than 90% compared to only 45-50% for alternative energy forms, so the overall cost to heat a comparable amount of food product is similar.

The Ohmic heater cannot be used for all food products. The applicability of Ohmic heating depends on each product's electrical conductivity and on whether the product is an insulator or a conductor. Insulators cannot be heated with the Ohmic heater. Some insulators include non-ionized fluids such as bone, fats, oils and alcohols. The Ohmic heater generally cannot be used to heat tap water unless some salts are added to increase the conductivity. Fortunately, a great many foods contain moderate amounts of free water with dissolved ionic salts and are therefore conductors. Most pumpable food products with water in excess of 30% conduct sufficiently for the Ohmic heater to be applied.

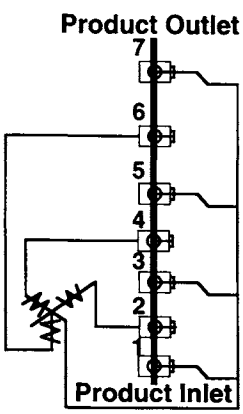

Figure 2. Diagram of Ohmic heater electrode wiring. Source: APV

Pre-sterilization of an aseptic processing system utilizing an Ohmic heater is done by recirculating a solution of sodium phosphate or sodium citrate at a concentration which approximates the electrical conductivity of the food product to be processed. The temperature necessary to achieve sterilization is reached by heating the recirculated solution with the Ohmic to the superelevated temperature with an aseptic back pressure valve in the system.

Although there are obvious advantages to this new technology, there are also some drawbacks. The Ohmic cannot be used to create a phase change such as gelatinizing starches or other stabilizers. Starches must be pregelatinized prior to processing. The optimum temperature the food product should be at prior to processing is 55°C. Therefore, the heat-stable stabilizer must be heated to between 80°C and 85°C to effect gelatinization and then cooled back to 55°C by more efficient alternate heat exchangers. The particulates are then added and the temperature brought to equilibrium prior to entering the Ohmic for sterilization. This is very time consuming and not energy efficient.

The obvious advantages of Ohmic heating are:

* Particulates and carrier medium are heated virtually simultaneously
* Very rapid heating (1°C per second)
* No moving parts
* No hot heat transfer walls
* No obstruction in the heating area like scraped surface heat exchangers
* No fouling of the heat transfer walls
* No noise
* Low maintenance

There are 12 commercial installations in the world heating and sterilizing such products as strawberries, ready meals, meat, poultry and pet food. Expect many more installations as the learning curve is expanded. Commercial systems are available in 75 kilowatt (kW) and 300 kW sizes rated at 1650 and 6600 lbs. per hour, respectively [5,6].

2.2. High voltage pulsed electric field heating

Another heating/sterilizing system under development is the use of high voltage pulsed electric field process heating. Research centered around this technology is taking place in both the UK and the United States [6]. In the UK, Sale and Hamilton found that pulsed high voltage electric fields (up to 25 kV/cm) produced different stages of kill to vegetative bacteria cells in yeast depending upon pulse length, number of pulses and field strength [7]. The lethal effect was not due to heating and was independent of current density and energy output; damage to the cell membranes appeared to be the cause. The pulsing action of the electric field causes an irreversible loss of membrane function because of selective osmotic barrier between the cell and its environment.

Although electric field sterilization has not been commercialized, the research continues in the United States, the UK and Japan. Electric field sterilization offers the possibility of cold sterilization followed by aseptic packaging.

2.3. Isostatic high pressure sterilization

One of the most exciting new technologies for commercial sterilization of food products is the use of isostatic high pressure. Isostatic means the pressure is equalized from all directions so that neither the product nor the package is damaged. High pressure

sterilization is in it's infancy, but it is being researched in the UK, Sweden, France, Germany, Belgium, Spain, Japan and a number of places in the United States [8]. There are a couple of high acid products on the market in Japan and the first low acid product will soon be introduced in France [9].

High pressure processing not only destroys microorganisms, but also deactivates enzymes and can gel diced fruit to create preserves. Depending upon the product to be processed, commercial sterilization with pressure can be affected with as little as 3,000 bar to as much as 200,000 bar of pressure. Vegetative microorganisms can be inactivated at pressures as low as 3,000 bar at room temperature.

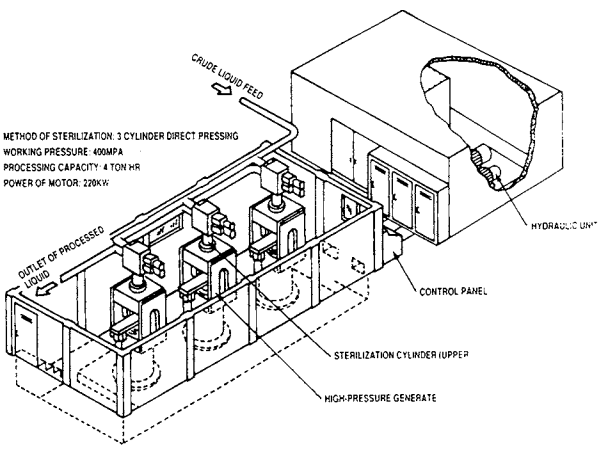

Figure 3. Schematics of high-pressure processing of food. (Courtesy of Yamauchi, Food Eng. Magazine)

The lethal effect is strongly influenced by the food product's composition (especially pH and water activity). Certain proteins, polysaccharides and organic acids can have a protective or synergistic effect on inactivating microbes. Bacterial spores, however, are extremely pressure resistant at room temperature. Research indicates that high pressure coupled with a moderate temperature of 70 to 80°C is very effective (Chapter #9 in this book deals exclusively with high pressure processing) in producing a commercially sterile food (10).

2.4. Corrugated tubular heat exchangers

Tubular heat exchangers have been used for aseptic processing for years to sterilize and cool liquid products such as milk and coffee creamers. It was not until recently that design changes allowed tubular heat exchangers to be used for viscous products and products containing particulate matter.

In the late 60's there was a boom in the number of aseptic processors packaging puddings. All the systems utilized scraped surface heat exchangers to sterilize and aseptically cool the pudding prior to packaging. Scraped surface heat exchangers were used to facilitate the high pressures incurred by other types of heat exchangers and to

prevent protein burn-on in the systems. Aseptic product with particulates was not an issue as there were no aseptic systems for processing particulates until the mid 70's. The first aseptic systems for processing acid products such as fruit for yogurt and ice cream mix also were designed and installed using scraped surface heat exchangers. Scraped surface heat exchangers are expensive, costly to maintain and are not very energy efficient is not feasible to capitalize on regeneration with them.

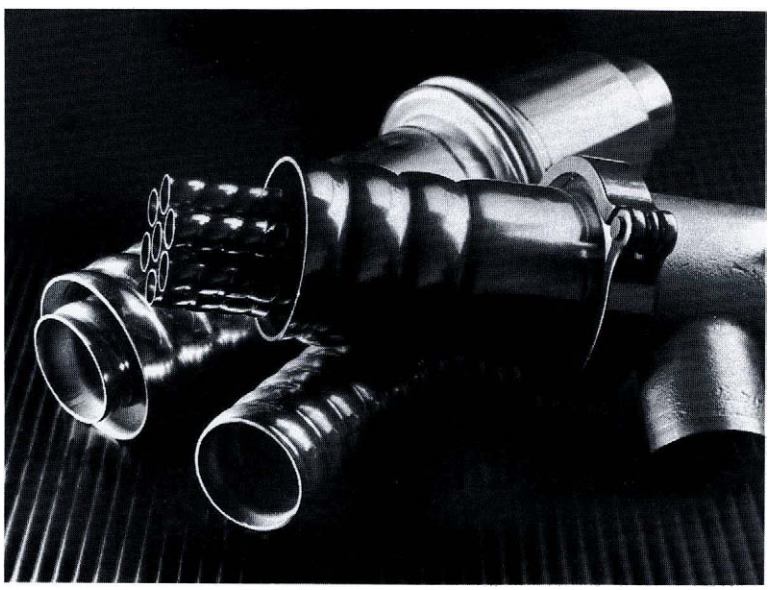

Figure 4. Corrugated tubular heat exchangers (Courtesy of Cherry Burell Corp.)

In an effort to lower the capital and operating costs of aseptic processing systems, improvements were made in tubular heat exchange designs. One of the most significant design changes has been the use of corrugation [11]. Corrugation has allowed higher heat transfer rates, less burn-on resulting in longer production runs, and wider tube design allowing the tubular heat exchangers to be used to aseptically process significant particulate. Temperatures up to 185^{o}C and pressures up to 100 bar can now be attained in aseptic systems with the use of corrugated tubular heat exchangers [12]. These newly designed tubular heat exchangers are now replacing most of the systems using scraped surface heat exchangers that were installed in the 60's and 70's. The new tubular heat exchangers are also being used to aseptically process products such as diced tomatoes (2 centimeters), sliced and diced peaches, sliced strawberries, blueberries, etc. [13]. The corrugated design also facilitates the processing of viscous products containing protein, such as pudding, without the loss of efficiency due to protein burn-on that resulted with older designed tubular heat exchangers. Product-to-product and water-to-water regeneration is easily attained with tubular heat exchangers, thus making them far less costly to operate than scraped surface heat exchangers.

2.5 . Marlen pump

The Marlen reciprocating piston pump is doing more for the growth and renewed interest in the aseptic processing of particulates in the food industry than any other new technology. The inherent design of the Marlen has allowed the largest particulates to be processed. It also has zero slip regardless of viscosity of the product being pumped and it has made the change from initial sterilization water to product extremely simple by the changing of one switch on the control panel.

The operating principle of the hydraulically driven Marlen is unique. It consists of two reciprocating stainless steel pistons. Each of the pistons has a stainless steel sleeve around it. With either water or product in the hopper of the pump, one sleeve loads while the other piston is pumping. During loading the sleeve and piston retract together, filling the cavity with product. The sleeve then moves forward to fill and trap the product. The product is also automatically precompressed to the operating pressure of the system. When the pumping piston finishes its stroke, a valve automatically switches and the other piston starts forward while the first sleeve reloads with product. During the switching of the pistons there is negligible upset in pressure. The Marlen pump comes with either 15 or 18 cm diameter pistons which pump up to 5,442 and 8,164 kgs/hr respectively, at up to 30 bar pressure.

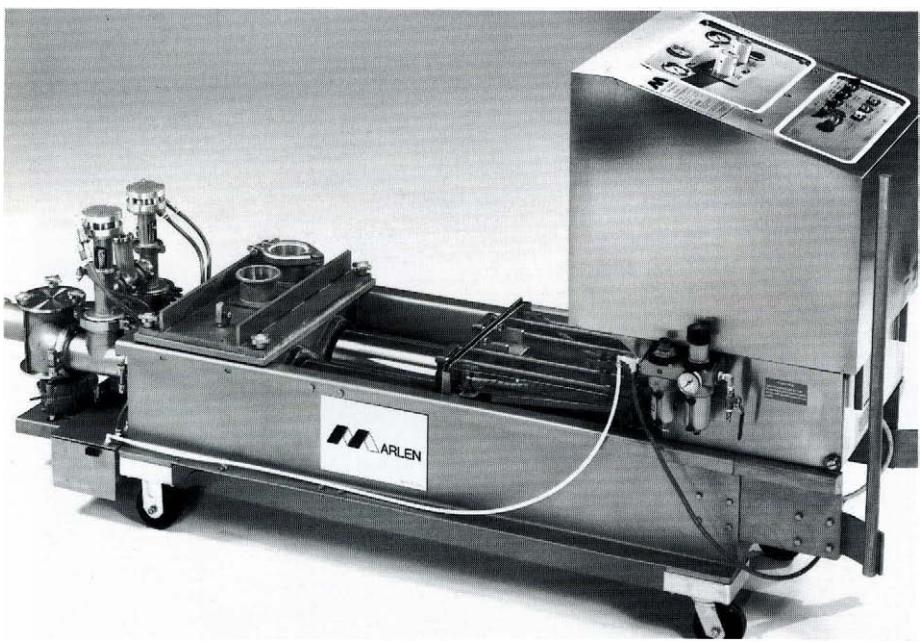

Figure 5. Marlen pump (Courtesy of Marlen Research Corp.)

Another unique feature of the Marlen which facilitates aseptic processing is a sterilization mode in which the sleeves stay sealed shut, but the pistons are in operation. During loading, instead of the sleeve retracting, a valve in the front manifold opens and allows water to fill the sleeve while water is being pumped out the other side. This feature allows the Marlen to be used for pumping the initial sterilization water with zero slip and allows the operator to set production flow rate and temperature with the sterilization water.

Product to be processed is in the hopper of the Marlen while sterilization is taking place. When sterilization is completed and product is ready to be processed, all the operator must do is change one position of a switch on the control panel. On the very next time to load a sleeve the water valve remains shut and the sleeve retracts to load the product. When the other sleeve pushes the last water out, the product immediately follows the water making the switchover extremely easy.

2.6. Combibloc Comitop

PKL Verpackungssysteme, manufacturer of the Combibloc aseptic filler for pre-formed cartons, announced a new technology in a resealable fitment. The fitment is an injection molded, single-piece fitment that is attached to the carton after filling. This allows the carton to be opened, some product poured out, and the carton resealed. Of course, after opening, the remaining product is not shelf-stable and in most cases must be refrigerated.

Figure 5. CombiTop carton Courtesy of Combibloc USA

Trademarked and patented, the ComiTop fitment consists of an easy open clip closure with a sealing tongue, a vent hole, unsealing flap, channels, and a pouring lip. All one has to do is "lift" the lid and "push" on the tab. The internal tab serves as an opener to break the package seal. The resealing is strong enough to sustain stacking, shaking and even being stored on it's side without leaking. It's also strong enough to protect the contents from migration of other flavors.

The ComiTop fitment is applied to the package by downstream equipment after filling and sealing. The carton is specially manufactured to accommodate the ComiTop. The outer polyethylene layer is score-cut with a special tool that leaves the inner layer of aluminum and polyethylene untouched. Sterility and shelf life are not compromised. After scoring, filling, and sealing, the cartons are conveyed to the applicator.

Although not available yet, competitor Tetra Laval advises they will have a reclosable container in the near future. Tetra will start with new technology with the 1 liter size package.

2.7. Aseptic pouches

Within the last several years, two packaging manufacturers have developed and commercialized aseptic pouch fillers. Inpaco, located in Nazareth, Pennsylvania, USA and Robert A. Bosch located in Waiblingen, Germany, introduced aseptic pouch fillers for food products. Bosch's first introduction was for acid food products, whereas Inpaco's first introduction came as a result of building an aseptic pouch filler for pharmaceutical products. Each now has US Food and Drug approval for aseptic packaging of low acid foods.

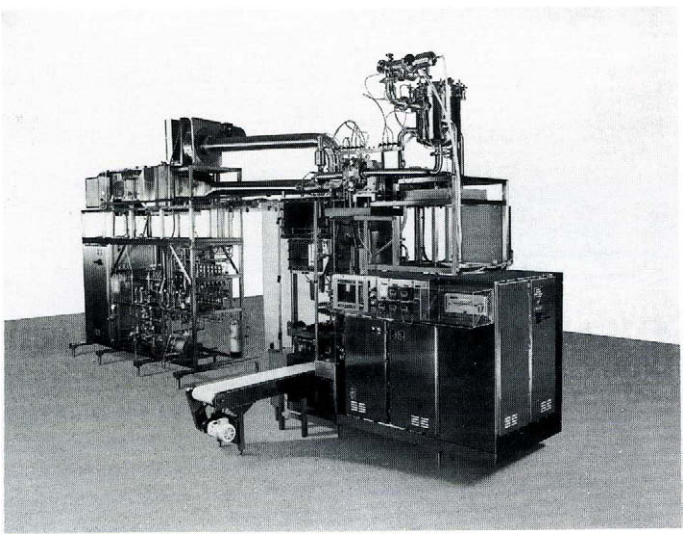

Figure 7. INPACO aseptic pouch filler (Courtesy of INPACO Corp.)

Each of the fillers sterilizes the packaging material with hydrogen peroxide followed by sterile hot air for drying. Filling rates are approximately the same with Inpaco filling at 32 #10 size pouches per minute and Bosch at 24/25 pouches per minute. Each of the fillers can fill from 1 to 5 liter packages.

As the cost of rigid containers continues to rise, it is expected that the demand for aseptic pouch fillers will be the fastest growing segment of the aseptic packaging industry. This market is economically driven, as the cost of #10 (3 liters) sized pouches is 45 to 50% less expensive than a case of #10 cans. With this kind of cost difference economic justification of the cost of the filler is usually in months depending upon production use.

There are other advantages of aseptic pouches that will contribute to the worldwide demand for these filling machines. Some of the other more paramount advantages of flexible pouches compared to #10 cans are:

* Greater safety
* Lower warehousing cost
* Lower shipping costs
* Less space required
* Less residual product loss
* Easier to open
* Disposal savings

2.8. Aseptic ship

One of the newest innovations in aseptics was introduced in 1993 [14]. The Ouro do Brasil is a ship built and dedicated to aseptically transport citrus juices for Citrosuco Paulista of Brazil to Europe, Japan and the United States. The ship was built in Norway with technology that was originally developed at Purdue University in West Lafayette, Indiana, USA.

Figure 8. Aseptic ship (Courtesy of Citrus Coolstores, Inc.)

The ship can aseptically transport single strength juice or concentrate in 16 vertical stainless steel tanks each holding 200,000 gallons. The ceiling, walls and floor in the hold of the ship are all stainless steel. In that, all 3.2 million gallons can be shipped. The Ouro do Brasil is 564 feet long. It is so large that it had to be built in two sections and welded together in the water. The success of this venture has been so great that another ship is being built.

3. CONCLUDING REMARKS

Aseptic processing and packaging is one of the most dynamic areas of food processing. Even before this chapter goes to print, there assuredly will be many more innovations to report as research around the world in aseptic processing will be rewarded with positive results.

There are many reasons for the interest in aseptic processing. Some of the more important include energy savings, enhanced nutritional and organoleptic properties of the food products, the lack of need for preservatives and reduced packaging costs. The microwaveability of most aseptic containers will also contribute to the growth of aseptic packaging.

The number of aseptic systems being installed every year continues to increase. It is very possible that those food processors choosing not to apply aseptic processing techniques to their product whenever they can will be competing with those that do use this method of processing. Most likely, these processors will be competing at a disadvantage.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 11

The use of ionizing radiation in the preservation of food

Wassef W. Nawar

Department of Food Science,
University of Massachusetts, Amherst, MA 01003 U.S.A.

1. INTRODUCTION

The preservation of food is an ancient practice. Man used fire, sun-drying, salting and fermentation to keep his food longer. Today, sophisticated techniques of dehydration, freezing, heat and chemical treatments are established and accepted. Food irradiation is a relatively new revolutionary process with essentially the same objectives as those of the traditional methods of food preservation i.e., extending shelf-life and reducing the incidence of food-borne disease. Obviously, not all methods of preservation are applicable to all foods. Heat treatment, for example, cannot be used to preserve "fresh fruit." Other factors, e.g., cost, complexity, packaging and transportation must be considered in the choice of an appropriate method. Radiation-preservation is intended not to replace but to compliment the existing more conventional methods. It provides a remarkable technological versatility with far reaching implications to the important issues of world hunger, health and disease, and political unrest.

The irradiation treatment involves exposure of the food, under controlled conditions, to high speed electrons or x-rays from machine sources or to radiant energy from gamma rays. The amount of energy absorbed (dose) has been usually measured in rads (1 rad = 100 ergs absorbed per gram of matter). The rad is, however, superseded by the Gray (Gy) in the International System of Units (1 Gy = 1 joule/Kg = 100 rad). Chemical change resulting from radiation can be expressed in terms of the amounts of substrate change or new products formed. The G-value is used to express the number of molecules formed or destroyed per 100 electron volts (ev) of energy absorbed.

Although extensive research over several decades has provided ample evidence for the efficacy and the safety of this process, its widespread acceptance has been hampered by concerns based on emotions, misconceptions and erroneous association with the issues of nuclear reactors, nuclear weapons and radioactivity. It is hoped that the forces of education, public forums and media communications, can join in overcoming this problem. This article is an effort to provide a brief account of the various aspects involved in this new process.

2. ADVANTAGES

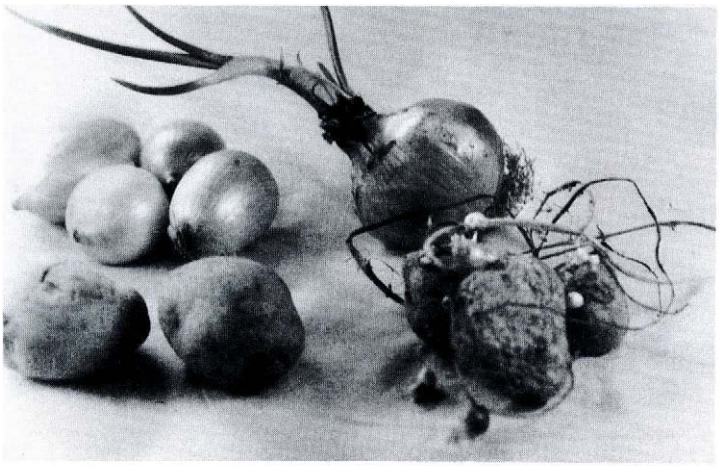
The irradiation treatment is a "cold" process because there is at most only a few degrees temperature rise in foods from the radiation energy absorbed, even at the higher doses used for sterilization. Consequently, radiation treatment causes minimal changes in appearance and provides good nutrition retention. It leaves no chemical residue and thus can replace chemical fumigation and reduce the need for chemical additives. It allows the treatment of products of a wide range of sizes and shapes, e.g., a truckload of produce, large carcasses, thin slices of meat. Food irradiated in flexible packages can be more easily stored and/or transported.

3. APPLICATIONS

As mentioned above, the two major objectives of food irradiation are elimination of pathogenic microorganisms and extension of shelf life. In addition, food irradiation has been effective in a variety of other applications (Table 1). Figures 1-3 show examples of the remarkable effectiveness of the irradiation treatments on the quality of onions, potato and strawberries.

Table 1
Applications of Irradiation Treatment of Food

Application	Food	Dose (kGy)
Control of Pathogens	Poultry, Meat, Seafood	1 - 10
Sprouting Inhibition	Potatoes, Onions, Garlic	0.05 - 0.15
Delay of Ripening	Fruits and Vegetables	0.01 - 1.0
Disinfestation of insects	Grain, Dried Fruit, Dried Fish	0.2 - 1.0
Control of Food-Borne Parasites, e.g., Trichinae	Meat, Fish	0.1 - 1.0
Reduction of Spoilage Microorganisms	Meat, Fish, Fruits, Vegetables, Spices	0.4 - 10.0
Sterilization	Meats, Poultry, Seafood	10 - 50
Improvement in Physical and Technological Properties, e.g., Reduced Cooking times		1 - 10

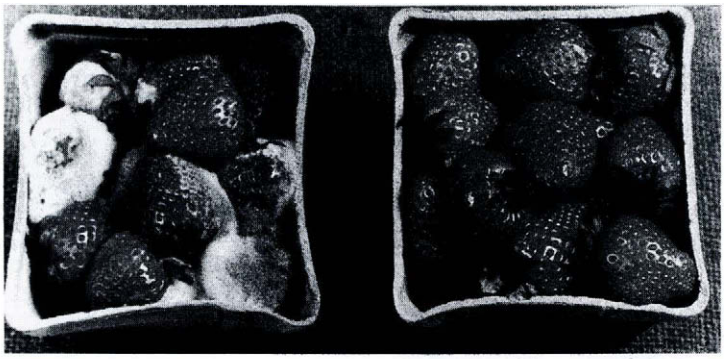


IRRADIATED UNIRRADIATED

Fig. 1. Irradiated potatoes and onions exhibit a longer storage life because of inhibition of sprouting.

IRRADIATED UNIRRADIATED

Fig. 2. Comparison of irradiated potatoes with untreated potatoes after 6 months of storage.



NON-IRRADIATED IRRADIATED (2 kGy)

Fig. 3. Comparison of irradiated strawberries free of visible mold spoilage with untreated strawberries after 15 days of storage at 2°C.

4. IRRADIATION SOURCES AND FACILITIES

Two types of radiation sources are used in food irradiation, radionuclide and machine radiation. Radionuclides are radioactive elements which upon decay release deeply penetrating gamma rays. Cobalt-60, produced by exposing Cobalt-59 to neutrons, and cesium-137, a by-product of nuclear reactors, are the only radionuclides permitted in food processing. However,cesium-137 is less widely used because of its limited availability. The machine sources are electron beam generators or electron accelerators and x-ray machines. In an electron accelerator, a hot filament emits electrons into an evacuated chamber. The electrons are attracted by a positive electrode and focused into a narrow beam. The high-energy electrons penetrate the "window", a very thin metal foil, which scatters the beam focus allowing a more uniform exposure of the food surface. In contrast to gamma rays, accelerated electrons have a limited penetrating capability, less than 8 cm and are therefore only useful for the treatment of foods which can be processed in thin layers e.g., grain and thin slices of meat. X-rays are generated by high speed electrons from accelerators, which are made to hit a metal target causing it to emit x-rays. Like gamma rays, x-rays are deeply penetrating. However, their use in food processing is more expensive and impractical. Recent advances indicate that new types of x-ray generators may be developed which would be better suited for food irradiation.

Both radionuclide and machine sources must be provided with adequate shielding to protect personnel. In case of radionuclide sources, the source is usually stored in a deep pool of water, and raised above the water during irradiation of food.

The irradiation treatment can be conducted in either a batch or continuous mode. The batch method is simpler to design, easier to operate and is more flexible. The continuous operation is more suitable for large volumes. It may involve a single-pass or a multiple pattern, the design of which allows a more uniform exposure of the food and a more efficient

use of the source. Mobile irradiators are also available. These are compact units which are best suited for certain foods where season, location, transport and interval between harvest and processing, may be limiting factors, e.g., seafoods.

5. GENERAL MECHANISM

Ions and excited molecules are the first species formed when ionizing radiation is absorbed by matter. A high-energy charged particle loses energy, in a series of small steps, by electric interaction with electrons in the absorbing material. Such interaction involves a large number of atoms or molecules, distributed along the particle's track, raising them to excited levels. If these levels are above the ionization potential of the atom or molecule, ions are formed. The positive charge may be distributed over a part of the molecule or localized in one atom or group of atoms. If the excited levels are below the ionization potential, excited atoms or molecules are produced. Excited molecules could also result indirectly by neutralization of the ions formed. The protons of electromagnetic radiation, unlike charged particles, tend to lose a large amount of energy upon interaction with matter and the ionization or excitation occurs largely via secondary electrons. The primary effect of a single gamma ray, for example, may be to produce a 1 Mev electron (and a positive ion). Such an electron may produce 30,000 - 40,000 additional ionization processes and 45,000 - 80,000 excitations. The secondary electrons produced in this process are called "δ-rays" when they have sufficient energy (100 ev) to produce further ionizations. Only about 5% of the primary fast-electron interactions result in δ-rays, but δ-rays cause about half the total ionization from fast electrons. Primary processes, which result in the formation of ions and excited molecules, occur in the first 10^{-14} sec on the time scale for events in radiation chemistry.

Chemical breakdown is brought about by the decomposition of these primary species (excited molecules and ions), or by their reaction with neighboring molecules. The free radicals formed by the dissociation of excited molecules and by ion reactions are largely responsible for the observed chemical changes and generally dominate the mechanisms postulated for the formation, upon irradiation, of stable radiolytic products. They may combine with each other in regions of high radical concentrations, or may diffuse into the bulk of the medium and react with other molecules.

The ability of ionizing energy to break certain bonds in organic molecules is the reason behind its special value. The splitting of critical bonds in microorganisms or other pests will result in their death or dysfunction.

6. EFFECTS ON FOOD COMPONENTS

Radiation chemistry of the major food components has been reviewed in great detail in the literature (Diehl, 1982; Dauphin and St. Lebe, 1977; Delincee, 1983a, b; Nawar, 1977). Only a brief summary is given here.

6.1. Water

In food, or parts of food containing little water, direct action of the radiation on the organic molecules is the major source of chemical change. In many foods, however, water is the major constituent. Since fast electrons interact indiscriminately with molecules along their track, much more excitations and ionizations of the water molecules will result than of the other components. The products formed in pure water and dilute aqueous solutions by irradiation can be summarized in the following general equation:

$$H_2O \longrightarrow 2.7\ {}^{\cdot}OH + 2.7\ e^-_{aq} + 0.55\ {}^{\cdot}H + 0.45\ H_2 + 0.71\ H_2O_2 + 2.7\ H_3O^+$$

The primary radiolysis products of water disappear in fractions of a second by reacting with each other or with other food components. The hydroxyl radical, ·OH, is a powerful oxidizing agent. It can add to aromatic and olefinic compounds and abstract hydrogen atoms from carbon-hydrogen and sulfur-hydrogen bonds. The hydrated electron, e^-, is also highly reactive. It adds rapidly to most aromatic compounds, carboxylic acids, ketones, aldehydes and thiols. H_2O_2 is formed by recombination but in the absence of oxygen its formation is extremely low. Hydrogen atoms can abstract hydrogen from C-H bonds or add to olefinic compounds. They are produced in a relatively low yield.

6.2. Proteins

The radiolysis of proteins can be largely ascribed to reactions of their constituent amino acids, with the sulfur and aromatic amino acids being the most sensitive. In addition, the structural and conformational features of the proteins influence their response to radiation. Reactions of the electrons lead to deamination, reduction of disulfide and peptide linkages, addition to aromatic groups (Delincee, 1983a; Garrison, 1981; Taub, et al 1979). The OH radical reacts readily with aromatic heterocyclic and sulfur-containing residues. Volatile products resulting from radiolytic decomposition of protein include ammonia, fatty acids, ketoacids, aromatic compounds, amides and mercaptans. Sulfur-containing compounds are important because of their off-flavor characteristics. In addition to degradation, the protein may undergo unfolding and aggregation, particularly in the case of globular proteins.

In cases where meats are irradiated at sub-freezing temperatures, the radiolytic effects on the proteins are generally very small. At -40° C, the proteins are fixed in a rigid matrix and indirect effects by radical diffusion are minimized. Radicals from direct effects undergo recombination reactions with little or no degradation or aggregation detected. Consequently, under such conditions, changes in amino acid profiles are found to be negligible and the amount of volatile compounds produced markedly reduced. Very little structural alterations occur in the protein molecules and no free radicals persist in the meat.

The effects of irradiation on enzymes is responsible for certain distortions of physiological processes during the storage of irradiated fruits and vegetables. The doses normally used for the irradiation of meat are not sufficient to inactivate enzymes which cause autolysis during storage. Heat inactivation before irradiation is thus necessary for long-term stability.

6.3. Carbohydrates

Although the major products formed by irradiation in many pure sugars and saccharides have been studied (Dauphin and St. Lebe, 1977), little research has been conducted on the radiolytic products derived from the carbohydrate portion in complex foodstuffs. However, reaction mechanisms in simple bound sugars are similar to those in the more complicated polymeric materials containing these subunits.

In aqueous systems, radiolysis of carbohydrates occurs mainly by indirect action of OH radicals which react primarily with C-H bonds. The carbohydrate radicals thus formed react further by dismutation, dimerization and dehydration. The deoxycarbonyl radical can undergo further reactions of dimerization, dismutation and saturation.

Radiolytic products of glucose, for example, include gluconic acid, glucuronic acid, D-glucono-1,5-lactone, saccharic acid, D-arabinose, D-xylose, D-erythrose, glyoxal, dihydroxyacetone, formol and H_2O_2, if irradiated in oxygen atmosphere, and deoxygluconic acid, erythritol, deoxymannitol, deoxyarabinohexitol, deoxyribohexitol, and D-mannitol, if irradiated under nitrogen.

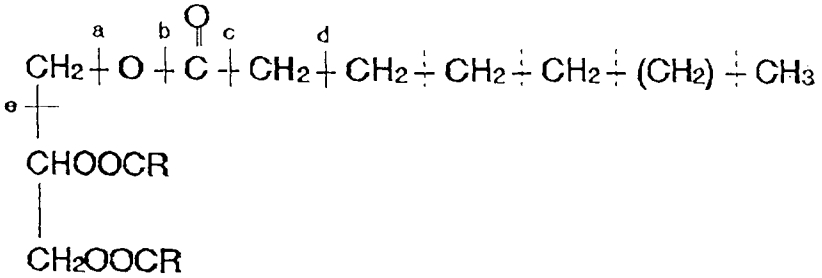
Polysaccharides such as starch, cellulose, pectin, and glycogen undergo cleavage of the glycosidic linkage producing lower molecular weight fractions such as glucose, maltose and dextrin. Further decomposition gives rise to radiolytic products of smaller molecular weights including formic, gluconic, acetic, glyoxylic, pyruvic, malic and oxalic acids, acetone, acetaldehyde, malonaldehyde, glyoxal, glyceraldehyde, dihydroxyacetone, hydroxymalonaldehyde, furfural, hydroxymethylfurfural, diacetyl, acetoin, methyl formate and methyl alcohol.

Detailed quantitative data for the products from various sugars and saccharides irradiated under different conditions can be found in the comprehensive review by Dauphin and Saint-Lebe (1977). Obviously, the amounts of radiolytic products from carbohydrates vary with composition, dose, and irradiation parameters. When corn starch, with a water content of 12-13%, was irradiated under O_2, the concentration of the malondialdehyde produced was 0.2 μg/g/kGy. The concentrations of formol, acetaldehyde, acetone, glyoxal, methyl alcohol, glucose, and ribose were 2, 4, 0.21, 0.35, 0.28, 0.58 and 0.06 μg/g/kGy, respectively. The presence of other nutrients in complex foods, e.g., proteins and lipids, are known to provide protection against radiation damage in carbohydrates. Diehl (1982) calculated that in a model food consisting of 80% water and 6.6% each of carbohydrates, proteins and fats, the maximum concentration of the total products produced from the carbohydrate component by irradiation at 5 kGy would be 0.5 mg/100g.

Although the possible cytotoxic effects of malondialdehyde have received particular attention, the yields of this product are minimal under the normal pH encountered in foods.

6.4. Lipids

Fatty-acid-containing compounds typically undergo preferential cleavage at locations in the vicinity of the carbonyl group and randomly at the other carbon-carbon bonds (Delincee, 1983b, Nawar, 1977). Primary ionization in oxygen-containing compounds involves the loss



of a non-bonding electron with the result that the unpaired electron is highly localized on the oxygen. Preferential bond cleavages near the carbonyl group are facilitated by the tendency of the oxygen atom to complete its valence shell of electrons. Thus, in a triacylglycerol molecule-ion, preferential cleavages (solid lines) result in the formation of alkyl, acyl, acyloxy, and acyloxymethylene free radicals and, in addition, the free radicals representing the corresponding glyceryl residues. Free radical termination may occur by hydrogen abstraction, and to a lesser degree by loss of hydrogen with the formation of an unsaturated linkage. Thus, depending on fatty acid composition, specific compounds of smaller molecular weight than their substrate, e.g., hydrocarbons, aldehydes, esters, are produced.

Alternatively, the free radicals may recombine, giving rise to a variety of radiolytic products of longer-chain or dimeric nature. Table 2 provides a list of radiolytic compounds identified in model systems of triacylglycerols. Most of these compounds were also identified in irradiated meats.

Irradiation and subsequent storage in the presence of oxygen accelerate lipid oxidation probably by enhancing the formation of free radicals which can combine with oxygen, the breakdown of the hydroperoxides and/or the destruction of antioxidants. The products thus formed are identical to those usually present in unirradiated but oxidized lipids.

6.5. Vitamins

The radiation stability of vitamins has been studied and reviewed (Tobback, 1977; Basson, 1983). Much information on the radiosensitivity of individual vitamins has been obtained from experiments with pure model systems in simple solutions. In foods, however, the effects of irradiation depend, to a large extent, on a number of factors as for example composition of the food and its physical structure, water activity, temperature, gas atmosphere, etc.

In general, vitamin B_1 has been reported to be the most radiation-sensitive among the water-soluble vitamins. Of the fat-soluble vitamins, vitamin E is recognized as the most sensitive. Whole milk irradiated at approximately 1.5 kGy caused a loss of 35% in vitamin B_1 but no losses were observed in the thiamin content of milk powder irradiated at doses 28 and 56 kGy. Thiamin loss of 20% in wheat flour irradiated at 0.35 kGy and 35% in oat flakes irradiated at 0.25 kGy, were reported. No significant loss of vitamin B_1 was found in egg powder irradiated at doses below 5 kGy and stored for 15 months. The observation that vitamin B_1 exhibits much more resistance to radiation when present in food has been

Table 2
Radiolytic Products of Triacylglycerols

A. Breakdown products of smaller molecular weight than substrate

CO_2, CO, H_2	Propanediol diesters	Carboxylic acids
n-Alkanes	Propenediol diesters	2-Alkylcyclobutanones
1-Alkenes	Oxopropanediol diesters	Monoacylglycerols
Alkadienes	Aldehydes	Diacylglycerols
Alkynes	Ketones	Triacylglycerols
Esters	Lactones	Ethanediol diesters

B. Adduct Products

Glyceryl ether diesters

Propanedioldiester dimers

Propanedioldiester-triacylglycerol adducts

Triacylglycerol dimers

attributed to the fact that it is usually bound to proteins which protect prosthetic groups against radiation. The sensitivity of vitamin C to irradiation treatment is believed to be similar to its sensitivity to heating or oxidation. Under practical conditions of irradiation, losses rarely exceed 30%. Niacin is known to be very resistant to radiation.

The radiosensitivity of vitamin E is influenced by its initial concentration and varies markedly from one food to another. It can be significantly minimized by exclusion of oxygen. Vitamin D in food is relatively stable to irradiation.

Although some reports concerning the degradation products of some vitamins are available, the characterization of these compounds and their significance in irradiated food have not been fully explored. Since some of these products may play a significant role with regard to food quality and wholesomeness, this aspect merits further research.

6.6. Complex Foods

In general, the products formed by irradiation in a complex food such as meat are qualitatively the same as those which arise from the radiolysis of its constituent components, i.e., lipids, proteins, and carbohydrates. However, there is a quantitative difference. While dilute solutions of pure sugars, amino acids, vitamins, and enzymes are relatively sensitive to radiation, these compounds are more stable when exposed as constituents of food. This is attributed to the distribution of radiation damage among the many constituents in food and to the protective effects of certain constituents. For example, amino acids were found to protect trehalose against radiolytic decomposition. This phenomenon was explained by their ·OH scavenging properties and/or their ability to act as hydrogen donors (Adam, 1977 a,b; Diehl, et al 1975, 1978; Dizdaroglu, et al 1977; Taub, et al 1979).

6.7. Comparison with Heat

Heat processing of food is an accepted practice. The energy absorbed by food when exposed to irradiation at legal doses is much less than that absorbed when the food is heated. For example, an absorbed dose of 1 kGy would increase the temperature of aqueous foods by 0.24° C.

It is not surprising, therefore, that for most practical applications, the chemical and physical changes which occur in food by irradiation are often less than those observed by cooking, frying, canning, baking, etc. (Fig. 4).

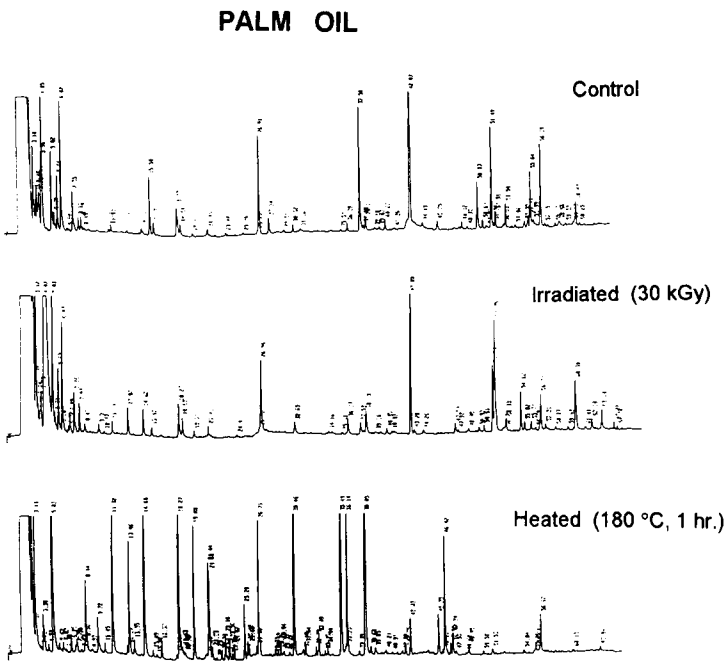


Fig. 4. Comparison of the Volatile Compounds Generated from Palm Oil by Heat and Irradiation as Analyzed by Gas Chromatography.

7. Methods of Identifying Irradiation in Foods

Reliable methodology for the detection of irradiation treatment in food is important to enforce compliance, enhance consumer confidence and facilitate international trade. A good detection method should meet certain criteria. These include specificity, reproducibility, sensitivity, accuracy, discrimination, dose dependence, simplicity, speed of measurement and low cost.

A number of methods based on the measurement of chemical, physical or biological changes which can be detected in irradiated foods have been studied (Anon, 1991a). To date no single method is available which can be universally applied to all foods. This is to be expected since most of the detectable effects are often extremely small and are not necessarily unique to the irradiation treatment. Radiolytic changes in food are influenced by other factors, e.g., food composition and physical structure and processing parameters. Another difficultly is caused by the lack of uniformity among agricultural products and their handling practices.

At present three techniques, the lipid-derived volatiles, electron spin resonance and thermoluminescence, have reached the stage of practical application. At various stages of development are several other detection methods. Only a brief listing of these and/or the principles upon which they are based, is given here.

7.1. Physical Methods

7.1.1. Electron Spin Resonance (ESR)

This method involves the detection by ESR of paramagnetic centers produced by irradiation in rigid matrices, e.g., bones, shells, seeds (Derosiers, 1989; Derosiers and McLaughlin, 1989; Gray, 1995; Gray and Stevenson, 1989; Raffi and Angel, 1989). The resulting asymmetric signal is believed to be derived from the hydroxyapatite mineralized component of bone and is different from the endogenous symmetric signal commonly detected in the unirradiated product and is assumed to originate from the organic component of the bone tissue.

In addition to whole bones of fresh and frozen meats, the method is applicable to sub-millimeter bone fragments recovered from mechanically deboned meat; fish bones, teeth, fins and scales; and the exoskeleton of shrimp, lobster and crab. The ESR method can also be applied to spices, and dry fruits and vegetables in which case the free radicals derived from cellulose or sugar are measured, and to egg shells where ESR signals have been attributed to carbonate ion radicals.

The ESR signal is specific, stable and dose-related. The measurement is sensitive, rapid, and non-destructive. However, it requires specialized analysts and relatively expensive instrumentation.

7.1.2. Thermoluminescence (TL)

The TL method is based on the emission of light when energy trapped in crystalline lattices during the irradation is released by heat. The measurement of a TL curve is simple and rapid. It is mostly applicable to spices and dry foods. Several investigations, however, confirmed that the principal origin of the signal is the minerals adhering to the food (Sanderson, et al 1989). Techniques have been described to improve the reproducibility and discriminating power of the method by TL measurement of these minerals after their isolation from the irradiated food.

Thermoluminescence analysis has been performed on the minerals isolated from the intestine of shrimp and silicates extracted from fruits and vegetables.

More recently photostimulated luminescene techniques (PSL), in which the energy to release trapped charge carriers is provided optically, were developed. This is highly radiation specific since energy conservation principles require that the quantum energy differences between stimulation and luminescence are balanced by energy stored in the form of trapped charge carriers. The method can be used for fast screening of irradiated spice and shrimp products. A PSL instrument is now commercially available and can be easily used by non-scientific personnel.

7.1.3. Impedance

The ratio of magnitude and phase angle of electrical impedance at two different frequencies can be used as parameters for the detection of irradiated potatoes (Hayashi, et al 1982). The method is only applicable to fresh potato tubers.

7.1.4. Viscosity

The viscosity of some spices is lowered by irradiation. Although irradiated black and white pepper were clearly distinguishable from unirradiated controls, the applicability of the method for other spices requires more study (Farkas, 1987; Farkas, et al 1990).

7.1.5. Near Infrared Analysis

The near infrared spectrum of spices is influenced by irradiation. However, in some cases the effects of storage on the spectra were significant. The applications of the method have been studied with black and white pepper, paprika, allspice and cinnamon (Barbassy, et al 1992).

7.2. Chemical Methods

7.2.1. The Lipid-Derived Volatile Method

This method relies on the measurement of "key" volatile radiolytic compounds which result form the preferential cleavage in the carbonyl region of fatty acids (Nawar and Balboni, 1970; Nawar, et al 1990). In particular, two hydrocarbons from each fatty acid, are especially useful as markers. One has one carbon atom less, and the other has two carbons less than the substrate fatty acid and an extra double bond. The formation of these compounds increases linearly with dose. The technique involves solvent extraction of the lipids,

collection of the volatiles and quantitative measurement of the hydrocarbons by gas chromatography.

The reliability of this method was extensively studied with chicken, beef and pork with excellent results (Nawar, et al 1990). Its application to seafood, spices, egg powder and other lipid-containing foods is promising (Anon, 1994).

7.2.2. Ortho-Tyrosine

This method is based on the measurement of o-tyrosine produced by irradiation via hydroxyl radical attack on the phenylalanine component of proteins. Since o-tyrosine has been found in the fluid, but not in the fiber of unirradiated meat, measurement of o-tyrosine in the water insoluble fraction, i.e., muscle fiber, can be used to detect irradiation treatment (Karam and Simic, 1988; Meir, et al 1988). In meats, improvements in the separation and measurement of o-tyrosine by GC, HPLC have been recently described.

7.2.3. DNA

Various techniques based on DNA fragmentation, or modifications of pyrimidine or purine bases and sugar moieties are being developed and tested. These techniques include the use of conventional agarose electrophoresis of mitochondrial DNA, micro-gel eletrophoresis (Delincee, 1993) and the development of antibodies to dihydrothymine, a reductive radiolytic product of thymine. Application of these techniques has been tested on meat, fish, fruits and vegetables (Anon. 1994).

7.2.4. Gas Evolution

Some of the hydrogen and carbon monoxide produced by irradiation is trapped in frozen meats and dry grains. The gases can be released by heating and detected by gas chromatography or other gas sensors (Dohmaru, et al 1989). Research is needed to assess the effect of storage on the applicability of the method to specific foods.

7.3. Biological Methods

7.3.1. Shift in Microbiological Composition

Irradiation de-activates microorganisms but does not affect their ability to be stained. The difference between the viable bacterial count, obtained by conventional aerobic plating (APC), and the total count (dead and alive), obtained by the direct epifluorescent filter technique (DEF) or by Limulus Amoebocyte Lysate test, indicates the number of organisms deactivated by irradiation.

The method has been applied to the detection of irradiation in spices (Betts, et al 1988, Anon, 1994). Conclusive evidence of irradiation treatment, however, can not be obtained if the spice has been treated by fumigation or heat.

7.3.2. Half-Embryo Test

This test is based on the inhibiting effect of irradiation on the ability of seeds to germinate (Kawamura, et al 1989).

The shell of the seed is removed and half-embryos are incubated at optimum conditions of temperatures and humidity. Samples are judged as irradiated if shooting is less than 40%. The method is applicable to citrus fruit, apples and cherries.

7.3.3. Immunochemical Detection

Fragments resulting from radiolytic decomposition of egg white proteins can be separated and detected by immunoblotting using specific antibodies. (Bugyaki and van der Strichelon-Rogier, 1970).

8. SCOPE FOR FUTURE RESEARCH

The past 20 years have seen a significant increase in our understanding of the chemical, physical and biological effects of ionizing radiation on food and methodology of irradiation detection, as well as remarkable advances in industrial irradation technology, design of food irradiation plants, dosimetry, and commercial applications. Today the use ionizing energy in the processing of various food items is approved. That the irradiation treatment is a major food preservation method of great potential is quite clear. However, major research efforts are needed to find the combinations of parameters required to maximize achievement of the desired objectives while minimizing unwanted side effects, e.g., softening of certain fresh fruits and vegetables, discoloration, off-flavors. Combinations of irradiation with heat treatment or with the addition of certain additives, e.g., antioxidants, should be explored further.

Much research is also needed in the area of new applications, e.g., increasing the rate of drying of fruits or hydration of dehydrated vegetables, increasing the yield of juice from grapes for wine making, reducing cooking time of certain products, tenderizing beef.

In this writer's opinion our greatest future attention must be in the area of education and communication. Correct and reliable information, free of political pressure or competitive fears, must be disseminated to enable the consumer to make informed, independent choices and industry to make responsible decisions.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 12

Emerging-Freezing Technologies

Celso O. Bejarano Wallens
Kraft General Foods, Technology Center, 801 Waukegan Rd., Glenview, Illinois 60061, USA

Jim Venetucci
Liquid Carbonic, Freezing Systems Division, 135 South LaSalle Street, Chicago, Illinois 60603-4282, USA

1. INTRODUCTION

The main objective of this chapter is to describe the evolution of the Food Freezing Processing Industry, its equipment improvement, and the review of freezing methods, technologies and concepts used in the Frozen Food Industry. The "Emerging Freezing Technologies" chapter is developed in two parts. The first part is a general overview of freezing methods and terminology used in this industry. The second part is focused on the description of new, evolving technology concepts and its applications.

The depletion of the ozone layer in the atmosphere caused by the use of chlorofluorocarbons, CFCs, and the concern for the world's environment, together with the high costs involved in freezing, high energy consumption, finished product quality, consumer convenience and other factors initiated the frozen food industry to investigate new alternatives and opportunities to improve the quality of frozen products, equipment and manufacturing procedures and efficiencies.

The twenty-first century for the Frozen Food Processing Industry will open new opportunities and challenges to the food freezing equipment manufacturers, product development scientists and engineers that will be based on the following points:

- Improved finished product quality and product development
- Environmental concerns/acceptance and safety
- Reduce processing costs
- Technological competitive advantage among food processors
- Maximum equipment efficiency and flexibility
- Energy efficient systems, (CIP systems, automated computer control systems, self defrosting, etc.)
- Human skills' level
- Finished product distribution and storage
- Consumer acceptance

2. FREEZING TECHNOLOGY: AN OVERVIEW

Many factors are involved in the process of freezing food products that determine and affect the finished product quality and the freezing equipment performance. To achieve the desired freezing results, it is necessary to have the entire product mass at the same temperature throughout. This equilibrium condition in which the surface temperature and the center of the food mass are the same is dependent upon the freezing rate, heat transfer coefficient and the amount of heat removed from the food product.

This change is very important and it is directly related to ice crystallization, loss of moisture and microbial growth which becomes the basis for determining the quality of the frozen product. Some of the factors are:

- Freezing methods
- Equipment
- Product ice crystallization
- Freezer burn
- Freezing rate
- Specific heat
- Heat transfer coefficients
- Packaging
- Moisture losses and content
- Finished food product components and product shape

The freezing process time (dwell) is proportional to the freezing rate, packaging prior to freezing, the freezing method used, incoming product temperature to the freezer, final temperature, food product shape, specific heat, thickness and the food product components. The amount of heat removed and the rate at which it is removed by cooling depends on the chemical composition of each component. Larger amounts of sugars, salt and/or alcohol require longer freezing times and/or lower freezing temperatures. The finished product freezing point is related to the food components' molecular weights, their chemical structure and composition and the food product's low vapor pressure. It is known, that the lower the vapor pressure of sauces, liquid blends or solutions, caused by the dissolved components, the lower the freezing point. (Raoult's second law) (1). Large ice crystal formation and finished product damage could be reduced depending on the type of freezing system used, its efficiency, and freezing residence time. A simple definition of a freezing process is that it consists of lowering the product temperature to -18ºC (0ºF), a generally accepted industry target temperature, at the thermal center of the product, resulting in crystallization of most of the water and some solutes (2).

The freezing rate is defined by the International Institute of Refrigeration as the difference between the product initial and final temperature divided by the freezing time (2). Currently, several numerical mathematical model studies exist on freezing processes for foodstuffs. Freezing time, rate of cooling, heat transfer methods, temperature distribution in regular and/or irregular food products are very important factors used in the design of freezers and freezing processes. Several assumptions are considered when developing a mathematical model that includes irregular shape, chemical composition, heat transfer coefficient and the type of freezing media to be used. In this chapter, no consideration to mathematical modeling for freezer and freezing foodstuffs will be covered. A few references (3, 4) of numerical mathematical modeling are recommended in the bibliography. The freezing methods can be classified depending on their refrigeration medium, processing system (batch or continuous system) and the heat transfer methods that are used. To simplify the description of the freezing methods, we will classify them regardless of their refrigeration medium and heat transfer method, as follows:

a. Mechanical methods
b. Cryogenic methods and
c. Combination of mechanical and cryogenic methods

2.1. Mechanical Freezing Methods

Worldwide environmental concerns have been raised regarding the use of CFC's products as cooling media in refrigeration systems. ***Due to the World environmental concerns, the Montreal Protocol and the EPA regulations, the use of chlorofluorocarbons (CFCs) will be banned by the year 2000.*** Consequently, it will no longer be used for refrigeration. An alternative for most of the mechanical freezers that used CFCs as a refrigerant may be to change to ammonia or to Hydrofluorocarbons (HFCs).

Currently various industries that use CFCs in their refrigeration systems are converting to ammonia. Ammonia is less expensive and its performance coefficient is better than other available refrigerants (5). Examples of the ammonia refrigeration system are water chiller generation, static ice storage system, ice-makers, and brine-chilling system (6). These refrigeration methods currently utilize ammonia, CFC's, air and brine solutions as a coolant medium.

The heat transfer method used in mechanical freezers is convection heat transfer. In freezing food products, a few factors influence the identification of the freezing method and equipment. Some of the factors to consider in choosing the right freezing method and equipment are: product dimensions, shape, specific heat, thickness, freezing rate, packaging and food components' chemical characteristics. These mechanical freezing equipments are produced by several US and International freezer manufacturing companies (7).

2.1.1. Blast Freezer. One of the oldest and most commonly used methods in the food industry to freeze food products is the blast freezer. The blast freezer consists of a insulated room in which high velocity air moves over cooled refrigerated coils and then the cold air is circulated in the room by fans which use convection heat transfer to freeze the product. The refrigeration medium used in this type of equipment can be ammonia or CFC's and the heat transfer method used is convection heat transfer.

Food products and materials can be frozen in batches or in trays. The blast freezing process is slow, with high moisture loss and large ice crystal formation. The freezing temperature generally is about -40ºF (-40ºC). The blast freezer is one of the most flexible methods used to freeze food products and it has been improved by increasing the cold air velocity.

2.1.2. Plate Freezer. Plate freezing equipment was invented by Clarence Birdseye (8). This method is still used in the frozen food industry. Major equipment improvements had occurred in this type of freezing method that have transformed a batch system into a continuous automatic freezing system. The plate freezer system can be classified as single or double plate freezers. Initially, food products were placed on the refrigerated plate and the heat transfer was transmitted from the bottom-up. The heat transfer principle used in this type of freezer is conduction heat transfer. Technology, energy concerns, freezing time and other factors forced the plate freezer manufacturing industry to develop and improve this freezing method.

The double-contact plate freezer ***sandwiched*** the food product between the two refrigerated plates, consequently, it accelerated the freezing process. Currently, automatic, continuous plate freezers with improved heat transfer capabilities and reduced energy consumption can be obtained in the freezing equipment market. Another type is the vertical plate freezer (9). The refrigerant medium used is ammonia and the refrigeration temperature could reach -40ºF, (-40ºC),. This type of freezer is mainly used in freezing flat package of foods, ice cream, whole fish and packaged vegetables.

The finished product moisture losses are minimum because the food product is usually frozen inside of the package. One disadvantage of this method is the layer of air that remain inside the package, which causes a longer freezing time.

2.1.3. Spiral Freezer. This mechanical freezing equipment is one of the most currently used in the freezing industry for large production needs because of its convenience, reduced floor space, flexibility and efficiency. The spiral freezer consists of a continuous moving stainless steel belt in spiral form that carries the food product to different heights within the chamber allowing the product to freeze very fast due to the high air velocity.

The spiral freezer is a versatile and flexible method that is used to freeze a wide variety of food products such as: chicken, fish, meat, pizza, bread-dough, bagels, prepared and processed foods on open trays or packaged containers. The heat transfer method used is convection heat transfer. The refrigeration medium is ammonia and the spiral freezer could be designed and equipped with a horizontal or vertical air flow circulation. Variable speed drive motors provide freezing flexibility and allows the freezer to be used for various food applications that require different dwell times.

Finished product moisture losses for cryogenic spirals could be less than 1% depending on the freezing time (dwell), product components and incoming product temperature, and the design of the spiral freezer. Higher moisture losses above 2% can be expected with mechanical spirals due to warmer operating temperatures.

2.1.4. Air Impingement Blast Freezer. The air impingement freezer is a type of blast-tunnel freezer that blast cold air at higher velocities directly on top and bottom of the foods. The high air velocity applied directly on the food breaks the insulating boundary air layer that covers the product, thereby allowing a very fast freezing (10).

This freezing technology is based on the maximization of the surface heat transfer coefficient and the high cold air velocity blasted on the food product. Dwell freezing time is reduced and is dependent upon the food product thickness and its mass surface area for heat convection. Surface moisture losses are low and freezing times are reduced. (This method is energy and cost efficient). This freezing method could freeze food products faster than any other mechanical method. The heat transfer method used is the convection heat transfer. The refrigeration medium is usually ammonia.

The air impingement freezing method is a versatile method used to freeze different types of food products such as: chicken, fish, meat, bread-dough, and compact processed foods which do not have small surface particles or toppings on open trays or packaging containers. Variable speed drive motors allow the freezer to be used for various applications. It is most commonly used for freezing whole or large particles of foods such as: Corn, meat patties, fruits, hamburger, vegetables, sea food, whole chicken and large pieces of meat, etc.

2.1.5. Belt Freezer. The belt freezer is a modification of a tunnel/blast freezer that consists of a long solid stainless steel moving conveyor belt, two large diameter drum wheels move the belt and a few pans are located under the belt. Cold brine solution -40ºF, (-40ºC), is continually sprayed under the belt and the brine solution is then recirculated back to the ammonia refrigeration system. To increase the system efficiency and capacity, cold air or a spray of cryogenic gas could be used to add more refrigeration. The cold air or gas moves at high velocity in the opposite direction of the moving food. The liquid or solid food product is deposited on top of the belt and its is frozen by conduction-convection heat transfer.

The liquid product is frozen from the bottom and top. The frozen product is scraped from the belt by mechanical action, or the frozen product pops-up by itself because of its frozen-brittle state, releasing it from the belt surface as the belt begins to turn. The belt freezer system is versatile and cost efficient depending on its application. This system could also be used for solid product such as: meat patties, shrimp, hamburgers, vegetables, pizza and semisolid food products.

2.1.6. Fluidized Bed Freezer. This freezing method uses the air blast concept to individually quick freeze, IQF, food products that are diced, loose or have a consistent shape and size. The system consists of a perforated bed in which cold air at high velocities is circulated and forced by the action of powerful fans through the holes in the perforated bed. Fresh or blanched food product is fed continually into a vibratory conveyor that releases the

product into the fluid bed. The high air velocities, perforated bed, and the cold air allow the product to be kept in suspension and freeze the food product rapidly.

The turbulence and the agitation generated help to improve the heat transfer coefficient and reduces the overall freezing time. The suspended product continually moves along the fluidized bed. Convection heat transfer is applied in this freezing method. The production of frozen product varies depending on the air velocity, food thickness, food layer on the perforated fluidized bed, and the food product density. This is one of the best methods to IQF small food particles and vegetables and it is commonly used to freeze peas, corn kernels, small onions, diced food products, and other vegetables. The refrigeration medium is ammonia and the cold air temperature is -40ºC, (-40ºF), or lower depending on the air velocity and equipment efficiency (7, 11).

2.1.7. Immersion Freezing - CFC's. The use of liquid CFC's in the 80's as the freezing media presented greater advantages compared to a blast or mechanical freezer. The heat transfer methods used are conduction heat transfer during the immersion and convection heat transfer in the equilibrium chamber. The heat transfer coefficient for the liquid refrigerant is higher than that for a gas. The CFC's immersion process is not considered a cryogenic process. The residual CFC's in the foods and the destruction of the ozone layer are concerns that have forced the Frozen Industry to reduce its use.

This freezing method may become extinct due to the ban on CFC's. This freezing system was used during the 80's to IQF vegetables, pasta, and other food products. The freezing process consisted of transferring the food product into a liquid CFC's bath. The frozen product is moved forward by action of a stainless steal mesh belt conveyor. To improve the equipment efficiency and freezing rate, liquid CFC's is sprayed on top of the food in the equilibrium freezing chamber with any excess liquid returning to the bath.

2.2. Cryogenic Freezing Methods

The introduction of cryogenic gases in the early 1960's as an alternative to improve freezing processes in the frozen industry was a major product quality and process improvement. Air, liquid nitrogen and carbon dioxide are the most common and safe cryogenic liquids and gases used in the food industry. The physical properties of the cryogenic gases provided an important tool to help the food and non-food Industry to improve their plant automation, versatility, efficiency and manufacturing costs. The application of cryogenic liquid/gases in the frozen industry presents many advantages over the mechanical freezing system that, certainly, have revolutionized the frozen industry and some of the benefits are:

- Reduction in the freezing time,
- Reduction in moisture and flavor losses,
- Reduction in ice crystal formation during the freezing process,
- Low maintenance equipment service,
- Elimination of the ammonia refrigeration system and its maintenance implications,
- Small space required for the frozen equipment,
- Minimum product cell damage,
- Low capital investment, electrical cost and risk factor,
- Quick installation, convenient and portable,
- Flexible and versatile system,
- High heat transfer.

Cryogenic methods allow the food product to be frozen extremely fast, reduce the moisture losses to a minimum and increase the equipment flexibility and efficiency. The low temperatures of the cryogenics gases/liquids allow them to be used for different applications and for different food products.

The cryogenic low temperatures maintain the high quality of the finished product. However, if the freezing process is not properly controlled, the frozen product can be damaged.

The disadvantages of the cryogenic freezing methods are related to the cryogenic gas/liquid costs in the long term, the extended residence time in a liquid Nitrogen immersion bath that may cause product cracking, the need of pressurized tanks to keep the Carbon Dioxide as a liquid. Semisolid, diced and sliced products with high content of water such as sauces, sauce with particles, thin fresh pasta, etc. could become brittle if immersed for long periods of time in the liquid nitrogen. The cryogenic freezing optimization is related to the food product thickness and it is directly proportional to the freezing time, food physico-chemical structure and composition and its heat transfer efficiency. The cryogenic liquid/gas freezing method could be divided in two freezing processes:

2.2.1. Immersion and Spray Freezing. Liquid Nitrogen Immersion. This freezing system consists in allowing the food product to be in direct contact with the liquid nitrogen. The freezing equipment is comprised of a stainless steel tunnel-freezer with an immersion pan in which a conveyor belt travels through the bath. Level controls maintain the liquid nitrogen level. Variable speed drive motors provide the freezing system with a lot of flexibility in attaining different freezing times, temperatures, capacities and product freezing uniformity.

The food product is fed by a shuttle or vibratory conveyor to the freezing tunnel. The food product falls by gravity into the liquid nitrogen and within seconds its surface is hard frozen. To reach frozen temperatures inside the food an equilibrium time is needed. This step is achieved later in the last part of the freezer. Food freezing in liquid nitrogen will generate cold nitrogen gas because of the heat exchange that occur between the boiling liquid nitrogen and the food product. The cold nitrogen gas which is produced is then blasted on the food product while in the equilibrium chamber allowing the food product to continue to be frozen completely.

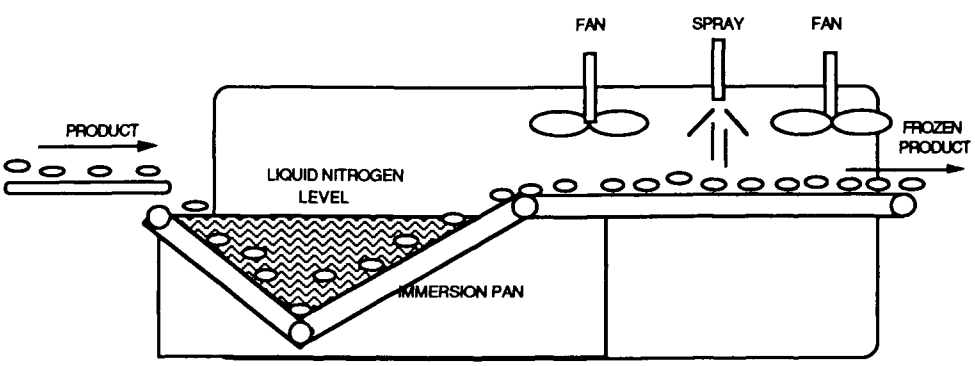

Figure 1. Schematics of immersion and spray freezing process.

The heat transfer methods used in these systems are conduction and convection heat transfer. Spray manifold systems and fans are located inside the equilibrium chamber to improve the heat transfer and the equipment freezing capability. (see Figure 1)

2.2.2. Spray Freezing. In this freezing method (see Figure 2) nitrogen and carbon dioxide are the most common cryogenic gases used to freeze food products.

The spray freezing system consists of a freezing tunnel that has spray nozzles that dispense the cryogenic gases on top of the food.

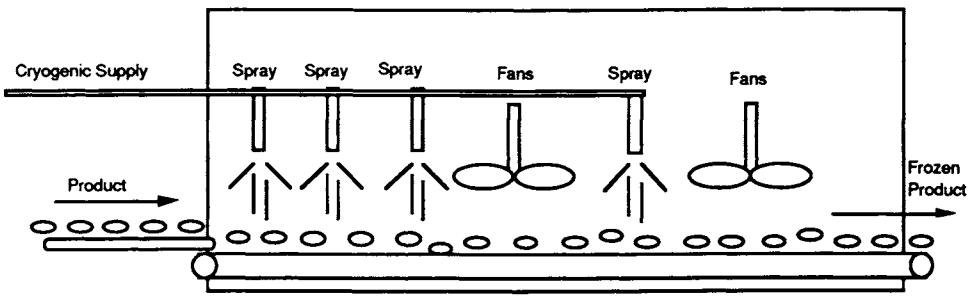

Figure 2. Schematics of a spray freezing process

The direct contact of the sprayed cold gases and the blasting action of the fans on the food enables the product to reach the freezing state in a few minutes. The heat transfer methods used are conduction and convection heat transfer.

Commercial spray freezing units are available as small to very long tunnel freezer to fit almost every need. The cryogenic gas (liquid nitrogen or CO_2) blows down the cold gas on top of the open trays containing food or the food is placed directly on the conveyor belt, thus freezing the product faster. Food product thickness is directly proportional to the freezing time and the heat transfer efficiency. The thicker the food product the longer the freezing time. The cold gas can be directed in either direction of the moving trays or food product. The cryogenic gas is sprayed in fine drops of liquid nitrogen or as solid particles of CO_2, commonly called snow flakes, on top of the open trays or directly on the food product to be frozen. This tunnel freezing system is a flexible method that is used to freeze chicken, fish, meat, pizza, bread, pies, cakes, entries and other processed foods on open trays or packaging containers. Moisture losses could be 1% or less depending of the freezing time.

2.2.3. Cryogenic Air-freezing System. This new air-freezing technology is used to freeze food products simulating the freezing effect that cryogenic liquid and gases have. This freezing blasting system uses compressed fresh air as a refrigerant. This new COLDBLAST™ system takes air from the environment and cools it through a three stages compression process, heat exchanger and a Turbo-expander (12). The cold air temperature reached is -250ºF. Cold air at a -100ºF is returned to the heat exchanger to be used as coolant, and the warm air is released to the atmosphere at 99ºF (See figure 3).

The cold air is fed into a tunnel or spiral freezer to quickly freeze the food product. This procedure reduces moisture losses to a minimum of 0.5% of product weight. The heat transfer method used is convection heat transfer (13). This air freezing system is environmentally safe and eliminate safety and residual concerns related to the ammonia and/or a CFC's system. The system advantages are similar to the benefits of the cryogenic freezing with liquid nitrogen and carbon dioxide gas. Compression, expansion and heat exchanger process unit operations are involved in this cooling-freezing method.

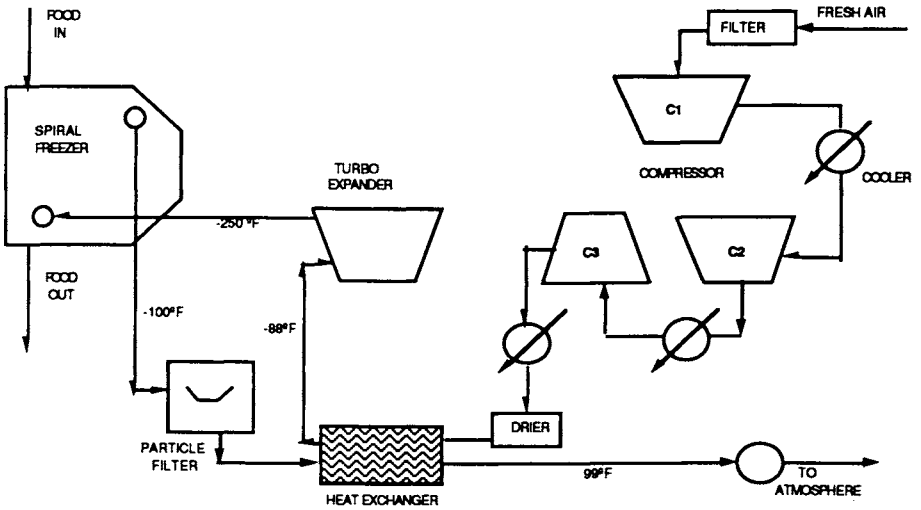

Figure 3. Schematics of a Coldblast System™. (Courtesy of Air Products and Chemicals, Inc.)

This air freezing system can be used for meat, fish, poultry, pizzas, processed and prepared food and baked goods. The use of cleaning in place, CIP, systems, defrosting units, air-filter and temperature control devices could be implemented in the freezing system to improve efficiency and savings.

2.3. Mechanical-Cryogenic Freezers

The frozen industry has taken advantage of the benefits of the cryogenic liquid and gases and the mechanical freezing equipment to improve the energy consumption, freezing times, equipment flexibility, food production rates, finished product quality and manufacturing costs. Combination of the best of the mechanical and cryogenic technologies has allowed the frozen food industry and freezing equipment manufacturers to develop new freezing equipment alternatives to improve finished product quality, procedures and capabilities. An overview of the cryogenic-mechanical freezer consists of freezing the food products using different systems that complement each other.

2.3.1. Use of Cryogenic Elements in the Integration of Mechanical and Cryogenic Freezing Methods Into a Single Freezing Unit. This system is used for its versatility, space requirement, capability, minimum freezing residence time to reach freezing equilibrium and for the high volume of frozen food products produced. The method consists in integrating the cryogenic elements and a mechanical freezer into a single processing unit with two major steps. Step one is a fast surface product frozen, and step two is an equilibrium frozen product temperature. Liquid nitrogen, LN_2, and carbon dioxide, CO_2, are the cryogenic mediums used for cryogenic freezing.

• **Carbon Dioxide Freezing System, CO_2.** The carbon dioxide cryogenic and mechanical freezers system is based on the physical properties of using pressurized liquid carbon dioxide. Carbon dioxide is stored as a liquid generally at a pressure of 300 psig. The liquid carbon dioxide is transferred to the mechanical freezer and released to atmospheric pressure generating an intensive cooling effect. The mechanical freezer could be spiral, tunnel, belt or spray.

The carbon dioxide snow produced is applied on top of the food and quickly develops a frozen crust in the processed food surface, and consequently, ice crystal formation and moisture losses are minimized. The high air-cold gas velocities, generated by the fans, complement the convection freezing process. The IQF product reaches its freezing equilibrium later in the tunnel or spiral freezer (See figure 4.). The cryogenic carbon dioxide system is very flexible and it is currently used by the Food Industry to freeze a wide variety of food products, such as chicken, fish, hamburgers, bake goods, cookies, meat patties, pizza, vegetables, entree, ethnic foods, etc. The heat transfer methods used are conduction and convection heat transfer.

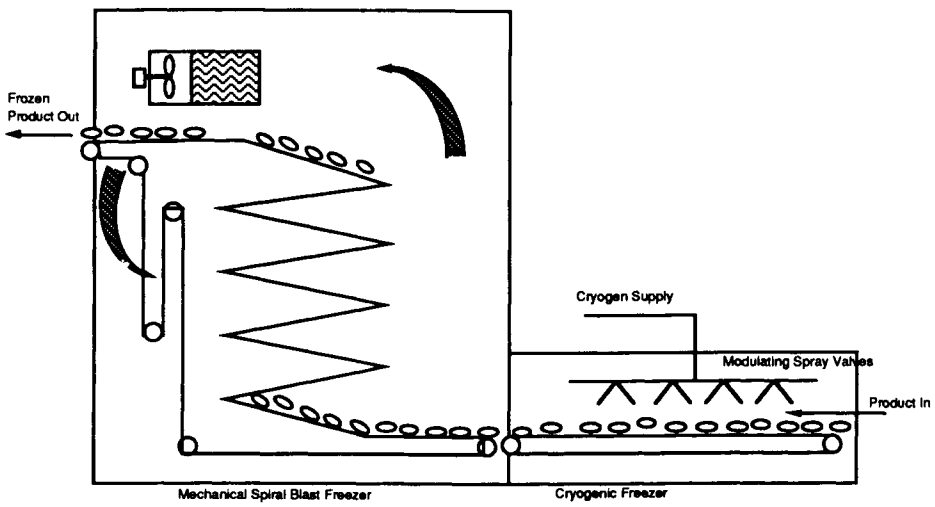

Figure 4. Schematics of a Cryogenical-Mechanical Freezer. (Courtesy of Liquid Carbonic Corp., Chicago)

• **Liquid Nitrogen (LN_2) Freezing System.** LN_2 works as follows: the food product is dispensed by gravity or by a vibratory conveyor into a liquid nitrogen bath, which is at its boiling temperature at atmospheric pressure of -320ºF (-195ºC), and remain there for a few seconds reducing the dehydration losses. The LN_2 level is automatically adjusted depending on the food product components, thickness, water content, water and LN_2 boiling activity, etc. The food product's surface is instantly frozen and by action of an open mesh conveyor belt, the frozen food product is transferred from the cryogenic bath to a mechanical freezer, (It could be spiral, tunnel, belt or spray freezer), for product freezing temperature equilibrium.

The use of manifold spray systems and fans in the mechanical freezer help to improve the heat transfer and equipment efficiency. The heat transfer methods used are conduction and convection heat transfer. Overall freezing residence time is reduced to a few minutes. Product through put and equipment efficiency are high and the maintenance required is minimum. The space required by this single integrated unit is very small compared with other freezing systems. Storage tanks and continuous liquid nitrogen feed system are needed to supply the cryogenic liquid to the processing line.

To increase the dual freezing system efficiency, the sensible heat of the liquid nitrogen transformed in gas could be recycled back to the mechanical freezer and blown at high speed on

the food product. The refrigeration media used in the secondary freezer could be based on an ammonia cooling system or a sub-cooled closed loop system.

3. NEW TECHNOLOGIES / APPLICATIONS / EFFICIENCIES

From the prior description and definition of the different methods and equipment used in freezing food products , it is apparent that in the 21st century most of the new technologies will be based on improving equipment efficiency, reducing energy costs, maximizing the advantages of ammonia as refrigerant, cryogenics, cooling efficiencies, dealing with the world's environmental concerns, identifying equipment flexibility and maximizing technical resources.

One of the biggest challenge of the freezing and refrigeration industry is the research and development of new alternatives in the refrigeration media equipment that are economical and efficient. The marketing future of a new self contained refrigeration media for the frozen food and refrigeration industry (other than CFC's) that could be compiled in a single small unit, is unlimited. The application opportunities are tremendous because it could be used in the home, supermarkets, transportation and distribution systems, warehouses refrigerators and freezers. A few ideas currently developed and potential opportunities for the freezing and the refrigeration manufacturing industry in foods and equipment are described below:

3.1. Cleaning in Place, CIP, System

The implementation of CIP systems into freezing units presents an opportunity to reduce costs of overall sanitation and freezing equipment's start-ups. The CIP systems could be automated, designed or retrofitted in tunnels or spiral freezers. A few of the advantages of a well designed freezer CIP system are:

Easily reach, sanitize and clean the mesh belts spiral conveyor, coils, fans, walls and floor of the freezer. Computerized CIP system controls the water temperature, consumption, and pressure, allowing savings of 1/2 to 2/3 of the water consumed with a manual freezer wash down. The CIP computer control system is automated by using programmable logic control systems, specially designed to any freezing equipment need. The use of a CIP recirculating system maximizes the utilization of water and chemicals. Recirculating 500 gallons of water, with a CIP system, provides the effect of using 9000 gallons of water in a one hour cleaning period (14). Savings in energy, sanitation crew, maintenance, chemicals and cleaning time. Updated CIP options could be accommodated to old freezing systems.

3.2. Preventive Freezer Maintenance and Computer Control

Energy consumption savings, Refrigerators account for 20% of the electricity consumed in US homes (15), improvements in equipment efficiency, low maintenance costs, and higher through put, could be achieved by implementing preventive maintenance, freezer manufacturing Technical Services programs, the use of self-defrosting system, variable speed drive motors, and the installation of process temperature sensors and computerized control automation systems.

3.3. Technological Improvements in New Valves, Spray-manifold, Cryogenics Gases Recirculation and Supply Systems

- Technological improvements in new valves and manifold systems used in cryogenic freezers allow better efficiencies and application of the cryogenic liquid and gases to pre-cool the food product or as a coolant in the freezer equilibrium layers or chambers.
- Improvements in equipment efficiency and capacity, by modifying the recirculating designs and systems, that maximize the utilization of the cryogenic gases' sensible heat generated during the immersion processes.
- Cold CO_2 supply system for large refrigeration uses that reduces cryogenic consumption. (Strata-Cold™) (16). This supply system is used when large volumes of cryogenic

refrigeration are needed. The storage vessel system, at -50ºF, (Standard systems store LCO_2, liquid carbon dioxide, at 0ºF), and its controls generates 15% more snow by delivering colder liquid CO_2 to the refrigeration process.

The system works based on the density of the LCO_2 at different temperatures. The cold CO_2 at -50ºF is more dense than the LCO_2 at 0ºF, consequently, it remains in the bottom of the storage vessel. The cold LCO_2 is fed to the refrigeration application as required. The LCO_2 at 0ºF is cooled to-50ºF and returned to the storage vessel (16). Applications for this system are: Food mixing, pneumatic cooling, food freezing, refrigerated trucking and freight car, and crusting.

3.4. Technological Improvements in Freezer's Wall Insulation

The improvement in the freezer insulation material will increase the operating efficiency of refrigerators and freezers for home or industry, will reduce its overall weight and may make it portable and easy to relocate according to the equipment and food process manufacturing needs and space availability. Wall insulation has been improved by developing a fiber glass vacuum panel and it could be used in refrigerators and freezer doors, adding more available space for storage. Currently Owens-Corning has a fiber glass insulation concept that gives six times the R value as that of the urethane foam insulation (17).

3.5. Technological Improvements in Equipment Design and Flexibility.

One current technological advance in equipment design is the self-stacking freezing belt spiral unit. This new freezing equipment does not need the steel structure required to support the spiral freezing belt and it works on a no-tension principle. In this new design, the tier is placed above another and the self stacking turns and work as a single unit. In this equipment improvement, there are not stationary parts in the freezing zone, consequently its freezing maintenance is minimum. The GYRoCOMPACT System ™ is a very flexible unit that operates with ammonia. The width and height of the belt self-stacking unit could be changed without changing the freezer structure because there are not stationary parts (18, 19).

3.6. Technological Improvements in Low-Temperature Mechanical Refrigeration System

This refrigeration system uses convection heat transfer to obtain faster freezing, reduce moisture losses, freezing costs and freezing time during its operation. This Low Temperature "non-expendable" CO_2 Mechanical Refrigeration System is an alternative to the current ammonia mechanical refrigeration system. The system operates at -55ºF, internal chamber freezing temperature, compared with -35ºF in typical ammonia systems (20). This system could use the ammonia system to condense the vapor CO_2 to a liquid.

4. FUTURE TECHNOLOGIES AND CHALLENGES

The world energy consumption, conservation, environmental concerns, worldwide market globalization, frozen, refrigerated, shelf stable processed food products competition and the high manufacturing costs will force the frozen food products industry to increase R&D challenges and to develop new technologies. These new technologies could be based on:

1. **Equipment Automation.** Improvement in equipment automation, by using sensors and computer control systems to increase equipment efficiency and maintain product consistency, quality and providing, at the same time, low freezing processing costs.

2. **Equipment Flexibility.** Improvement in equipment flexibility, efficiency and energy consumption. The designing of small self-contained refrigeration units which could be used in food freezing or food products distribution and storage.

3. **Better Understanding of Frozen Product and Freezing Processes.** Better understanding of freezing processes, food components physico-chemical properties, mathematical modeling, freezing equipment, and cooling heat transfer medium and its effects on food products, (21), will enlighten the path in improving finished frozen product quality, freezing processes and freezing equipment design.

4. **Refrigerated Shipping Containers for the Frozen Food Industry.** One current technological advance is the use of liquid CO_2 as an alternative to CFC's in shipping containers for the frozen food industry. The liquid CO_2 is transformed into a solid and it is used as the refrigerant in a system that has no mechanical moving parts. The system reduces the overall shipping costs, is cost competitive to build, efficient, and very economical (22). The system consists of pumping the liquid CO_2 into a tank located on top of the container where the liquid CO_2 is released to atmospheric pressure turning into dry ice snow. The dry ice snow is sublimated and because of its low gas density compared with the high warm air, the tendency is to flow downward thereby maintaining the freezing temperature inside the container.

5. **Manipulation of the Freezing Point.** The manipulation of the freezing point of food ingredients and raw materials, by using biotechnology principles, ingredients and/or changes in its physical characteristics and chemical structure without affecting its nutritional, quality and flavor values, open new R&D opportunities. Major improvements could be accounted, (in the finished product quality, food product shelf-life, the savings in energy conservation, and the frozen products price), if the food industry could produce, store, and distribute refrigerated and frozen raw materials, vegetables and processed food products at higher temperatures.

   Other freezing opportunities are related to the freezing point depression of the finished product. The food quality improvement, ice crystallization reduction, better texture and flavor would be a few of the results of the depression of the freezing point. Using recombinant DNA techniques, or chemical methods the suppression of ice crystal growth is a reality. Application opportunities of antifreeze protein and polypeptides technology could be used in vegetables, frozen dessert, and frozen dough (23).

6. **Other Liquid Nitrogen Freezing Opportunities**. Utilization of liquid nitrogen, boiling point -320ºF, (-196 ºC), to develop small self contained units for home, distribution and food manufacturing industry use. This unit could be manufactured using compression, expansion, heat exchangers, convection heat transfer methods, and other mechanical and thermodynamic principles to freeze food products, or, use similar refrigeration cycle procedures that are currently used.

7. **Improvement in New Freezing Techniques and Processes.**
   - The freezing of food products involving the application of sound waves (24).
   - Food products freezing using a cryogenic cooler with an inclined rotating drum (25).
   - The freezing of food products by improving thermal energy storage systems to keep up with increasing capacity or as a backup refrigeration.

8. **Low Temperature Thermal Energy Storage System** ($SECO_2$). This revolutionary system is a low temperature thermal storage system that uses the latent heat of carbon dioxide, 84 Btu/lb, at its triple point (26).

   The system is described as follows: "This thermodynamic state for the CO_2 exists at -70ºF and 60 psig. In the $SECO_2$, Stored Energy in CO_2, the solid is formed with a conventional cascade chiller and it is accumulated in a storage vessel which initially contains all liquid.

The chiller operates at full load and draws CO_2 vapor directly from the vessel vapor space as the low stage refrigerant. Cooling is supplied from the vessel by circulating CO_2 liquid from the vessel through a process heat exchanger. The vaporized CO_2 returns to the vessel and condenses directly against the solid/liquid slush" (26). This versatile and flexible thermal storage refrigeration system could benefit the Chemical, Petrochemical, Food Freezing Pharmaceutical, Gas liquefaction processes and Industries.

9. **Freeze Flo Technology**. It consists of producing food products that remain soft, firm in texture, high in stability, and ice free, maintaining its integrity, shape and consistency. Free water activity which encourage microbiological concerns is eliminated by the freeze flow process (27). This technology is currently used in developing freeze flow fruits with application in ice cream, desserts, and other frozen food product dishes. This technology could also be used in developing enrobed frozen food products, cakes, pies, pastries, and other added value food products.

10. **Microwave use in Freeze Drying Process**. This technology consists of using the microwave heating technology in subliming the freezing water as the food product is freeze-dried. Improvements in finished product quality, drying processing times, and organoleptic characteristics are obtained. The U.S. Army Natick RD&E Center, Mass., tests on peas and beans showed reduction in drying processing time that is proportional to the microwave wattage levels (28).

    The effectiveness of the process is based on the sublimation of the ice caused by the microwave absorption and conduction and convection heat transfer (28). This new technology could be expanded to other vegetables or food products that require freeze-drying procedures.

11. **Freezing Extrusion.** The application of extrusion principles and freeze flow technology in generating frozen food products and equipment flexibility to be used in the Frozen Manufacturing Industry.

12. **Liquid Concentration by Freezing.** This technology is based on the separation of the ice crystal formed from the concentrated liquid during the freezing concentration process. Challenge and success of this technology reside in the optimization of the process and equipment scale-up. This improved technology could be widely applied in concentrating liquids without using thermal evaporators. Better organoleptic attributes, finished product quality, lower handling and transportation costs could be obtained with the application and mastering of this technology (29).

5. CONCLUSION

The success in increasing worldwide market globalization of frozen products and overcoming the 21st century challenges will be characterized and measured by the improvements in the environment, bio-technology, product quality, transportation, distribution system, equipment design and flexibility, and the review of freezing technologies and concepts. Economic results, maximization of technical resources, joint ventures among academic organizations, equipment suppliers, and food manufacturers will be the pattern to follow for R&D in identifying and developing new freezing technologies. Better understanding of food components, freezing processes, equipment, mathematical modeling, and heat transfer mediums by the freezing Industry, scientists, product developers and process engineers will open opportunities to achieve better finished frozen product quality, consumer satisfaction, and it will create a healthier Frozen Food Industry.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 13

Role of Extrusion in Food Processing

D.R. Gray and R. Chinnaswamy
Midwest Grain Products, Inc.
1300 Main Street
Atchison, KS 66002-0130

1. INTRODUCTION

Extrusion cooking is the process of forcing a material to flow under a variety of conditions through a shaped hole (die) at a predetermined rate to achieve various resulting products (Dziezak, 1989). Extrusion cooking of foods has been practiced for over fifty years. Initially, the role of an extruder was limited to mixing and forming macaroni and ready to eat (RTE) cereal pellets. Today, the food extruder is considered a high temperature-short time bioreactor that transforms raw ingredients into modified intermediate and finished products (Harper, 1989). Extrusion cooking technology today is used for the production of pasta, breakfast cereals, bread crumbs, biscuits, crackers, croutons, baby foods, snack foods, confectionery items, chewing gum, texturized vegetable protein (TVP), modified starch, pet foods, dried soups and dry beverage mixes (Linko et al, 1983).

There are three major types of extruders used in the food industry; piston extruders, roller-type extruders and screw extruders (Thorz, 1986). Figure 1 shows examples of both the piston and roller extruders. The piston extruder can consist of a single piston or multiple sets of pistons that deposit a precise amount of product onto conveyers or trays. Piston extruders are primarily used for forming product shape and are used in confectionery as well as bakery production facilities. One example of the function of a piston extruder is cake, cookie or muffin dough being deposited onto a sheet with the use of a wire cutter, or into individual cups in an already shaped pan, and being conveyed to an oven for baking. Another example of a piston extruder is the depositing of fillings into doughnuts, cupcakes or chocolate type products.

Roller extruders are used to form the shape of a product. A roller extruder consists of two counter-rotating rollers that either turn at similar or differential speeds. This process is also refered to as calendaring in the dough industry. The roller surfaces can be smooth to create a long thin strip or can be perforated to form the dough into shaped products. The roller extruder can be altered to control the width of the layer of product moving in between them. Products such as crackers and hard cookies can be formed by creating the desired shape within the rollers and conveying the dough in between the rollers. The dough is forced into the pattern on the roller and is then conveyed to an oven for baking. Excess dough can be collected and reused. Products such as graham crackers or saltines are created using smooth roller systems to form thin layers.

Screw extruders utilize single, twin or multiple screws rotating within a metal cabinet called the barrel. The screws convey the material forward and through a small orifice called a die which can take on many shapes and sizes. Several external parameters such a screw

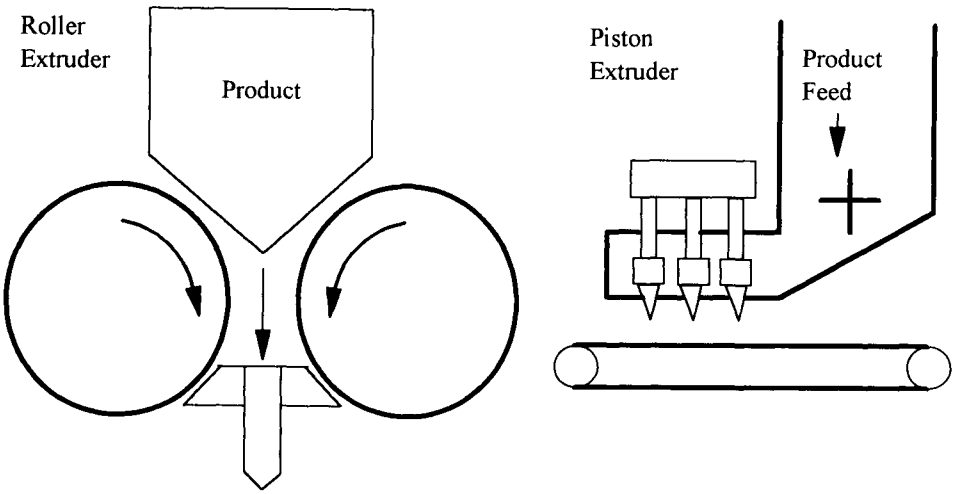

Figure 1. Examples of a roller extruder (left) and a piston extruder (right) (Dziezak, 1989).

speed and configuration, temperature of the barrel, die size and shape, and the length of the barrel effect the properties of the final product. The first food application for extrusion occurred in the 1800's for the production of ground sausage and meats stuffed in natural casings. The common meat grinder is a screw extruder that forces meat down a barrel with a single screw and through a multi-holed die plate. The size openings in the die plate give various widths to the final ground product. The piston extruder or a "ram type" extruder was used to stuff the ground sausage into casings (Dziezak, 1989). The same principle is used today for meat emulsions for the production of frankfurters and bologna-type products (Rust, 1987).

A brief history of the screw extruder shows that the pasta press was introduced in 1935 for forming and shaping pasta dough. Screw extruders providing both cooking and forming capabilities came in around 1950 for the production of animal feeds. The Collet extruder which is a short barreled cooker extruder was developed in 1946 and was used then and now for grain-based snack products (Dziezak, 1989). Because of the demand for pre-cooked cereals and starches in the 1960's, larger machines were required. These larger cooker extruders led to new applications in RTE cereals, snack products, as well as expanded the dry pet food market. Precooked infant foods were also developed (Harper, 1989). Improvements to the cooker extruder in the 70's led to the development of soft-moist pet foods, co-extrusion and the use of two extruders, one for cooking and the second for forming, were developed. The 80's has seen expanded use of twin screw extruder due to their versatility and productivity (Harper, 1989).

2. SINGLE SCREW EXTRUDERS

Extruders are further classified into single and twin screw extruders depending upon the number of screws in the barrel. Single screw extruders are the most popular because of

their low processing costs (Dziezak, 1989). Rossen and Miller (1973) reported that there are essentially five types of single-screw extruders used in the food industry. They are the pasta extruder, high pressure forming extruder, low shear cooking extruder, collet extruder, and the high shear cooking extruder (Harper 1986; Dziezak, 1989). The pasta extruder is one example of a single screw extruder and is used for forming various types of pasta products from a dough. The pasta extruder utilizes low temperatures and creates relatively low shear. It somewhat resembles a meat grinder in configuration in that the product is conveyed forward with a single screw to a flat, smooth die with several openings. The shape of the die determines the final shape of the product. The high pressure forming extruder is used for compressing and shaping pre-gelatinized dough into "half products" or products that will require another step in processing. An example of this is a snack product that after extrusion, would need to be fried, microwaved or processed by some other method before consumption. Also ready-to-eat (RTE) breakfast cereal pellets that need to go to a flaking roll, for example, can be formed using this type of extruder. The low shear cooking extruder is a continuous cooker for high moisture dough. The cooked product can then be processed further by forming or drying. Soft moisture pet foods and gelatinized starches are examples of the products made with this type of extruder. The collet extruder, as mentioned earlier, is a high shear, short barreled extruder used to make highly expanded snack food products. The collet extruder has no external heating source. Its energy comes from the viscous dissipation of mechanical energy. The low moisture input creates a highly expanded product with little or no additional processing necessary. The high shear cooking extruder is similar to the collet extruder, however a longer barrel is used to increase residence time and excess heat is removed by cooling. The final product is usually less expanded and requires a final drying process. A wide variety of dry ingredients and moisture contents leads to a variety of uses. Products such as texturized vegetable protein, dry pet foods and modified starches can be processed using this type of extruder. Table I compares the contrasts the different types of single screw extruders. The first step necessary in using any extrusion process is the mixing of the dry ingredients. After the dry ingredients have been mixed thoroughly, they are conveyed to the preconditioner. It is at this point that the ingredients are mixed with water or steam to the appropriate moisture content. The preconditioner can be either pressurized or a atmospheric chamber depending upon the specific needs of the process. The preconditioner utilizes steam when another heating source is necessary for the process. A preconditioner used with a extruder will increase residence time, reduce mechanical power consumption, increase capacity, optimize product quality and potability, reduce barrel wear and increase extruder efficiency (Harper, 1989; Wenger, 1992).

The preconditioner then deposits the ingredients into the screw barrel. The single screw extruder is composed of three distinct sections in the barrel; the feed section, the transition section and the metering section as shown in Figure 2 (Rossen and Miller, 1973; Harper and Harmann, 1974; Harper, 1989). The feed section receives the product from the preconditioner, compresses the feed material, works the material into a dough and convey it to the transition section (Harmann and Harper, 1974). The feed section has deep flighted screws and this aids in conveying the material forward. The transition zone further works the material into a dough that is partially cooked (121-177°C) and subjected to high pressure (Harmann and Harper, 1974). The metering zone function is to receive the compressed feed material, homogenize it and force it through the die at a constant pressure. The pressure is increased in this zone due to the die orifice and the small depth of the screw channel (Rossen

Table 1.
The typical operating data for the various types of single screw extruders (Harper, 1989).

Measurement	Equipment				
	Pasta Press	High-Pressure Forming Extruder	Low-Shear Cooking Extruder	Collet Extruder	High-Shear Cooking Extruder
Feed moisture, %	32	25	28	11	15-20
Product moisture, %	30	25	25	2	4-10
Maximum product temperature, °C	52	80	150	200	180
Screw diameter to flight height, D/H	3-4	4.5	7-15	9	7
No. of parallel screw flights, p	1-2	1	1	2-4	1-3
Screw speed (ω), sec^{-1}	4.5	6.5	10-30	50	70
Shear rate in screw (γ), sec^{-1}	5	10	20-100	140	165
Net mechanical energy input, MJ/kg	0.11	0.14	0.14	0.36	0.40
Steam injection ($m_s\lambda$), MJ/kg	0	0	0.11	0	0
Heat transfer (q) through jackets MJ/kg	(0.04)	(0.04)	0-0.11	0	(0.11)-0
Net energy input to product, MJ/kg	0.07	0.10	0.25-0.36	0.36	0.29-0.49
Product types	Pasta	RTE@ pellets, second-generation snacks.	Soft moist products, starch, soup bases, RTE@	Puffed snacks	TVP#, Dry pet foods, Modified starch.

@ Ready to Eat-Cereals
# Texturized Vegetable Protein

and Miller, 1973). Pressure at the die can be obtained by decreasing the size of the die orifice, i.e. the smaller the die orifice, the more pressure placed upon the die plate. Conversely, the feed zone has a large depth screw channel or large flow area while the metering zone has a small depth screw channel or small flow area. The ratio in screw channel depth in the feed zone to that of the metering zone is known as the compression ratio (Harper, 1989). The larger the difference in channel depth between the feed zone and the metering zone, the more pressure exerted at the die. Problems could occur if the die has a large resistance to flow, and result in pressure build-up in the feed zone. This leads to plastification of the ingredients in

that zone and instable working of the extruder. However, if the die has a low resistance to pressure, it leads to too much pressure build up in the metering zone and no pressure in the feed zone. This results in surging of the extruder (Janssen, 1989).

Heat input to the product during extrusion cooking comes from both internal and external forces. Steam can be injected into the barrel of the preconditioner, heat can be transferred from a steam jacket encasing the barrel, or frictional heat can develop on the barrel wall and in the die area during the rotation of the screw and flow of the product (Clark, 1978; Dziezak, 1989).

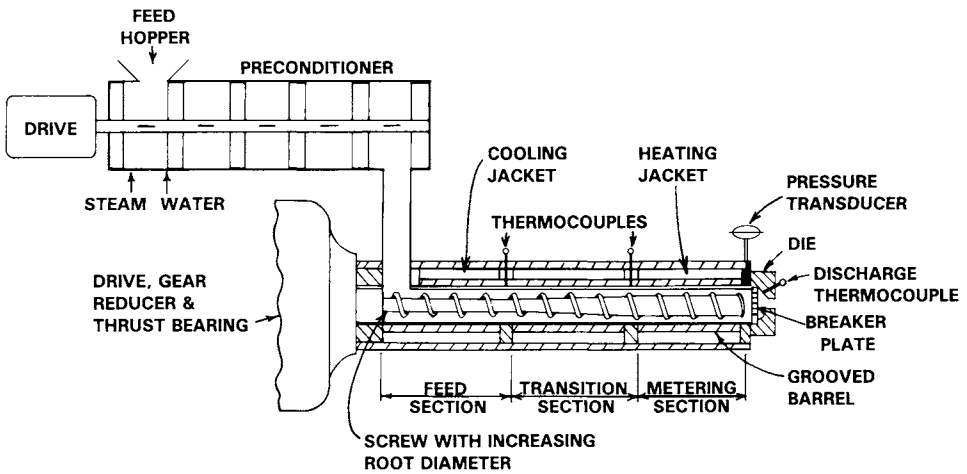

Figure 2. A diagram of the typical single screw extruder (Harper, 1989).

3. TWIN SCREW EXTRUSION

Single screw extruders are practical for simple, low cost extrusion operations. The demand for more diverse food quality products that require a expanded range of cooking applications, ingredients, and better control of processing variables has led to a increased use of twin screw extruders (Harper, 1989). Twin screw extruders are generally classified in two ways; by the degree of intermeshing between the two screws and by the direction in which they rotate. The screws are either rotating in the same direction (co-rotating) or in opposite directions (counter-rotating). Twin screw extruders are more complex and more expensive than single screw extruders, but they allow the user more control over extrusion parameters such as residence time and shear (Schuler, 1986).

Twin screw extruders can also be classified by the path of flow in the extruder channel. The flow can move either across or lengthwise in the channel or both depending upon the screw configuration as shown in Figure 3 (Schuler, 1986). In a lengthwise open extruder, the material is conveyed from inlet to outlet moving from the channel of one screw to the

channel of another. In a crosswise open extruder, there is an area common to both screws consisting of a path across the flights. Material may be exchanged from one flight to the other flight in a direction perpendicular to the screw channel (Schuler, 1986; Harper, 1989). Whether a screw configuration is lengthwise or crosswise opened or closed, will effect the conveying conditions, mixing action, and the pressure build-up capacity of the screw system (Schuler, 1986). As shown in Figure 3, co-rotating intermeshing screws can not be lengthwise and crosswise closed, and partially intermeshing co-rotating screws can not be lengthwise open and crosswise closed. In contrast, a counter-rotating intermeshing lengthwise open and crosswise closed system does not exist either (Schuler, 1986; Harper, 1989).

The action of the intermeshing counter-rotating screw and the intermeshing co-rotating screw differ significantly. In the counter rotating system, the screws roll off each other in the wedge area, whereas the co-rotating screws are always in contact with each other creating a natural wiping action (Schuler, 1986). Counter-rotating fully intermeshing twin screw extruders have a positive displacement characteristic, but they require reduced screw speeds to lower the separating forces that cause wear on the screw by the calendaring effect at the nip between the screws (Harper, 1989). These types of extruders are most commonly used in the plastic industry for their ability to process low viscous materials requiring low screw speeds and long residence times. Counter-rotating fully intermeshing twin screw extruders are not widely used in the food industry, but are utilized in the production of gum, jelly and licorice confectionery products which have plastic-like characteristics (Dziezak, 1989).

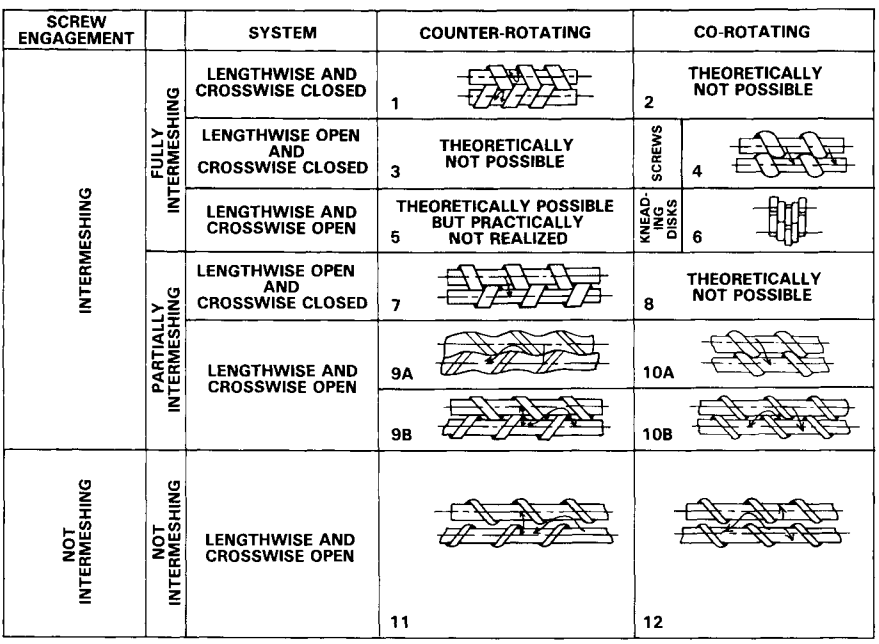

Figure 3. The various types of twin screw mechanisms (Ziminski and Eise, 1980).

For the counter-rotating twin screw extruder, non-conjugated screws are useful for mixing sections while conjugated screws and used where pumping is needed (Martelli, 1983). Co-rotating twin screw extruders are the most popular extruders used in the food industry. The advantages of this design are its pumping efficiency, residence time control, self-cleaning mechanisms and uniformity of processing (Schuler, 1986). Intermeshing co-rotating screws convey four to five times more material than intermeshing counter-rotating screws due to the interaction of the two screws that move the material from one screw to the other, resulting in a well mixed product (Hartley, 1984). Hartley (1984) discussed the advantages of the co-rotating screws. The intermeshing co-rotating screw configuration allows for one crest edge to wipe the other screw channel. This occurs at high speeds and allows for a efficient self-cleaning. Hartley (1984) reported that co-rotating screws have a translational motion in which one crest edge wipes a screw flank tangentially with a constant velocity. This can occur at a relatively high speeds and without producing a calendar effect. Therefore, the screws achieve a morre efficient and uniform self cleaning. Kneading discs are also used to aid in conveying the material forward to increase pressure at the die or conveying in reverse to reduce pressure by slowing down the passage of material (Hartley, 1984). Kneading discs also improve the mixing capabilities of the extruder barrel (Schuler, 1986).

There are three different types of screw designs for the fully intermeshing co-rotating twin screw extruders; the single lobe, two lobe and the three lobe. The three lobe design has a low volumetric capacity and is used to obtain high average shear rates and high energy input. The two lobe system is more common to the food industry. It has high conveying rates and relatively low average shear rates which result in a "gentler" treatment of the ingredients (Schuler, 1986). Single lobe designs have very low shear rates and are used when extruding materials that do not have free-flowing characteristics.

As the products flows down the channel, it moves from screw to screw in a "figure eight-like" motion. This results in better heat transfer between the product and the barrel, and better mixing capabilities and temperature control (Hartley, 1984; Schuler, 1986).

The most important feature to the success of the co-rotating twin screw extruder is the sealing profile formed by the intermeshing of the screws. It is this profile that makes the twin screw so unique. Self-cleaning characteristics directly result from the sealing profile, simply because it allows the crest of one screw to wipe the flanks of the other screw at a constant velocity. This provides a uniform shear velocity high enough to effect a wiping action for the bounding layers (Schuler, 1986).

4. COMPARISON OF SINGLE AND TWIN SCREW EXTRUDERS

Twin screw extruders are 1.5 to 2.5 times more expensive at a given throughput than single screw extruders. This is due primarily to the complexity of the screw, drive, and heat transfer jackets (Harper, 1989). The extra cost of a twin screw extruder can be partially offset by the ability to run at lower moistures which would require less energy for drying (Harper, 1989).

In single screw extrusion, the use of steam for heating provides about one half of the energy necessary for heating. The other half comes from mechanical energy. Twin screw extrusion heat is provided primarily with mechanical energy inputs. The use of steam will lower energy costs as it is a inexpensive source of energy giving single screw extruders the

advantage. However, single screw extruders run at higher moisture contents than twin screw extruders and with dryer effeciencies only at 40%, the production of low moisture products are becoming more attractive to processors. Twin screw extruders have a increased capability for the production of low moisture products (Dziezak, 1986; Harper, 1989).

Mechanically, twin screw extruders are more complex and have limited operating ranges than their counterpart single screw extruders. Twin screw extruders have limitations on torque, pressure, and thrust that are necessary to prevent excessive damage to the drive train (Harper, 1989).

Twin screw extruders are easier to control in terms of processing variables. This makes them easier to control and operate. Twin screw extruders are also capable of handling a wider range of ingredients due to the greater conveying angle and the self-wiping feature of the screws (Dziezak, 1986; Harper, 1989). Twin screw extruders also improve processing by increasing the amount of mixing in the channel, narrowing the residence time distribution which refers to the amount of time the product spends in the barrel, and the uniform shear rate across the channel depth (Harper, 1989). Pitch angles in the feed section are approximately 15° and produce an effective conveying angle in excess of 30°, or about three times the conveying angle of single screw extruders with similar pitch. The conveying capability of twin-screw extruders allows them to handle sticky and/or otherwise difficult-to-convey food ingredients.

Both twin screw extruders and single screw extruders have many applications in the food industry. Twin screw extruders offer a increased range of cooking applications and allow for better control and operating flexibility. If this is not a requirement for your food application, than the more economical single screw extruder is suitable for your processing.

5. MECHANISM OF FLUID FLOW

Flow inside a extruder is complex. Levine (1988) describes three types of extruder flow; drag flow, pressure flow and leakage flow. These types of flow can be found in any screw design (Levine, 1988). The drag flow refers to velocity of the screw in relation to the barrel. The velocity of the product being conveyed down the barrel by the screw is essentially 0 at the surface of the screw and gradually increases as you move away to the barrel wall. Figure 4 demonstrates the drag flow. Drag flow is like a liquid between two plates. Movement of one of the plates causes the liquid at the surface of the moving plate to move at the same speed the plate is moving. The further the liquid is from the moving plate and the closer to the stationary plate, the velocity of the liquid decreases as the force exerted to that area decreases (Levine, 1988). Drag flow results in a forward moving of material (Rossen and Miller, 1973).

Pressure flow refers to the pressure gradient between the feed zone of the extruder and the metering zone where the product is being discharged. The extent of this flow can be controlled by the size of the die orifice and the screw configuration. During normal operation, this flow is from the die to the feed section as shown in Figure 4 (Rossen and Miller, 1973). The leakage flow is similar to the pressure flow in that it is driven by a pressure gradient and results in flow going from the die plate to the feed zone (Levine, 1988). For this example, leakage flow would be the gap between the barrel and the screw. In a twin screw extruder, leakage flow can also exist when screws do not fully intermesh. When calculating the net

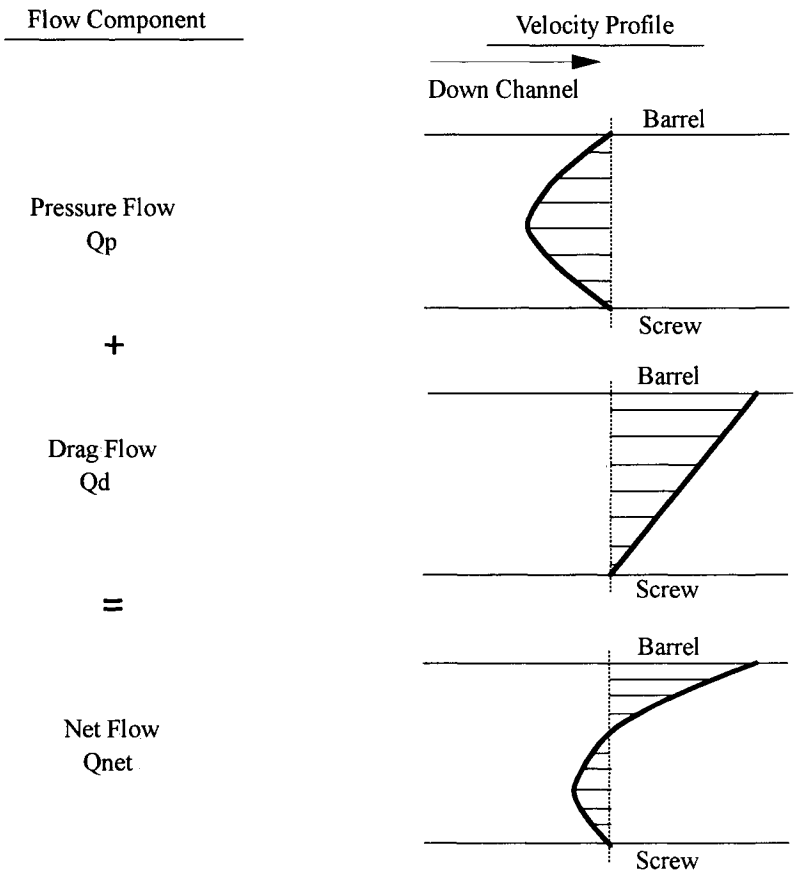

Figure 4. The types of flow existing in a screw type extruder (Rossen and Miller, 1973).

flow, the leakage flow is either not considered, or it is combined with the drag and/or pressure flows (Levine, 1988). The net flow of the extruder can be summed up in the following equation;

$$Q_n = Q_p + Q_d + Q_l \quad (1)$$

where

Q_n = net flow,
Q_d = drag flow,
Q_p = pressure flow, and
Q_l = leakage flow.

All extruders produce some or all of these types of flows. Intermeshing counter-rotating screws do not have drag flow. Forward movement occurs due to displacement of volume and this flow replaces the drag flow in the above equation. This same screw system also exhibits a small flow by the "calendaring" action of the parallel shafts. Similar to a roller extruder, it forces the flow to the bottom of the barrel and slightly forward. This is considered to be drag flow and is added in the above equation as such.

Viscosity also plays a important role in the flow of an extruder. Most food substances are thixotropic in nature, that is, they tend to shear thin. As the shear increases on the food substance, the viscosity decreases (Levine, 1988; Rossen and Miller, 1973).

6. EXTRUSION PROCESSING

6.1. Starch Modification

Transformation of starch in an extruder to a molten plastic-like product (destructurized starch) occurs in three steps; plasticizing the solid starch into a molten liquid through melting, shaping of the molten state with the use of a die, and cooking and texturization (Colonna et al, 1987). Starch undergoes strong chemical reactions during extrusion processing that result in depolymerization. The viscoelasticity of the starch is increased due to the sudden pressure drop, the molten mass expands. The is referred to as the "Weissenberg effect" and is independent of the "flash-off" of the super-heated steam which occurs during the sudden pressure drop as starch leaves the die (Clark, 1978; Colonna et al, 1987).

The fluid flow of starch inside the extruder can be described in terms of food dough rheology. Under isothermal conditions, molten starch in the extruder is said to be pseudoplastic (shear thinning) in nature and is described by the power law:

$$\tau = (K\gamma^n) \tag{2}$$

where

τ = shear stress (N/m^2),
K = consistency index ($n*s/m^2$),
n = flow behavior index (dimensionless), and
γ = shear rate (s^{-1}).

Clark (1978) found a good fit between the experimental data and the above model, but did not account for a large range of shear stresses. Other models have been developed, but are too complex to be discussed in this chapter. Other methods take into consideration temperature and moisture content since they are both controllable parameters of extrusion operations (Colonna et al, 1987).

The granular and crystalline structure of starch disappears during extrusion cooking. The starch phase is homogenized by the shearing of the molten granules. Extrusion destroys the organized crystalline structure either partly or completely, depending upon the amylose-amylopectin ratio and on extrusion variables such as moisture content and shear (Mercier et al, 1979; Colonna et al, 1987; Chinnaswamy and Hanna, 1990). In a waxy maize starch, a reduction of crystallinity is observed at extrusion temperatures of 70°C. At a higher

temperature the structure is destroyed and gave an X-ray diffraction pattern typical of an amorphous state (Colonna et al, 1987).

Starches with a typical amylose content of about 27% behave in a much different fashion. Two different structures are formed according to X-ray diffraction patterns. Below 170°C, the characteristic "V" starch structure appears, whereas when the temperature rises above 185°C, a new structure called "extruded" or "E" type structure appears (see Figure 5). The "E" type is characterized by three diffraction peaks slightly displaced from those of the "V" type. Both structures occur at 170°C, but reconditioning the starch to 30% moisture always transforms the "E" type to a more stable "V" type (Mercier et al, 1979; Mercier et al, 1980; Colonna et al, 1987; Watson and Ramsted, 1987). Because of the similarity between the "V" and "E" patterns, the assumption is made that the extruded starch structure is helical with six glucose residues per cycle in a hexagonal symmetry. The differences between the two are said to be due to the different interaxial distances in the two helices. The interaxial distance for the "V" structure in 1.38 nm while the "E" starch is 1.50 nm (Mercier et al, 1979). The results of X-ray diffraction suggest that during extrusion, the structure of the cereal starches is reorganized into the "V" structure and that the "E" structure is caused by the variation in the inter-helix distance rather than by the presence of another helix (Mercier et al, 1979; Colonna et al, 1987).

The amylose-amylopectin ratio determines the properties of the final product. Amylose provides lightness, elasticity, surface and texture regularities, but a sticky surface (Chinnaswamy and Hanna, 1988a). Amylopectin leads to a harder and less expanded extruded product (Mercier and Feillet, 1975; Colonna et al, 1987). Matz (1976) recommended an amylose content of 5% to 20% in starch to give an adequate crispness and acceptable texture in snack type products.

The gelatinization of starch is affected by several conditions set in extrusion processing. Chaing and Johnson (1977) reported that moisture content does not significantly affect starch gelatinization at low temperatures (65 and 80°C), but gelatinization increased at higher temperatures (95 and 110°C) when the moisture content is between 18% and 27%. Gelatinization of starch occurs at a higher rate when the moisture is between 24% and 27% than at 18% to 21% as the temperature was increased. Increasing the shear rate (screw speed) and /or the die size decreased starch gelatinization. Chaing and Johnson (1977) also reported that the (2,1) glycosidic bonds of sucrose and raffinose and the (1,4) glycosidic bonds of malto-oligosaccharides and starch are broken when cereal products are extruded.

In an extruder, liquefaction of starch occurs without the use of enzymes due to the development of high pressure and high shear. Starch is broken down into small molecular weight sugars at high temperatures and pressures (Chinnaswamy and Hanna, 1988a,b,c). However, the formation of monosaccharides has not been observed (Lorenz and Johnson, 1972; Chaing and Johnson, 1977; Colonna et al, 1987). During extrusion cooking, amylose and amylopectin are degraded into lower molecular weight materials. However, the percentage of α (1,6) linkages as compared to α (1,4) does not change during the extrusion process. Therefore, only the α (1,4) links are not affected. This could be explained through the theory that there are fewer α (1,6) linkages or that they are not as accessible as the α(1,4)(Mercier and Feillet, 1975). It is known that the macromolecular degradation occurring in the extruder is a function of extrusion parameters such as temperature, moisture and screw speed (Colonna et al, 1987).

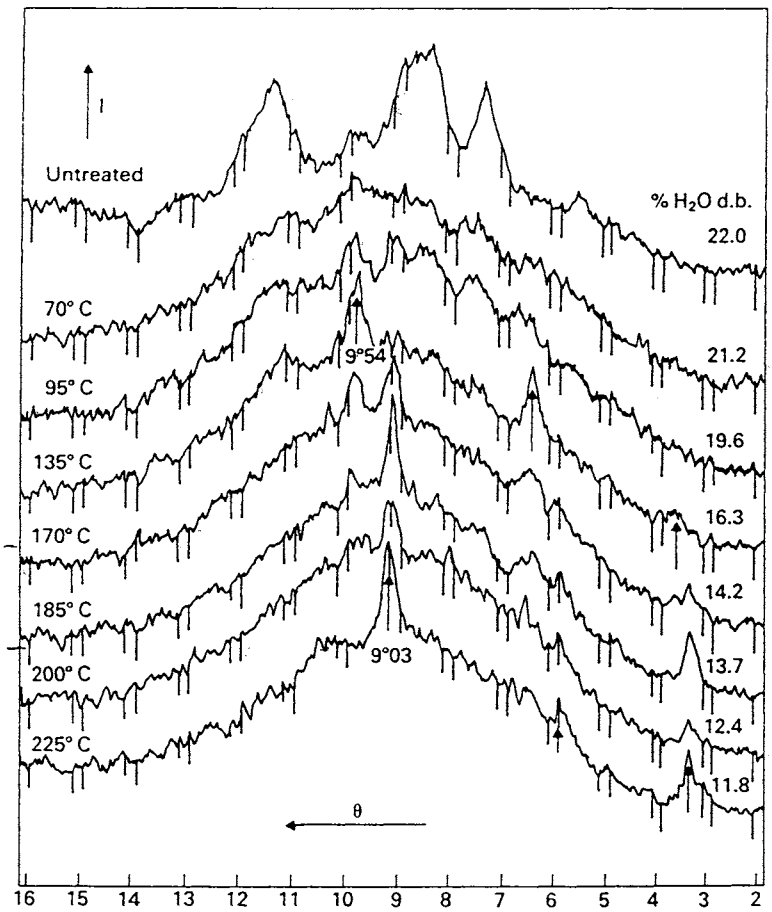

Figure 5. The X-ray diffraction patterns of corn starches extruded at the temperatures indicated. Initial moisture content before extrusion was 22% (db). Final moisture contents are indicated on the figure (Colonna et al, 1987).

The overall functional properties of starch after extrusion vary with the amylose-amylopectin content (Chinnaswamy, 1993). The water absorption index decreases with increasing extruder temperatures for waxy maize starch, while little change is observed for amylose until the temperature increases above 200°C. At this point the water absorption index increases sharply with increasing temperatures (Mercier and Feillet, 1975). The water solubility index also decreases with increasing amylose content. Native starch is, of course, insoluble in cold water. During extrusion, the molecules are broken down into smaller segments which make the overall solubility greater, and the starch becomes soluble in cold water (Mercier and Feillet, 1975). Chinnaswamy and Hanna (1988a) reported that the

optimum expansion ratio for starch was at 160°C for 70% amylose starch. The expansion ratio decreased at 140°C when amylose content of starch was greater than 50%. Chinnaswamy and Hanna (1988b) also found the optimum moisture content for expansion of starch to be 14%.

Chinnaswamy (1993) studied the effect of length to diameter ratio (L/D) of the die nozzle and how that affected the final expansion of extruded starch. Starch consisting of 25% amylose was used for this study. The expansion ratio increased sharply from 4.5 to 13 as the nozzle L/D ratio increased from 2.5 to 3.4 and then declined gradually to 8.5. The extruder pressure (measured in the extruder compression section), however, increased with increasing L/D ratios from 4.6 to 14.6 MPa. The increase in expansion ratio with L/D ratio and extrusion pressure may be due in part to a increase in the degree of starch gelatinization. It has been published that starch expansion is dependant upon the degree of gelatinization. The operating pressure increased with increasing length of the die and decreasing diameter of the die orifice. The results indicated that the operating pressure for maximum expansion of extruded starch was 7 MPa. However, it should be noted that L/D ratios for the die nozzles are not the best method for judging maximum expansion and pressure for the extrusion of starches because different combinations of length and diameter can give the same expansion and pressure results.

Chinnaswamy (1993) also reported the effect of chemical additives to the extruded starch at various amylose concentrations. It was found that the addition of NaCl increased the expansion ratio of starch as compared to the native starch at both 25 and 50% amylose contents with the expansion ratio of 50% amylose being slightly higher than the 25% amylose starch. The addition of sodium bicarbonate slightly lowered the expansion ratios for 25 and 50% amylose starches, but was higher than the native starch at 0% amylose content. Urea was also studied for its effect on expansion and was found to have the lowest expansion ratios for all four (0, 25, 50, 65%) amylose contents studied.

Chinnaswamy (1993) and Sokhey and Chinnaswamy (1992) further studied the effect of radiation treatment (10, 20 and 30 kGy) on expansion ratios of extruded starched with various amylose contents. At amylose contents of 25 and 50%, the expansion ratio increased with increasing radiation treatment. Chemical additives such as ceric ammonium nitrate, potassium persulfate and hydrogen peroxide which are known to induce or enhance free-radical formation were mixed with the starches treated with 20 kGy and all of the chemically treated irradiated samples showed significantly less expansion than the irradiated starches with no chemical treatment. All these chemical modification treatments have altered the starch molecular profiles which had irreversible effects on starch functional properties.

The partial depolymerization of starch in extrusion cooking leads to a low viscosity at 95°C compared to native starch, and subsequently signifies an absence of gelling ability. The paste stability at 95°C, however, is improved and starch retrogradation is reduced (Colonna et al, 1987). A loss of paste viscosities in extruded starches presents a major disadvantage in some product formulation. However, their slow dispersion in cold water and rapid dispersion in hot water to form products of various consistencies permit the commercial use of this type of modified starch for instant foods (Colonna et al, 1987). The overall digestibility of the starch product is also greatly increased as the temperature of extrusion is increased. However, the potential substitution of pregelatinized starches by extruded starches is based only on the estimation of their functional properties, rather than their nutritional value. This is due to the lack of information available on the nutritional quality of extruded starches (Colonna et al, 1987).

These types of starches, referred to as pregelatinized starches, quickly rehydrate in water and can be incorporated into cold food products to increase viscosity and binding. They are useful in food products that do not require cooking (Whistler and Daniel, 1984). Other possibilities for modifying starches using extrusion cooking is the direct injection of chemicals such as acids, cross-linking agents, and phosphate groups into the extruder barrel. This decreases the processing time for the modification of such starches.

6.2. Ready to Eat Cereals

Extrusion cooking of ready-to-eat cereals (RTE) provides several advantages over conventional processing methods. Extrusion cooking allows for faster processing times, lower processing costs, less square footage of the plant required for processing equipment, and greater flexibility leading to more types of end-products (Bailey et al, 1991). One needs to go no further than the local grocery store to see the wide array of cereals currently available in the market place. The creation of a wide range of cereal shapes and sizes is possible by changing the processing conditions such as moisture, temperature, ingredients, die orifice and screw configuration. The ability to coat the final product with various colors and flavors increases the variety of extruded RTE cereals even more. Bailey et al (1991) summarizes the steps required to make either expanded RTE cereal or a flaked product using a forming extruder.

The cereal ingredients are first screened to remove any large fragments that may interfere with flow in the barrel. Next, the product is combined in a mixer to ensure uniform dispersion of all dry ingredients. From the mixer, the dry mix is moved to the bin discharger which is mounted to the top of the extrusion cooker. The discharger holds and maintains a predetermined amount of dry mix so the mix can then be moved to the preconditioner by the screw feeder. The screw feeder assures that the flow of product is uniform to the preconditioner. The preconditioner allows increased residence time and mixing in the extrusion process. Heat can be added to the system in the form of steam which also increases the moisture of the dry mix and allows for even distribution of that moisture and thermal energy through the extra mixing that takes place. The moisture of the product assists in overall flavor development and affects the final texture of the product. It also improves extruder efficiency.

Bailey et al (1991) recommends the fully intermeshing co-rotating twin screw extruder for RTE cereals because of its versatility. The twin screw extruder can be adapted to perform several different tasks including heating, cooling, conveying the product, feeding, compressing, reacting, homogenizing, melting or rendering amorphous, cooking, texturizing and shaping of the product (Bailey et al, 1991). However, considering the most RTE cereals are of low moisture and high expansion formed under high shear and pressure, single screw extruders can also be utilized for this type of processing. The initial moisture content would be higher which in turn requires more energy for drying, but the same end product can be produced (Harper, 1986). The screw configuration is designed so that the feed zone conveys the product forward to a processing zone. In this zone, the ingredients are compressed using shear locks and a decreased shear pitch to make a homogeneous dough. The temperature of the dough increases dramatically in the last 2 to 5 sec of the total residence time of 35 to 40 sec. The barrel has a length to diameter ratio of 16.5 to 1 and is typically the same for direct expanded cereals as well as flaked products. The moisture content of the expanded product is around 10 to 15% at the die whereas the flaked product moisture contents are from 20 to

30%. The direct expanded cereal is ready to be dried further or toasted and coated if necessary before it is ready to package. The flaked product goes through several other processing steps.

The processing of corn flakes without the use of extrusion cooking is a long process that is manufactured in batches, and not a continuous process as described by Fast (1990). First, the germ must be removed from the corn kernel and the endosperm broken into two or three pieces depending upon the size of the kernel (the process varies slightly for wheat flakes and rice flakes). The other processing ingredients are added next. The product is then moved to batch cookers which are filled to 50-67% capacity to allow for expansion of the cooking product. The cookers are generally 4 ft in diameter and 8 ft long and built to withstand direct steam injection under pressure. The product is normally cooked at 15 to 18 psi for 2 hours. The products is considered finished cooking when the grits have transformed from a "hard, chalky white to a soft golden brown product when is translucent". The product is then dumped and moved onto further processing (Fast, 1990).

The product moves along to a delumping stage to break the grits into single pieces. Delumping is important in that it insures uniform drying of the product. The grits next move to the drying stage. The temperature used is generally 121°C (250°F) under a controlled humidity. The humidity must be controlled to "impede the removal of moisture from the center of the grit" (Fast, 1990). Controlled humidity prevents this phenomena known as case hardening. It also speeds up the drying process to the desired end moisture of 10 to 14%. The product is then conveyed to a cooling and tempering step when the moisture of the grit is allowed to migrate from the center of the grit to the outside making for a more uniform moisture distribution. Even with controlled humidity drying, the tempering step takes a few hours to complete. The grits are now ready to be rolled out in the rollers which are hollow to allow for chilled water to pass through removing any excess heat. From here the final step is to toast the product at 274-329°C (525-625°F) for 90 seconds to drive the final moisture down to 1.5 to 3%. This step creates the blistering effect that is desired in the final flaked product.

Making corn flakes with extrusion processing eliminates the long cooking processes required with the grits, the several hours of tempering to equilibrate the moisture and the bottleneck created in production between the cooker and the controlled humidity drying. These steps are eliminated because extrusion processing begins with flour instead of a whole corn grit and follow the same processing procedures used for extrusion processing of RTE. The flaked product, after leaving the cooking extruder, goes to a forming extruder. This extruder has low shear and a deep flighted screw to allow the dough to be extruded through a die that makes little beads. The barrel temperature is controlled using a jacket to circulate water around the system to remove excess heat. This process has a long residence time and since the extrudate in hot and moist, it adds to the flavor development (Bailey et al, 1991). The beads are formed by cutting the product with a rotating blade at the end of the die. The same type of blade is also used to cut the direct expanded RTE cereal at the end of the cooking extruder. The beads are conveyed with a negative air conveying system to insure the outside of the bead dries significantly enough to prevent the product from sticking together (Bailey et al, 1991). The beads are then transferred to a long cylinder called the bead conditioning reel which allows air to cool the temperature of the product to 38 to 63°C. The residence time can be changed by altering the angle at which the reel sits. The steeper the angle, the shorter the residence time (Bailey et al, 1991). From the conditioning reel the

product goes to the tempering screw where the objective is to maintain proper moisture and temperature of the bead. If the beads are too cool, heat is applied to the wall of the tempering screw (Bailey et al, 1991). Corn and rice beads tend to flake best between 43 to 54°C while bran and multi-grain beads flake best at 46 to 63°C (Bailey et al, 1991). From here the product is passed through a metal detector to ensure product safety and eliminate sparks that may form on the flaking roll if metal fragments are present. This will also increase the wear life of the flaking roller. The beads go to flaking roller where the final shape is reached. From here, as in the direct expanded cereal products, the flakes go to ovens to be toasted for additional flavor and to coaters to add flavor and sugar to the outside of the product. The product is then dried and cooled and is ready for packaging (Bailey et al, 1991). This process cuts the final floor time needed to produce a flaked cereal from several hours to 30 to 60 minutes.

Another area where extrusion cooking can cut the time necessary to process a RTE cereal is in the production of gun puffed whole grain cereals (Fast, 1990). The traditional method calls for rice or wheat grain. Wheat requires a pretreatment step to avoid loosing the bran in a ragged manner. This can be accomplished by treating the product in a 4% brine solution (26% salt). The salt toughens the bran during preheating and make it adhere to itself better and makes it stronger.

In the traditional process, the product is then loaded into a steel pressurized barrel built to withstand 200 psi that has a internal volume of 0.4 to 0.5 ft^3. The puffing gun has one opening into which the product is loaded into and fired for puffing. The gun is closed and sealed after loading. Heat is applied to the walls of the barrel through the use of gas burners and the barrel is rotated to insure uniform heating. The water inside the grain is converted to steam. When the pressure is released, the internal steam is released from inside the grain causing puffing to occur. The final product is about 5 to 7% moisture and must be dried to 1 to 3% moisture in the final product.

Extrusion processing of puffed cereal products also begins with flour instead of a whole grain which eliminates the preconditioning step. The extruder cooks the product and forms it into the desired product with the use of a die, with cooking and extruding a one step process instead of a two step process. An example of a product made in this fashion is General Mill's Kix (Fast, 1990). The dough goes from a cooking extruder to a forming extruder to make the general shape for puffing in the same manner as before. This completely eliminates any pretreatment and also eliminates the worry of broken kernels not adequate for puffing using the whole grain (Fast, 1990).

The die used can also have an affect on the expansion of RTE cereals. Chinnaswamy (1993) experimented with 25% amylose starch to determine the effect of the die shape on expansion. As the length to diameter ratio (L/D) of the die increased, expansion increased until the L/D reached 3.4 mm. As the L/D increased further, expansion slowly decreased. Chinnaswamy (1993) also found that an increase in extrusion pressure increased the expansion of 25% amylose starch. Optimum expansion was achieved at starch blends of 50% amylose. This is very important in choosing optimum conditions for the desired expansion of an RTE cereal product.

6.3. Snack Products

Snack product are very similar to RTE cereals in that they are cereal starch based products. However, snack products are generally extruded at a lower moisture content, higher

shear and higher temperature to cause significant starch modification as opposed to the RTE cereals. This leads to a more expanded product that melts in the mouth (Harper, 1989). As mentioned previously, the collet extruder is primarily used for the forming a snack products with high expansion. The internal pressure, as well as the size and shape of the die orifice, leads to the final shape of the product. Extruded snack products are produced in a variety of shapes and sizes which include rings, stars, curls, balls, lattices and squiggles (Tettweiler, 1991). There has been little interest in producing corn-curl type products with a twin -screw extruder because of the high capital costs of these machines and the wear that occurs to the twin screws due to the low moisture content used during processing. Briefly, the single screw extruder performs adequately in making this type of product at a lower cost. Also, the control of temperature and shear are not necessarily desired in this case, so the use of a twin screw extruder is not cost effective (Harper, 1989).

Twin screw extruders play a role in second and third generation snack products that need to be fried, baked, microwaved, reformed, etc. by the consumer or processor before consumption. The twin screw extruder in these cases is used more for forming a product. The single screw extruder in this case eliminates processor control necessary to properly maintain a consistent product. Twin screw extruders are also responsible for creating a whole new line of low-calorie crispbread snack foods (Linko et al, 1983; Harper, 1986). Extrusion replaces the old baking lines used to make this type of product which were more expensive and required more drying and cooling than the extruded product.

Extrusion technology also plays a role in the processing of tortillas. After the dough has been mixed, extrusion can be used to form the tortilla. The extruder is designed for minimum kneading of the dough and works a sheeting mechanism. The extruder sheets the dough into a layer that is dusted with flour, rolled, and further thinned by a crossroller. From this point, the dough is cut to the desired size with a rotary cutting unit with the scrap returning to the extruder to be reprocessed (Serna-Saldivar, 1988).

Piston and roller extruders play a dominant role in the baking of cookies and other baked snack goods. Many bakeries use piston type dough depositors that either directly deposit dough into a pan or use a wire to cut a layer of dough to be baked on a cookie sheet. Generally, short dough is used for these types of products to ensure a clean cut. Roller extruders can be smooth and used to make thin sheets that are required to make cracker type products. Here, the rollers move back and forth perpendicular to the cookie sheet to layer the cracker dough. The layers are held together with docking pins during baking and are responsible for the holes found in saltines. The thin sheets of dough can also be used to make figure cookies, such as animal crackers, by cutting the dough layers into various shapes and sizes. They can also be perforated to various shapes and sizes to make formed products such as Oreo type cookies. Sugar and shortening are used at lower levels than those for conventional cookies to ensure the dough does not spread while baking and yet the design of the perforated roller is not lost. This leads to a crumbly dough that is just wet enough to work with.

6.4. Texturized Vegetable Protein

Texturization of vegetable protein is the restructuring of protein molecules (usually soy protein) into a layered, crosslinked mass which is resistant to disruption upon further heating and/or processing (Harper, 1986). Texturized vegetable protein (TVP) is divided into two classification; extrusion cooked meat extenders and extrusion cooked meat analogs. Meat

extenders are produced from defatted soy flour (52% protein) and soy concentrate (70% protein). These products are rehydrated to 60 to 65% moisture and are blended with meats or meat emulsions at levels of 20 to 35 % or higher (Rokey and Huber, 1987). Meat analog processing involves using one or two extrusion cookers in series to convert vegetable protein into varieties of meat analogs which can have both the texture and appearance of meat (Rokey and Huber, 1987).

When the mechanical and thermal energy of extrusion cooking is applied to proteins during extrusion processing, they tend to lose their structure, unfold and become denatured, forming a continuous visco-elastic mass (Harper, 1986; Rokey and Huber, 1987). The design of the extruder barrel, as well as the screw configuration, align the protein molecules in the direction of the flow. This realignment exposes bonding sites which lead to cross-linking and a reformed expandable structure (Harper, 1986; Rokey and Huber, 1987). The increase in shear, temperature and retention time will cause cross-linking to occur between the protein molecules. Texturization is resistant to disintegration upon rehydration and leads to a chewy end product (Rokey and Huber, 1987). The temperature in the barrel is usually between 140 and 160°C as that range leads to the best chemical cross-linking between vegetable proteins and gives the final fibrous meat-like structure. The final product can be rehydrated to approximately three times its weight (Harper, 1986).

In addition to retexturizing vegetable protein, Rokey and Huber (1987) discuss several other functions extrusion cooking serves. (1) Extrusion cooking denatures proteins which lowers solubility and makes the product more digestible. It also inactivates enzymes and destroys the activity of any toxic proteins. (2) Texturization with extrusion cooking deactivates heat labile growth inhibitors native to raw vegetable protein. (3) Extrusion cooking controls bitter flavors associated with soy proteins. Most of these flavors are volatile and are vented off during extrusion processing. Others are lost due to the compression of the protein at the die orifice. (4) Extrusion cooking also provides a homogeneous, irreversible bonded dispersion throughout a protein matrix. This ensures the final product is uniform and all ingredients have been incorporated thoroughly. (5) The shape and size of the extruded vegetable protein is convenient for packaging and shipping.

A typical setup for the processing of TVP includes a bin or feeder, preconditioner, and extrusion cooker (Rokey and Huber, 1987). The raw ingredients are added to the bin and are metered to the preconditioner after being thoroughly mixed. It is important that the flow is constant and the rate of flow controllable. The preconditioner controls the rate of flow and moisture through the injection of water or steam (Rokey and Huber, 1987). The addition of steam controls the raw material moisture content and temperature. The raw material requires a moisture content ranging between 10-25%. The preconditioner can either be pressurized or atmospheric. A pressurized preconditioner can create a higher outlet temperature but can destroy nutrients (Rokey and Huber, 1987).

Either single or twin screw extruders can be used for texturizing vegetable protein. In both cases, the texture is affected by screw speed, barrel, temperature, moisture, raw material quality, and die orifice (Rokey and Huber, 1987). The advantages and disadvantages of the twin screw and single screw extruders have already been mentioned. The protein is conveyed down the channel of the barrel and converted into a dough. The compression ratio, which refers to the volume in the flights of the screw, decreases as the product moves closer to the die which increases pressure. The product at this point has been plasticized. As the product passes through the die, the product suddenly reaches room temperature and expands (Rokey

and Huber, 1987). The die for processing TVP emits a smooth streamlined flow that eliminates shearing so to not disturb the newly cross-linked protein (Rokey and Huber, 1987).

6.5. Pasta Products

Pastas are generally wheat-based products that are formed from a dough, where no leavening is necessary. They are generally made of flour and water, although eggs are sometimes added (Hoseney, 1986). Durum semolina is the best material for making flour for pasta. Durum wheat is a hard wheat and gives pasta its yellow color and is generally different from common wheat. Most durum wheats are white spring wheats that are translucent and high in carotenoids which gives it a slight amber color (Hoseney, 1986). Generally, durums have poor wheat gluten and do not make good bread products.

Figure 6 demonstrates the extrusion equipment used for processing pasta products. The semolina is mixed with water until 31% moisture is reached. The product will form into small dough balls. This mixing occurs in a air tight mixer in order to control the incorporation of air (Hoseney, 1986). A degasser can also be used to aid in keeping air out of the dough (Cantarelli, 1985). Small air bubbles in the product will weaken the final dried pasta product. The incorporation of air also leads to the activation of lipoxygenase enzymes that bleach the dough white. Hence, elimination of air in the system will limit the activation of these enzymes (Hoseney, 1986).

A single screw extruder with deep flight channels to eliminate shear is used for the extrusion of pasta products. It acts as a mixer, kneads the dough and exerts pressure. The dough moves down the barrel to the extruder die. This process produces a lot of heat which needs to be eliminated. Generally, the pasta extruder is equipped with a jacket that is usually filled with cold water, keeping the temperature below 45°C. The low temperature combined with the low moisture content leads to little or no expansion which is desired in pasta production (Hoseney, 1986). A high level of extrusion velocity leads to less damage to the gluten network and better starch retention upon cooking while lower extrusion temperatures produce less damage to the protein fraction thus yielding better hydration upon cooking (Cantarelli, 1985). The smoother the die plate, the better the production. Therefore, stainless steel and teflon dies are preferred over bronze dies which are more expensive and tend to wear out faster. The die must be thoroughly cleaned and kept frozen after use to prevent the growth of bacteria which could greatly effect the final product (Hoseney, 1986). Several different die shapes can be used to make the various types of pastas available in the market.

The pasta then goes through a long drying step lasting 10 to 16 hours. Drying is the most important process in pasta production as cracking or checking can occur if the product is not dried properly (Hoseney, 1986; Cantarelli, 1985).

Due to the high cost of durum wheat flour and the availability of other types of starches in developing countries, there is a trend to develop pasta products based on cereal products other than wheat (i.e., rice, starch , potato flour, maize and legumes) (Giese, 1992). These technologies require a high temperature pretreatment of a fraction of the dough starches or flour, which is then mixed with the remainder of the ingredients. This could be done with a preconditioner and a cooking extruder and allows for proteins to form a coagulated network. The operation works well to improve pasta quality with raw materials with inferior protein quality (Giese, 1992). Extrusion cooking could also play a role in pregelatinizing starch in pasta products leading to gelatinized levels over 95%, a low microbial count, and the ability to rehydrate in cold water (Giese, 1992).

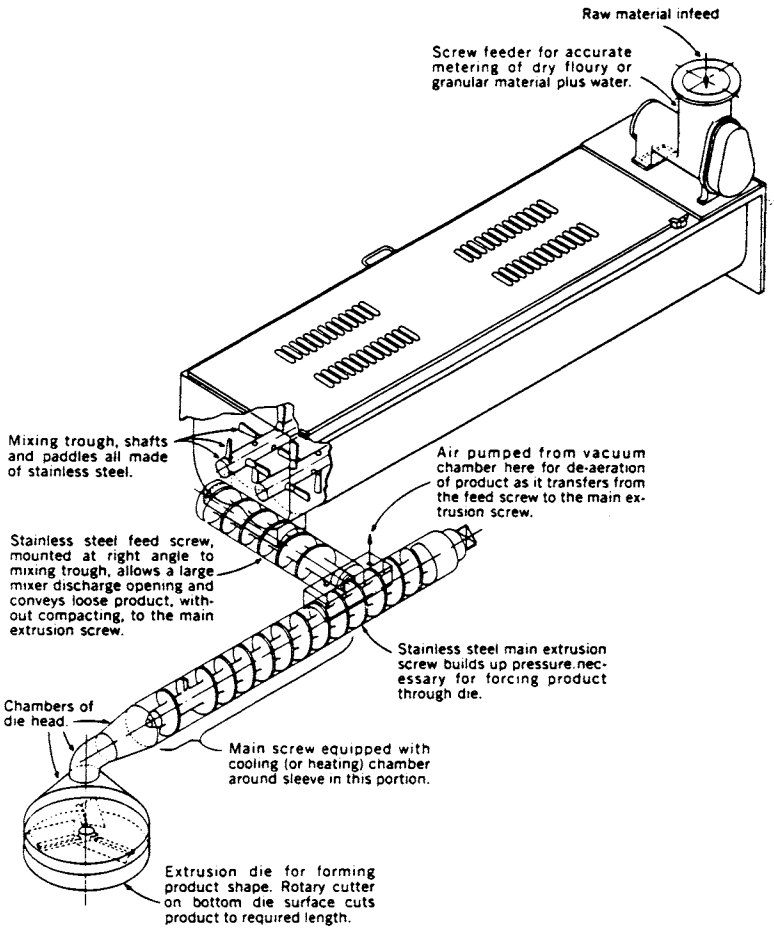

Figure 6. An example of a single screw macaroni extruder (Harper, 1981).

6.6 Meat Products

The best example of the meat product extrusion is the standard meat grinder. The meat grinder is similar to the pasta extruder in that there is one solid screw with deep channels used for conveying the meat forward, and a smooth die is used to save on wear and forces the product through the desired die size. Simply put, it is a forming extruder. One of the drawbacks to using a meat grinder is that more surface of the meat is exposed to the metal of the grinder. This leads to microorganism contamination of the product and incorporates oxygen thus decreasing the shelf life of the product (Urbain and Campbell, 1987). Besides the obvious production of hamburgers, the use of a meat grinder is the first step to the production of fresh sausage products. Spices and flavorings are added after grinding in a mixer.

Fresh ground sausage is packaged in either non-edible or edible casings. For both types of packaging, a piston extruder, screw extruder or what some call a ram type extruder are used. The piston and ram types are very simple in design. A certain amount of product is forced through an die opening and into the casing. These machines are designed to twist the sausage casings to a desired length after a designated amount has been forced in, leading to the long sausage links available in meat markets. At this point the fresh sausage is ready for consumption.

Another method in which extrusion is used in meat processing is the production of meat emulsions such as frankfurters and bologna. Although the simple piston extruder will stuff the casings in a similar fashion as the fresh sausage, co-extrusion is also used. Figure 7 demostrates that co-extrusion is essentially the extruding of two products at the same time (Potter, 1986a). A premixed collagen dough casing is extruded around the outside of the emulsion and formed around the product. Then the emulsion is twisted to desired lengths the same way the fresh sausage is and the frankfurters go to the smoke house to be cooked (Potter, 1986a).

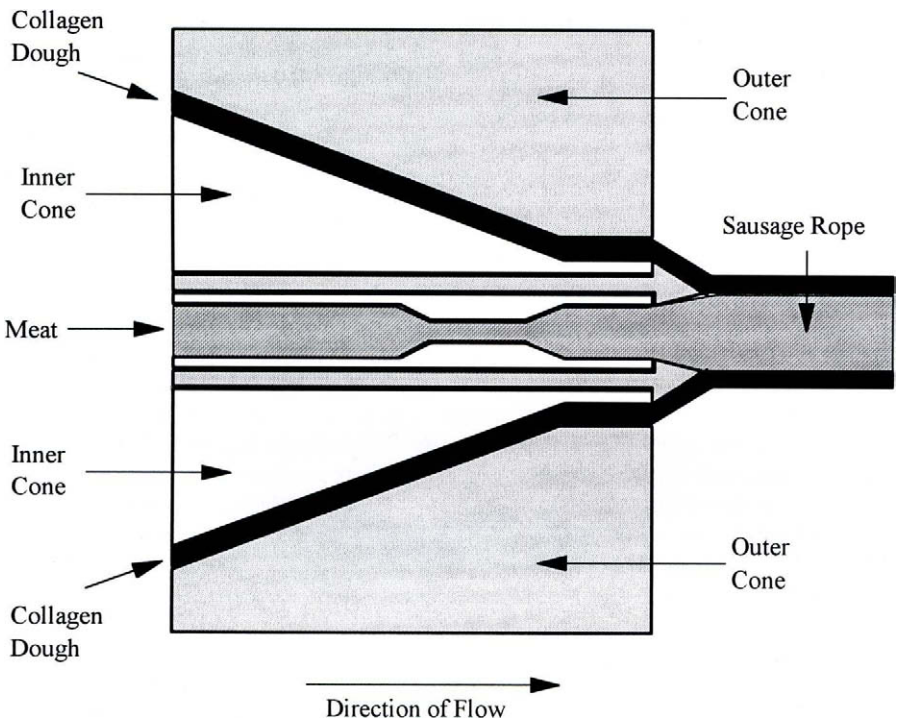

Figure 7. Co-extrusion processing set up for frankfurters (Potter, 1986).

6.7. Confectionery Products

Twin screw extruders are more common in the processing of confectionery products. This is due to the twin screw's ability to convey material, renew material at the heating surfaces, control temperatures of heat-sensitive materials, and incorporate fat, milk solids, nuts, color, and flavor portends (Harper, 1989). Twin screw extruders used for confectionery products generally have a longer length to diameter ratio (25:1) to increase the heat transfer area that is required to produce products such as licorice, peanut brittle, caramel and toffee (Harper, 1989). For example, licorice is produced by adding flour, starch, corn syrup, sugar, and a gum such as carboxymethyl cellulose (Harper, 1989; Schuler, 1986). The ingredients are blended together to make a low viscosity slurry before being pumped to the extruder. In the extruder, high pressure and temperatures dissolve the sugar and gelatinize the starch. Flavors and colors can be directly injected into the barrel to improve product characteristics. The finished product comes through the die and is cut to the desired length (Schuler, 1986). By producing licorice continuously, labor and energy is reduced compared to conventional methods.

Extrusion cooking is also used in the production of milk chocolate. After production of chocolate liquor from processing the cocoa beans, the liquor goes through several steps before it becomes milk chocolate. The chocolate liquor is first mixed with sugar, milk, cocoa butter and other flavors. This mixture is then subjected to a fine grinding step called refining by passing through revolving rollers (Potter, 1986b). The mixture then goes to a special heated mixing tank to be "conched" (Harper, 1989; Potter, 1986b). These tanks have pressure rollers that grind and aerate the melted liquor to improve smoothness, viscosity and flavor. This is usually performed at 60°C for 96 to 120 hours (Potter, 1986b). Chaveron et al. (1984) reported that this entire process is being replaced using a twin screw extruder and the total processing time runs between 2 to 3 minutes. A preconditioner is used as a mixer in this process and incorporate air into the system. The mixed product is then fed to the barrel where heat would be applied and melt the mass to form milk chocolate.

Extrusion can also be an alternative for liquid depositing of confectionery jelly materials such as "jelly bean" and "gummy bear" type products (Moore, 1989). The product is first cooked and evaporated down to its final moisture content with no extra drying being necessary. The cooked material goes to a forming extruder which is used to convey a viscous material that is thick and plastic like. Cooking extruders can also be used in this field. It has been demonstrated that jelly formulations can be cooked, extruded, and die face cut with a high speed rotary knife (Moore, 1989). This is done with instantized gelling starches that can be processed at moderate temperatures without boiling or flash-off (Moore, 1989). One problem with this technique is the temporary hot resting stage of belt travel, where the gel can most effectively form, is lost in this process. One way to prevent this is to use a chilled water spray on the die face, the emerging hot candy and the knife cutting edge. This can serve as a temporary release agent, preventing stickiness and buildup on the work surfaces (Moore, 1989).

A forming extruder is also used in the processing of jelly confectionery product that would be a "continuous rope" to be cut into pieces (Moore, 1989). The pieces are then coated with sugar or a glaze and be ready for consumption. The flavors and colors are directly injected into the preconditioner of the extruder and mixed thoroughly before entering the extruder barrel. The belt travel after extruding is cooled to allow the product to be workable. Sticking and hang-up in production will occur in this process and thus the reason to cool the

product immediately after coming through the die opening. As mentioned earlier, chilled water sprays in appropriate areas will aid in processing. The blades used are guillotine or ferris-wheel type cutters that are not at the end of the die orifice. They work after the product is cooled to a workable temperature (Moore, 1989).

6.8. Co-extrusion

The concept of co-extrusion was discussed briefly in the meat products section of the chapter. Since co-extrusion is used in several areas of food processing, we decided to separate it from the other sections and discuss it now. In co-extruded products, the outer layer is processed with a cooker extruder with a die that forms a hollow center. The center is then filled with a viscous filling which will not flow freely at ambient temperatures (Bass, 1985). The uniformity of cooking and operation of the twin screw extruder make co-extrusion more practical in the processing of snack type products as well as center filled confectionery goods (Harper, 1989). It is now possible to pump the filling directly into the center of the expanded outer product with the use of special dies developed for this process (Bass, 1985).

Several commercial products consisting of outer layers made by cooking extrusion and filled with pumpable centers have been introduced to the market. Examples of these types of products are Cadbury's "Criss Cross," Mars' "Cornquistos" and "Dooleys," and Frito-Lay's "Stuffers." No information was available on the success of these products (Abbott, 1987). It is assumed that these products are post filled or the filling is not introduced when the outer layer is expanded. The product handling and injection filling equipment necessary for these products is obviously complex and expensive.

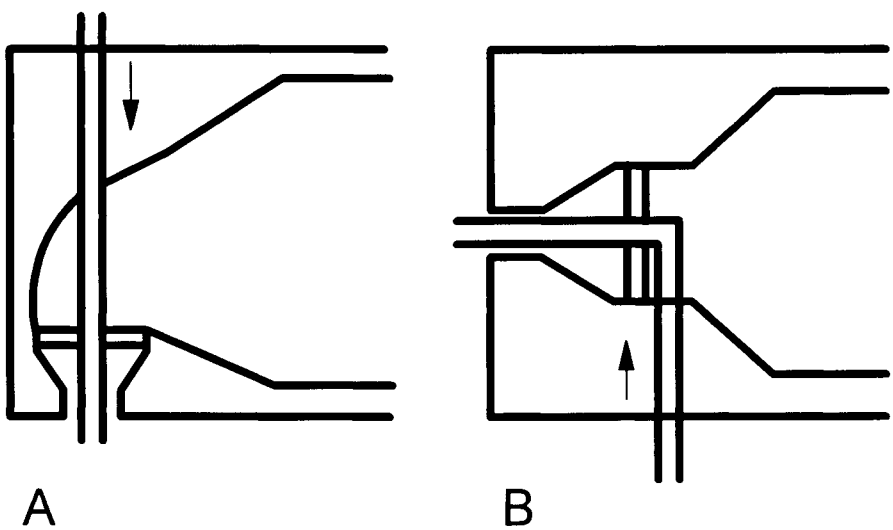

Figure 8. The two types of die designs used for co-extrusion. "A" demonstrates the extrudate being turned 90° and "B" shows the center filling being turned 90° (Abbott, 1987).

Figure 8 demonstrates the two methods available for die design. Both are based on turning the product 90 degrees in the final design. The design shown in Figure 8b is more preferred in that the smaller of the two components is turned 90 degrees as compared to designing a entire extruder to flow downward towards the floor. The overall principles behind co-extrusion is shown in Figure 9. Briefly, the crispy expanded outside of the product is kneaded into a cooked gelatinized dough forming around the smaller barrel inside the extruder that contains the filling. The inside barrel protrudes further out from the larger extruder barrel and fills the expanded outside with the desired filling. The product is then cooled enough to cut with a rotary blade before going to the dryer. To achieve the most space for remixing, it is desirable to have the center entry point as far away from the final die as possible. This, however, leads to significant passage of heat form the extrudate to the center filling, giving problems with cooling of the inner wall of the extrudate and excessive temperature rise of the center. A reasonable compromise would be a distance of 40 mm (Abbott, 1987).

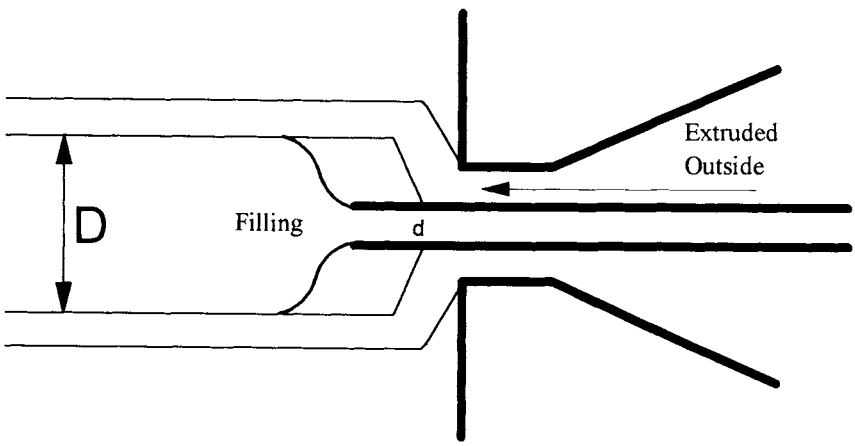

Figure 9. Cross section showing the expansion effect of a aerated filling in the process of co-extrusion. "D" refers to the diameter of the extruded outside layer and "d" is the diameter of the filling extruder barrel (Abbott, 1987).

Extrusion cooking technology has made great advancements in producing snack type products which are inexpensive to produce, made continuously and have a wide range of food applications. Co-extrusion cooking extruders can only enhance this field and continue to meet the demand for innovative snack, cereal, pet foods and candy products (Abbott, 1987).

7. CONCLUSION

Extrusion technology is starting to exert itself into all areas of food manufacturing. Several varieties of extruders are available for the productions of all types of RTE cereals, pasta, cereal-based snack foods, pet foods and aquatic feeds. More and more food companies

that used conventional methods for the production of these and other related products are slowly replacing their old processing systems with extruders. Processing speed is increased and production cost are reduced significantly.

Almost every type of ready-to-eat breakfast cereal process can be replaced with extrusion technology. With extrusion cooking being a continuous process, it is more economical than the batch type processing associated with most breakfast cereal processing. The wide variety of die shapes and sizes and cooking extruder types make any expanded RTE cereal easy to process.

The market for texturized vegetable protein has been growing steadily and is expected to do so over the next several years primary because of health concerns from consuming too much red meat. Extrusion cooking of vegetable proteins lowers the cost significantly for making TVP and makes it a economical substitute for vegetarians.

Extrusion cooking is also very useful in the pet food industry. Again, the multiple types of extruders and various shapes and sizes of extruder dies leads to a abundance of final product appearances and moistures. Both dry products as well as soft-moist pet foods can be produced with extrusion cooking. Co-extrusion can also be utilized to create a even wider variety of pet food products. Extrusion cooking can also be used to produce a wide range aquatic feeds. In this process, the density of the final extruded product can be controlled to produce a product that will either float or sink to the bottom to feed either top or bottom feeding fish (Wenger, 1992).

Cooking extruders are also being utilized as replacements for the current pasta extruders. The process for producing pasta, as mentioned earlier, requires as long drying procedure. A cooking extruder would produce a pasta product requiring minimal drying in comparison.

Current research with cooking extruders includes the extrusion of starch and starch/gum mixtures to modify the starch characteristics and possibly forming graft copolymers that would lower the cost of gums currently used in the food industry. Other current research includes using extrusion cooking to produce restructured meat products and using the extruder as a reactor for enzymatic hydrolysis of starch type products.

The possibilities are endless for extrusion cooking and their use in the food industry should continue to grow.

Acknowledgements

The authors would like to gratefully thank Wenger Manufacturing, Inc., Werner & Pfleiderer Corp., and Dr. Judson Harper and his staff at Colorado State University for their assistance in constructing this chapter. D.R. Gray would also like to thank his wife, Jean Gray, for her assistance in the preparation of this manuscript.

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Food Processing: Recent Developments
A.G. Gaonkar (Editor)

Chapter 14

Extraction technologies related to food processing

K. K. Tiwari

Department of Chemical Technology
University of Bombay
Matunga, Bombay - 400 019, India

1. INTRODUCTION

The separation of components from natural products is becoming an important part of the food, flavour, fragrance and pharmaceutical industries. The trend towards greater consumption of processed foods has created a dynamic food industry that converts raw materials into foods for which chemical and nutritional content, flavour, colour, texture and other properties are carefully defined and controlled.

Food processing industry involves handling of complex raw materials having complex molecules as chemical constituents. The need to process these materials without loss of aroma, flavour and taste is of paramount importance. Extraction is an excellent separation technique for recovery of active constituents from raw materials and for removal of undesirable constituents. The recent progress in extraction technology has been in the use of novel techniques for separation using supercritical fluid, aqueous two-phase and reverse micelle extractions.

This chapter briefly reviews the conventional solvent extraction techniques of leaching and liquid-liquid extraction used in food processing. The supercritical fluid extraction in which the dissolving power of a solvent can be manipulated to produce improved quality food materials is discussed in some detail. The aqueous two-phase extraction technique is presented, with emphasis on the separation of proteins. The other extraction techniques using reverse micelle systems and emulsion liquid membrane systems for extraction of biomaterials are discussed at the end of this chapter.

2. TERMINOLOGIES

Solvent extraction is a separation process which involves the removal of individual constituents from a mixture of solids or liquids upon addition of a solvent in which the original constituents have different solubilities. In **leaching** or **solid extraction**, a liquid solvent is used to dissolve soluble material from its mixture with an insoluble solid. In **liquid extraction**, a liquid solvent, usually an immiscible liquid, is used to separate miscible liquids by preferentially dissolving one of them. The extracted solute is separated from the solvent by distillation or evaporation.

The process of separating a mixture which is in solid state or in liquid state or in solid and liquid state by contacting it with a fluid maintained under conditions of temperature and pressure above its critical point is termed as **supercritical fluid extraction**. Separation of the dissolved substance can be accomplished either by reducing the pressure at constant temperature or by raising the temperature at constant pressure. Other terms used for this separation process are **dense gas extraction** and **supercritical gas extraction**.

Aqueous polymer/salt systems and aqueous polymer/polymer systems have the property of forming two immiscible phases. When high molecular weight, water-miscible polymers are used in these systems, biomaterials have a tendency to distribute with different concentrations between these phases. **Aqueous two-phase extraction** is the separation process which involves partitioning of solute between the two aqueous phases. The partition coefficient is manipulated by changing the type and molecular weight of polymers, type of phase-forming salt, and type and ionic strength of the added salts.

Reverse micelle extraction is a separation process where reverse micelles are used to extract solute from an aqueous phase to a reversed micellar organic phase. The reverse micelles are monodisperse aggregates of surface active molecules in an organic solvent. The Winsor type II system, a water-in-oil (W/O) microemulsion in equilibrium with an aqueous phase is of particular interest in extraction of biomolecules.

Emulsion liquid membrane extraction is a three phase process where extraction is performed by dispersing water-in-oil (W/O) emulsion in a continuous water phase (W/O/W) or dispersing oil-in-water (O/W) emulsion in a continuous oil phase (O/W/O). Usually the solute is selectively transferred from the continuous (outer) phase through a selective liquid membrane which separates the two miscible outer and inner phases. In contrast to the reverse micelle extraction where drop size may be in nanometers, the encapsulated inner droplets in the liquid emulsion membrane extraction are typically a few micrometers in diameter.

3. CONVENTIONAL SOLVENT EXTRACTION

Solvent extraction is the term used for liquid-liquid extraction as well as leaching since a solvent is used to preferentially separate one or more constituents from either a liquid or a solid mixture.

3.1. Leaching

Leaching is a widely used separation process for the following:

1) Extraction of edible oils from seeds, beans, nuts, rice bran, wheat germ, coconut and other sources.
2) Extraction of essential oils from flowers, leaves and seeds.
3) Extraction of oleoresins from spices.
4) Extraction of sugar from sugarbeet and sugarcane.
5) Extraction of coffee and tea.
6) Extraction of fish meal.
7) Extraction of active ingredients from leaves, pods, seeds, flowers and barks e.g., extraction of tocopherols, etc.

Owing to its widespread use in diverse industries, leaching is also known as lixiviation, percolation, infusion, elutriation, decantation, and settling. The two main operations in leaching are: (a) contact of liquid solvent with solid for transfer of solute from the solid to the solvent and (b) separation of the extract, from the residual solid. Other auxiliary operations are preparation of the solids for extraction and recovery of solute from the solvent by distillation or evaporation.

3.1.1. Preparation of solid material

The mechanism of leaching may involve simple physical solution or solution due to chemical reaction. The rate controlling step in leaching may be the diffusion of solvent into the mass to be leached, diffusion of the solute into the solvent or diffusion of the extract solution out of inert material of solid. Whatever the mechanism, the leaching process is favoured by the reduction in size of the solid.

The preparation of the material for extraction is to make the solute more accessible to the solvent by size reduction of solid. This gives increased surface area per unit volume of solids to be leached and reduced distance to be traversed within the solid by the solvent and the extract. The preparation of the material for extraction involves crushing, grinding, flaking or cutting into pieces or cosettes. Grinding to a very fine size may cause packing of solids during extraction such that free flow of solvent during extraction is impeded. In case of material with cellular structure, the cell rupture due to grinding may lead to extraction of undesirable components.

3.1.2. Selection of solvent

The selection of solvent for extraction of food material is made on the basis of (1) solvent capacity, (2) selectivity, (3) chemical inertness, (4) thermophysical properties of the solvent such as density, viscosity, boiling point and latent heat of vaporization, (5) flammability, (6) toxicity, (7) cost, and (8) availability. Special requirements for food products are concentration and toxicity of the residual solvent. Growing awareness of carcinogenic tendencies of certain solvents have restricted their use. Similarly, concentration of the residual solvent from the permitted category has also been fixed by the regulatory authorities. Desolventizing has to be carefully monitored to achieve the permissible residual solvent level.

Normally n-hexane, short chain alcohols (methanol, ethanol), ketone (acetone), esters (ethyl acetate, n-butyl acetate), chlorinated hydrocarbons (methylene dichloride, ethylene dichloride) and liquid carbon dioxide have been used as solvents for leaching. In view of the very low maximum permissible limit of solvents such as chlorinated hydrocarbons in food materials and regulatory restrictions, the use of chlorinated solvents is discouraged.

3.1.3. Selection of operating temperature

Temperature plays an important role in solid extraction. Higher temperatures give higher solubility of solute in solvents, permitting higher rates of extraction. However, higher temperatures may also mean high solvent losses, extraction of some undesirable constituents, and damage to some sensitive components in the plant material. A compromise is necessary in the selection of operating temperature.

3.1.4. Leaching equipment

Extraction equipment may be classified based on the mode of operation as (1) batch or (2) continuous. The equipments are divided into two principal classes with respect to the solids handled. They are: (1) fixed bed contact and (2) dispersed contact. In the former, the solids are kept in the form of a fixed bed while in the latter the solids are dispersed by moving them in a liquid solvent. The fixed bed devices may have the solvent contacting done by three methods: (a) percolaton, (b) full immersion, and (c) intermittent drainage. The dispersed contact is usually effected by suitable agitation and is used for materials which disintegrate during leaching. The extraction may be performed in one stage or multistage arrangement. Most multistage operations are performed in a countercurrent manner. Extraction equipment of various types are described in various handbooks and review articles (Coefield Jr., 1951; Brennan *et al,* 1976; Prabhudesai, 1988; Pratt and Stevens, 1992; Wakeman, 1993).

Some of the commonly used extractors described in the above-mentioned references are as follows :

Bollman extractor, Blaw-Knox extractor (Moving bed perforated basket type percolation systems)
Rotocel extractor (Multicompartment countercurrent percolation extractor)
Kennedy extractor (Multichamber unit with impellers)
De Smet extractor, Lurgi extractor (Endless belt percolation extractors)
Bonotto extractor (Vertical plate continuous dispersed solid extractor)
Hildebrandt extractor, DDS extractor (Total immersion screw conveyor extractor)
Sherwin-Williams extractor, Allis Chalmers extractor (Multistage mixer-settler disperse contact systems)
Dorr extractor (Multistage decantation system)
Diffusion battery (Multibatch countercurrent extraction system)

3.1.5. Applications

3.1.5.1. Extraction of edible oils

The major constituents of oilseed are oil, proteins, carbohydrates, crude fibre, moisture and inorganic matter. Some minor constituents are pigments, vitamins, antioxidants, and enzymes. The meat of the seed is predominantly composed of lipids and proteins, while the protective coating is largely composed of crude fibre and carbohydrates. Proteins and carbohydrates are insoluble in oil. Oil is a complex mixture of glycerides. Some esters exist as waxes which need to be recovered from the oil. Similarly, the undesirable complex lipids should not be present in the oil.

Preliminary operation before extraction consists of crushing the seed and dehulling. The meat is separated from the hulls. This is important as the oil content of hulls is very low (<1%). If hulls are not separated, they will absorb some oil as well as increase the material to be handled. The oil is distributed in the entire meat of the oilseed and is contained inside tough-walled cells. Size reduction helps in rupturing some of these cells to release the oil and also reduces the distance to which the solvent must diffuse to reach the oil.

The crushed, milled or flaked meat is charged to the extractors. Solvent, normally n-hexane, is fed to the extractors at about 50°C. Depending upon whether the operation is batch or continuous, the required quantity of solvent is contacted with the meal, using one or more contacts to reduce the oil content from the initial value of 15-19% to about 0.5% in the extracted mass.

After extraction the wet flakes contain about 40% solvent and 0.5% oil. These are carried to a desolventizer unit which may be steam jacketed paddle conveyor followed by an enclosed rotary drum dryer. The evaporator-dryer system removes the mixture of solvent and water which is condensed and the oil is separated and recycled.

The miscella, the oil-laden solvent, is fed to multiple effect evaporators and then a falling film evaporator. The evaporated solvent is condensed and recycled to extractors.

3.1.5.2. Extraction of tea and coffee solubles

Extraction of dried, blended tea leaves with hot water is accomplished in 3 to 5 stages in a diffusion battery. Temperature for extraction is raised from 70°C in the initial stage to 90°C in the final stage using intermediate heat exchangers. The final solution usually contains 2.5-5% solids. For preparing instant tea, the solution is concentrated to about 50% solids in vacuum evaporators. The volatile aroma stripped off during this stage is condensed and blended back. The solution is dried by spray, freeze or vacuum belt dryers. In place of a diffusion battery, other extractors like Rotocel or continuous countercurrent multistage extraction system with split feed may be used.

In the extraction of coffee solubles, ground and roasted coffee beans are extracted with hot water under pressure. The diffusion battery containing 5 to 10 percolators may be used. The percolator may operate in a batch or semicontinuous mode with countecurrent flow of hot water. The extraction temperature is raised from 100°C in the initial stage to 180°C in the final stage. Intermediate heat exchangers are used for this purpose. The final solution contains 25-30% solids. This is evaporated and dried to get instant coffee.

Decaffeination of tea and coffee may be done by using liquid-liquid extraction before drying.

3.1.5.3. Extraction of other materials

Spices such as black pepper, capsicum, celery and cumin and materials such as hops may be extracted by using solvents such as acetone, n-hexane, methylene dichloride, ethylene dichloride, ethanol or liquid carbon dioxide. Various patents, review articles and technical publications are available in literature. 10-15% yield of oleoresins is obtained from various spices. Flavours and aromas from fruits and fragrances from flowers are obtained by solid extraction followed by liquid-liquid extraction. It is necessary that the natural aroma, flavour, and fragrance be preserved by use of non-reactive solvents.

3.2. Liquid extraction

Liquid-liquid extraction is used in food processing to either selectively recover valuable components of a natural product or to remove undesirable components from it. Extraction of flavours and essences from crude extracts of citrus oils and separation of

monoglycerides and lecithins from edible oils are examples of recovery of valuable components. Removal of fatty acids from edible oils, partitioning of the crude oils to obtain higher unsaturation are examples of the latter category. Decaffeination using liquid-liquid extraction is an example of value addition to the coffee and tea as well as recovery of valuable caffeine.

3.2.1. Selection of solvent

The selection of solvent has been discussed in section 3.1.2 where the criteria are enumerated. In the case of liquid-liquid extraction, interfacial tension plays an important role. A low value of interfacial tension is desirable for getting a good dispersion with high interfacial area for extraction. However, a very low value will cause emulsion formation, which will create problems in phase separation. In the extraction of natural products some extracted material may also cause undesirable emulsification.

The solvent should have high solute capacity and selectivity. It should be inert to avoid formation of harmful artefacts. Robbins (1980) has given a table of solute-solvent interactions to be used for selection of solvents. Distribution coefficients may be predicted using UNIFAC or ASOG group contribution methods.

The toxicity is specially important in view of the extraction of food materials. The residual solvent concentration has to be within the specified limit. This reduces the number of acceptable solvents for extraction.

3.2.2. Liquid-liquid extraction equipment

The commercial extractors are classified in many ways. One classification is based on the manner in which the phases are contacted. The two classes of equipments are: (1) contact by gravity and (2) contact by centrifugal force. In the class of equipments where the countercurrent flow is produced by gravity, they are further classified by the type of agitation, viz., (a) unagitated columns, (b) pulsed columns, (c) mechanically agitated columns, and (d) miscellaneous devices. In unagitated columns, discrete stage contact is provided in sieve plate columns and differential contact is obtained in spray and packed columns. Similarly in pulsed columns, discrete contact is obtained in pulsed mixer-settlers and pulsed sieve plate columns and differential contact is obtained by mechanical or pneumatic pulsing. The mechanically agitated columns may be rotary devices or reciprocating devices. Mixer-settlers give discrete stage contact in this subcategory, while columns such as rotating disc contactor, Kuhni column and Schiebel column give differential contact. The reciprocating devices are typically reciprocating plate columns such as Karr extraction column. The miscellaneous equipment for extraction are static mixers, ultrasonic extractors, and pumping extractors. The centrifugal extractors also have discrete stage contact as in Luwesta and Robatel extractors and differential stage contact as in Podbielniak and Delaval extractors. Treybal (1963), Reissinger and Schroter (1978, 1984), Lo *et al* (1983, 1993), Robbins (1984), Walas (1988), and Pratt and Stevens (1992) discuss the constructional features, design, performance and selection of the liquid-liquid extraction equipment.

3.2.3. Applications

3.2.3.1. Extraction of edible oils

The hexane-extracted oil (described in section 3.1.5.1) is a mixture of triglycerides and fatty acids with small quantities of mono and diglycerides. The refining process for removal of free fatty acids is accomplished either by neutralization with aqueous alkali or by steam stripping. The neutralization process may be considered as extraction with reaction. Some suggested alternative liquid-liquid extraction processes are based on the use of solvents such as methanol, ethanol or acetone. The solvent may be an aqueous solution containing 85-95% organic solvent. Use of a mixture of hexane and aqueous ethanol for deacidification has been studied (Thomopoulos, 1971). Rotating disc contactors have been suggested for extractive deacidification. While in the case of alkali neutralization, mixer-settler units are used.

Monoglycerides present in the mixed glycerides have wide use as emulsifiers. Use of dual solvent system of ethanol and a straight chain hydrocarbon has been studied to get a fraction rich in monoglycerides (Arida *et al,* 1979).

Fractionation of triglycerides based on the degree of unsaturation of the fatty acid chains in the molecule was reported by Parsons (1976) using aqueous n-methyl pyrrolidone and dimethyl formamide as solvents. Use of propane at high pressure has been reported by Moore (1950). A special case of fish oil extraction using aqueous acetone to get enriched eicosopentanoic acid has been reported (Jap. Pat., 1984). Use of supercritical fluid extraction for the same purpose is reported by Krukonis (1988).

Separation of lecithin from crude soyabean oil using aqueous ethanol as solvent is reported by Liebing (1972). The extraction using supercritical carbon dioxide-propane (Peter et al, 1987) and supercritical carbon dioxide-acetone (Stahl and Quirin, 1985) for deoiling the lecithin have also been reported. Separation of alpha tocopherol from amaranth seed oil using conventional and supercritical carbon dioxide extraction have also been carried out (Nautiyal, 1995).

3.2.3.2. Extraction of caffeine

Caffeine content of the coffee beans is 1-2% (dry basis). The decaffeinated instant coffee contains 0.2-0.3% (dry basis) caffeine. The recovered caffeine finds ready market in the soft drinks industry. Hamm (1992) has given an excellent review of the different solvents for decaffeination, solubility of caffeine in different solvents, distribution coefficients of caffeine for water-solvent-caffeine systems at different temperatures (solvent: chloroform and dichloromethane) and solubility of caffeine in water and aqueous coffee extract (non-caffeine solubles). Solvents and extraction conditions for caffeine extraction from coffee beans are also summarized.

The liquid-liquid extraction of caffeine from the aqueous solution of coffee solubles is accomplished by using a chlorinated solvent such as dichloromethane. The first liquid-liquid extraction stage is maintained at high temperature (40-80°C). The extraction column may be a rotating disc contactor (RDC) or reciprocating plate contactor (Karr). The caffeine-rich dichloromethane stream is again contacted with water to back-extract the caffeine to the aqueous phase in a secondary extractor. The temperature is maintained

at 20-25°C with a very high phase ratio of aqueous to organic phases. The dichloromethane stripped off is recycled back to the primary extractor and aqueous stream is used to obtain caffeine. The extractor is again an RDC or Karr unit. The conditions of operation vary depending upon whether coffee solubles are obtained from green beans or from roasted coffee beans.

3.2.3.3. Extraction of other materials

Citrus oils such as lemon and orange oil contain a very large amount (>90%) of hydrocarbons and very small amount of flavour-imparting citrals, the monoterpene aldehydes. These aldehydes, neral and geranial need to be separated from the terpene and sesquiterpene hydrocarbons. Ethanol separates the citrals from the insoluble hydrocarbons. A solvent pair consisting of aqueous methanol with n-pentane may be used to separate and concentrate the citrals into the alcohol layer using a rotating disc contactor. Supercritical carbon dioxide with water as entrainer may also be used to separate the extract phase predominantly containing the citrals.

Extraction of aromas of fruit juices has been accomplished by using liquid carbon dioxide. The alpha-acids (humulones) are separated from the hop extracts by using either aqueous methanol with a hydrocarbon solvent or using aqueous potassium carbonate with petroleum spirit. Alpha-acids obtained in the polar solvent layer is stripped with n-butanol and isomerized to increase the flavour (Can. Pat., 1961; Brit. Pat., 1972).

4. SUPERCRITICAL FLUID EXTRACTION

Supercritical fluid extraction (SFE) is a unit operation that exploits the high dissolving power of fluids at temperatures and pressures above their critical values. In view of the recent advances in process and equipment design, SFE will have a significant impact on the food industry.

Advantages of SFE processes for the food industry:

1) Low temperature separations: It produces extracts from natural products where the heat-sensitive components remain undamaged.
2) Solvent residue: The extracted solute can be made almost solvent-free i.e., the residual solvent content can be reduced to almost zero ppm.
3) Solubility variation: The solubility variation of active constituents can be achieved by simply increasing or lowering extraction temperature and pressure.
4) Fractionated extraction and fractionated separation: The different constituents of natural products may be fractionally extracted and the extracted solutes may be fractionally separated.
5) Cheap solvents: Solvents can be completely recycled and also gases like carbon dioxide which are cheap may be used. This gives lower cost of solvents compared to conventional liquid-liquid extraction.
6) Low energy process: The SFE is a less energy intensive process requiring low utilities consumption.
7) Fast diffusion: The diffusivity of supercritical fluids is higher than that of liquids and viscosity is lower than that of liquids. This gives higher mass transfer rates.

8) Easy solvent recovery: Due to the high volatility of solvents they can be easily recovered.

In addition, there are no environmental risks and no fire hazards, when carbon dioxide is used as the solvent.

4.1. Industrial applications of supercritical fluid extraction in food industry

Commercial processes are available and used in the production of decaffeinated coffee and in preparing hops extract for beer brewers. These processes use carbon dioxide in the supercritical and in the liquid state.

In beer brewing industry there is a preference to carry out fermentation with hops extract, rather than using whole hops. This permits the preservation of raw material over a longer brewing season and provides a better quality raw material. Two variants of hops extraction process are used. One variant uses liquid carbon dioxide. This is offered for licence by the Brewers Research Foundation, London. Commercial plants using this process are at Pauls and Whites Brewery in U.K. and Carlton Ltd. in Australia. The other variant uses supercritical carbon dioxide. This is offered for licence by SGK of Mullheim, Germany. A plant using this technology is in operation at SKW AG, Trostberg, Germany. Pfizer in U.S.A. also uses this technology to separate the bittering agents from hops.

In addition, commercial activity or interest has been reported at over thirty companies including some end users of the technology, equipment suppliers and engineering companies. The activity is generally in the areas of extraction of spices, essential oils, oils and fats, cholesterol, flavours, pharmaceutical materials, and in supercritical fluid chromatography. All these process applications illustrate the wide range of applications of extraction with supercritical fluids in process industries. Excellent compilation for the use of supercritical fluid extraction are published from time to time. These give the status of the technology viz., laboratory scale, pilot plant scale or commercial scale. Brunner and Peter (1982), Randall (1982), Paulitis *et al* (1983) have presented some of the earlier exhaustive reviews. Koerner (1993) has given a listing of the pilot plants and commercial plants for supercritical fluid extraction. Table 1 highlights the use of supercritical fluid extraction in the processing of natural fats and oils (based on the information from various sources). Table 2 presents the operating conditions for the extraction of caffeine and coffee oil from coffee, humulones from hops, nicotine from tobacco and oleoresins from spices. As seen from the tables wide variation of conditions have been used for the extraction and separation.

4.2. Description of the process

A supercritical fluid extraction system consists of four basic components: an extractor, a separator, a utility section and a temperature/pressure control system. Additionally, other equipments such as system for the use of entrainer, valves, back pressure regulators, flow meters etc. are needed for the proper operation of the system. Schematic diagram of a supercritical fluid extraction unit is presented in Figure 1. The gaseous carbon dioxide is condensed and stored in the condenser, and brought to the required extraction pressure by a diaphragm metering pump. It is then heated to the required extraction temperature in a heat exchanger.

Table 1
Processing of natural fats and oils by supercritical fluid extraction

Mixture	Extracting agent	Entrainer	Operating conditions of	
			Extraction	Separation
Lanoline from wool grease	Propane, propylene	- -	100-115°C, 50-110 bar	100-115°C, 40-50 bar
Fat from milk, from oil seeds	Ethylene, CO_2 and (SF_6, CHF_2Cl, $CF_2=CH_2$, C_3F_8, N_2O, C_2H_6, C_3H_8)	- -	20°C, 200 bar 50°C, 350 bar	Decompression, increase in temperature, 65 bar
Oil from oil seeds	CO_2, ethane, propane	- H_2O	40°C, 220 bar 80°C, 50 bar	40°C, 20 bar 150°C, 50 bar
Acid removal, deodorization	CO_2	-	150-250°C, 100-250 bar	Adsorption on activated charcoal
Refining of natural oils	Propane, (CO_2, ethane)	-	130°C, 150 bar	40°C, 7.4 bar
Free fatty acids (oleic, stearic) removal	Ethylene	benzene acetone	100-110°C, 100 bar	125-130°C, 100 bar
Acid removal, deodoration	CO2, propane	Acetone, ethanol	70-80°C, 115°C, 135 bar	110-125°C, 135 bar
Removal of glycerides of oleic acid, purification of monoglycerides	CO_2, propane	Acetone,	70-80°C, 135 bar	110°C, 135 bar
Fractionation of cod liver oil	Ethane	-	55-90°C, 100-160 bar	Decrease in pressure

Table 2
Extraction of active substances from plant materials

Mixture	Extracting agent	Entrainer	Operating conditions of	
			Extraction	Separation
Caffeine from coffee	CO_2	H_2O	40-90°C, 160-220 bar	Absorption in water Adsorption on activated charcoal
	N_2, CO_2	Methanol, ethanol	40-80°C, 150-300 bar	Increase in temperature, absorption in water
	C_3H_8	H_2O	97-100°C 100-150 bar	Absorption in water, decrease in pressure and/or increase in temperature
	N_2O	H_2O	60-100°C, 200-300 bar	Ion exchange
	CO_2	H_2O	-	Ion exchange
	CO_2	H_2O	80-90°C, 200-300 bar	Adsorption on activated charcoal by temperature decrease
	CO_2, (SF_6, CHF_3, CHF_2Cl, CF_3Cl, C_3F_8)	H_2O	40-50°C	Decrease in pressure and/or temperature increase
Caffeine from water solution	CO_2	-	60-95°C, 250 bar	Absorption in water
Humulones from hops	CO_2, (SF_6, CHF_3, CHF_2Cl, $CF_2{=}CH_2$, C_3F_8, N_2O, C_2H_6, C_2H_4)	H_2O	35-80°C, 80-300 bar	Decrease in pressure
Oleoresins from spices	CO_2, (N_2O, SF_6, C_2H_6, C_3H_8)	H_2O	35-80°C, 80-300 bar	Decrease in pressure
Nicotine from tobacco	CO_2, Ar, SF_6, (N_2O, halogenated hydrocarbons)	H_2O	35-100°C, 80-300 bar	Decrease in pressure, adsorption on activated charcoal

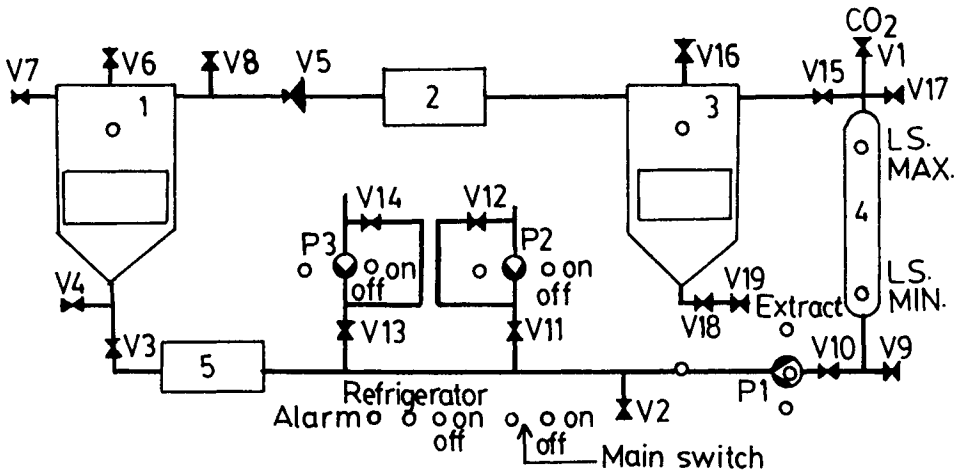

Figure 1. Schematic diagram of a supercritical fluid extraction unit

In the extractor, solid as well as liquid materials may be treated. The solid material is introduced in a basket with two filter elements at both ends. The liquid material may be pumped through the valve V-7. Stirring of the material may be done through a magnetic stirrer or a stirrer with double mechanical seal.

The pressure control of the extractor is achieved by a pressure controller. The gas from the extractor contains the extracted solute. The extract is separated in the separator either by reducing the pressure or by increasing the temperature. Two hand valves V-18 and V-19 are installed for the removal of the extract in case of batch extraction of solid materials.

The evaporated carbon dioxide from the separator is condensed in the precondenser and stored at low temperature in the condenser vessel. The pressure in the condenser is kept constant by a refrigerator.

Process conditions most widely recommended for supercritical extraction with respect to the extracting fluid are:

Extraction	*Separation*
$T = (1.2 \text{ to } 1.3)T_c$	$T = (1.3 \text{ to } 1.5)T_c$
$P = (2 \text{ to } 3)P_c$	P = P separation or
	$P = (0.5 \text{ to } 2)P_c$

The separation process is essentially determined by the capacity and selectivity of the extracting supercritical fluid and their dependence on temperature and pressure. The

superficial velocity of the extracting agent, the pressure and temperature in the separator and the time of extraction are the other variables which affect the extraction process.

Figure 2 presents a flow sheet for a multiextractor unit with parallel feed of supercritical carbon dioxide. The separation is done in two stages by using two separators in series. Thus, the components which have lower solubility at intermediate pressure are separated and the remaining solute is separated in the second separator. The fractionation of volatile oil from oleoresin can be obtained in this manner for spice extraction.

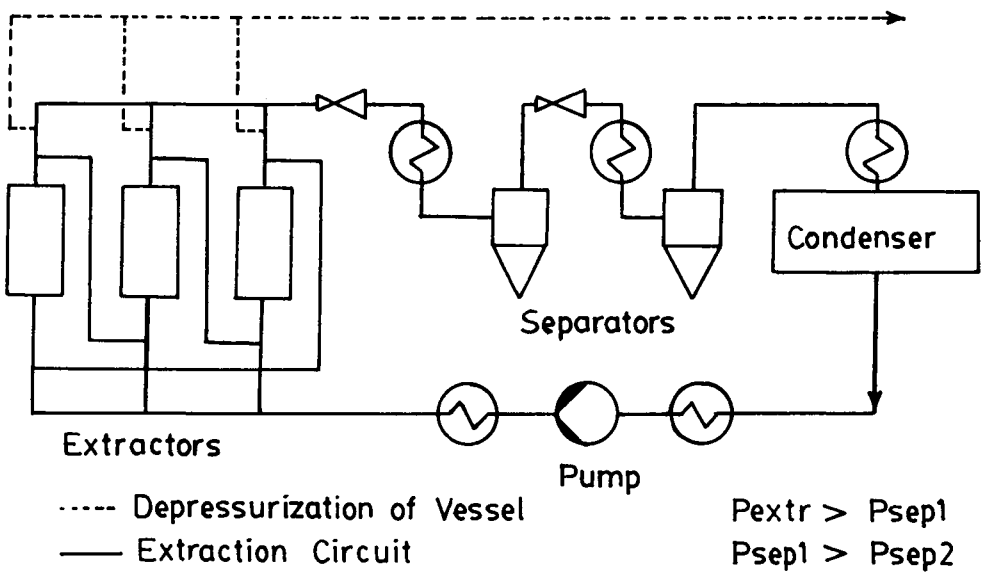

Figure 2. Flowsheet of SFE with two-stage separation

4.3. Design criteria

Design of a plant for high pressure extraction must be based on the required rate of production and the nature of employed feed. In principle, a distinction must be drawn between the following possibilities :-

1) Purification of the feedstock: The feed raw material is regarded as the product from which certain components must be removed during the extraction process. Examples being the production of decaffeinated coffee and of nicotine-free tobacco.
2) Recovery of an extract: In this case, bulk of the feedstock is regarded as a practically valueless carrier source of the extract. Examples are the production of hops extract, cocoa butter and various spice extracts.

Requirements of plant design differ only in relation to the mechanical treatment of the raw material, e.g., its feed into the extraction equipment or agitation. In the case of solids recovery mechanical effects should be avoided as far as possible, whereas in the production of a soluble extract such effects may be altogether desirable.

Furthermore, it may be required to recover both the solids as well as certain extracted components. In addition, there are cases where the plant must be operated in two stages; extracting one component from the feedstock, storing it temporarily and, after the extraction of second constituent, returning it to the bulk material. This procedure is adopted when a certain component A is to be selectively removed while retaining a component B in the feedstock which is more volatile than A. For example, in the high pressure extraction of tea, it is essential to remove the tea aroma from a batch fed into an extraction vessel in order to add this aroma to an already decaffeinated batch in another vessel. A similar situation exists in the case of tobacco, where nicotine and aroma are extracted separately and the aroma is subsequently blended back into the tobacco. In a multistage plant, a fractional extraction can be operated under precisely defined process conditions for the individual stages.

4.3.1. Parameters to be determined in process design

As a basis for sizing of the plant and of individual items of equipment, a prediction or prior knowledge of some process data is required. Information on the following is necessary:

1) Required rate of production.
2) Mode of operation of the plant (continuous or batchwise).
3) Bulk density of the solid feed. The bulk density depends not only on the density of the solids but also, to a large extent, on the form and consistency of the material and on its moisture content. It must, therefore, be determined in the course of preliminary experiments.
4) Ratio of solvent mass flow rate to the mass of solids treated.
5) The extraction time.

Out of these parameters, the required rate of production is specified. Depending upon this rate, the mode of operation i.e., whether batch or continuous, is decided. Bulk density of solids feed, as explained earlier, must be experimentally determined. These three parameters enable the volume of the extraction vessel to be calculated.

The quantity of solvent to be introduced into the plant is determined precisely from the required operating temperature and pressure. Then, by knowing the time of extraction the mass flow rate of solvent can be calculated.

As yet there is no model for a mathematical description of the predominantly non-steady state processes of extraction with supercritical fluids. Hence, one cannot predict the time of extraction from any model and one must rely on the results obtained in laboratory experiments. For a particular feed, the extraction time will differ for different solvents and entrainers as well as with different operating conditions. For predicting this extraction time or residence time of the solvent in the extraction vessel, mass transfer coefficients will have to be predicted. These values are not available at the present time.

Further, basic parameters required for the design are the operating pressures and temperatures. In addition to process data, e.g., the required supply and removal of energy, structural characteristics of the plant also depend on these variables. Thus, wall thicknesses must be determined and sealing problems solved, and this depends on the ranges of pressures and temperatures applied and on the associated load cycles in the plant.

Accurate knowledge of the physical properties of the solvent, the raw material and the extract are also required. For example, density and viscosity are required for knowing flow characteristics. The decomposition temperature of the raw material is particularly important from the point of view of determining operating temperature.

Data on the flow resistances and pressure losses in beds of the solid material and parts of the plant through which the solvent flows are also important with regard to choice of the conveying system for the solvent. These can be obtained either from theoretical considerations or from preliminary experiments.

Finally, mass and energy balances must be drawn up for the design of many plant components, e.g., for all equipments performing heat transfer function.

4.3.2. Thermodynamic considerations

To determine if an extraction of interest is technically and economically feasible, it is necessary to have an adequate quantitative representation of the phase equilibria between the solute(s) and the solvent(s) involved. Without this information, process models can not be made, and few conclusions can be drawn concerning equipment size, operating conditions, solvent flow rates, and extraction yields. Mixtures of materials at supercritical conditions exhibit highly non-ideal behaviour and do not lend themselves easily to quantitative data correlation. This is particularly true for materials of biological origin (King and Bott, 1982).

However, in simpler situations some progress can be made. The well known Soave-Redlich-Kwong and the Peng-Robinson equations of state are most frequently used for phase equilibrium calculations. Due to the difficulty in predicting phase equilibria, especially for natural products, only a crude estimate of operating pressures and temperatures can be made. For design, one must then rely upon the results obtained from experiments carried out with the material to be extracted.

4.4. Applications to food industry

Some of the applications of supercritical fluid extraction are discussed.

4.4.1. Oilseeds extraction

The extraction of various oilseeds like avocado, castor beans, corn germ (wet milled), cottonseed, peanut, rapeseed, sesame, soyabean and sunflower using supercritical carbon dioxide have produced good quality oil and undegraded meal in high yield. Figure 3 gives the solubility of corn oil in supercritical carbon dioxide as a function of pressure at different temperatures. It can be seen that high solubility of corn oil is obtained at higher pressures. The rate of increase of solubility with pressure is lower in the high pressure region. This is considered for the value of pressure to be used for extraction.

The overall yield of extraction with supercritical carbon dioxide compares well with that obtained by hot hexane extraction. The carbon dioxide-extracted oil has a very low phosphatide content. Therefore, it has the advantage of being equivalent to a degummed, hexane-extracted crude oil (Friedrich and List, 1982).

Some work using carbon dioxide at lower pressures has attracted a lot of attention. Various advantages are obtained by extracting the oil at lower or near critical conditions.

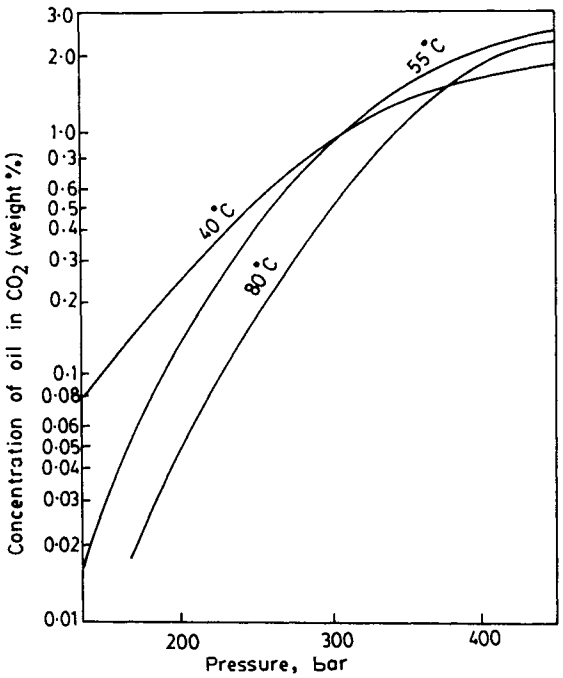

Figure 3. Solubility of corn oil in CO_2

4.4.2. Cholesterol extraction (Anon., 1988, 1989, 1990; Froning *et al,* 1990)

A high cholesterol diet can accelarate the development of atherosclerosis with its dual sequelae of thrombosis and infarction. In view of this, production of cholesterol-free butter, cheese, ice cream and egg yolk is drawing the attention of the food processing industry. NutraSweet (Deerfield, IL., USA) is commercially producing low fat, low cholesterol egg yolk powder. Various companies (Phasex Corp., USA; Supercritical Processing, USA; SKW, Germany) have set up pilot plant facilities for cholesterol extraction using supercritical fluids.

The process for cholesterol-free dairy products involves separation of fats from the milk in a centrifugal separator. The cholesterol is then removed from the fats by extraction with supercritical carbon dioxide. Use of methanol as entrainer increases the solubility of cholesterol in fluid phase by an order of magnitude. The cholesterol-free fats are reblended into the milk by conventional methods.

A typical plant for supercritical fluid extraction of cholesterol from butter oil is shown in Figure 4. Butter oil is fed into the extraction column between two packed sections. The supercritical carbon dioxide at 40°C and 175 bar is fed at the bottom of the column for countercurrent extraction. The cholesterol-laden carbon dioxide flowing up through the top packed section is contacted with the cholesterol-rich extract which is refluxed at the top. The cholesterol-rich extract is obtained as the top product and low-cholesterol butter oil is obtained as the bottom product. The carbon dioxide is recycled from the

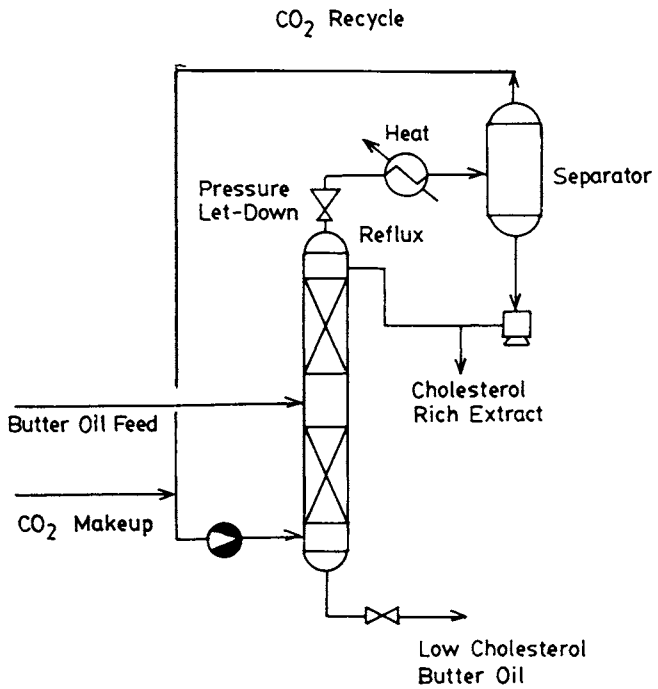

Figure 4. Process for supercritical CO_2 extraction of cholesterol from butter oil

separator to the bottom of the column. Conditions in the separator are subcritical for carbon dioxide and require the carbon dioxide to be recompressed with make up carbon dioxide for reuse in the process. In case entrainer is used for the separation, it is fed with the butter oil. Two columns may be used in place of two packed sections in one column. Some plants use sieve plate columns for the extraction process.

Froning *et al* (1990) have reported removal of approximately two-third of the cholesterol from spray dried egg yolk using supercritical carbon dioxide at 306 atm, 45°C and 374 atm, 55°C.

4.4.3. Decaffeination of coffee

A commercial process for removing caffeine from green coffee beans has been in commercial production at Hag AG (a unit of General Foods) in Bremen, Germany, for many years. The process was developed and commercialised under licence from SGK of Mullheim, Germany.

Cleaned, moisturized coffee beans are extracted to a caffeine level of about 0.1% with supercritical carbon dioxide at 120 bar and 40°C. The carbon dioxide after extraction passes over an activated carbon bed where the extracted caffeine is adsorbed. The carbon dioxide is then recycled to the extractor. Solids are charged and discharged in a batch manner.

4.4.4. Spices extraction

The standardization in the food industry has led to the spice extracts or oleoresins to be used in place of the spices. Hubert and Vitzthum (1978) have obtained extracts of spices like pepper, nutmeg and chillies. The degree of extraction of piperine from pepper was almost 98% and that of essential oil was 81%. The extract was yellow as compared to the olive-green with methylene dichloride as solvent. Similarly, the extraction of capsaicine was 97% from chillies. RAPS & Co. in Germany is commercially marketing oleoresins of a large number of spices (RAPS Bulletin, 1994; Hartmann, 1993).

Tables 3, 4, and 5 give results of the extraction of cumin, celery and ajowan seeds, respectively (Mishra and Tiwari, 1994). The extraction with supercritical carbon dioxide was performed at conditions: temperature, 35-55°C; pressure, 80-450 bar; batch time, 3 hours; and flow rate of carbon dioxide, 5-20 kg CO_2/(kg spice)(hour). Comparison is presented with hexane extraction (Soxhlet apparatus, 5 hours batch time) and hydro-distillation. Commercially available steam distilled volatile oil was also analysed. These results are presented in the tables. In all cases, the volatile oil could be obtained at 80-100 bar pressure and supercritical temperature. Good quality oleoresin was obtained at 200 bar and 35°C.

Table 3
Comparison of extracts of cumin seed extracted by different methods

Method of extraction	Oleoresin extracted (wt% of seed)	Volatile oil separated (wt % of seed)	Composition of volatile oil						Color of oleo-resin	Color of vola-tile oil
			1	2	3	4	5	6		
Supercritical fluid extraction	2.76-8.16	2.60-3.20	1.11	15.9	17.7	25.1	15.3	22.9	Yellow brown	Pale yellow
Solvent extraction using hexane	10.4	3.14	0.98	13.1	16.9	23.8	16.7	22.3	Dark brown	Pale yellow
Hydro-distillation of ground seed	—	2.96	0.89	15.8	17.8	24.6	19.2	19.4	—	Pale yellow
*Steam distillation	—	—	1.04	14.9	23.8	26.1	19.0	13.7	—	Pale yellow

1. Alpha-pinene 2. Beta-pinene 3. Para-cymene
4. Gamma-terpinene 5. Cuminaldehyde 6. 1,3- and 1,4-p-Menthaldiene-7-al

* Commercial sample obtained from Synthite Chemicals Ltd., India.

Table 4
Comparison of extracts of celery seed extracted by different methods

Method of extraction	Oleoresin extracted (wt% of seed)	Volatile oil separated (wt% of seed)	Composition of volatile oil				Color of oleo-resin	Color of vola-tile oil
			1	2	3	4		
Supercritical fluid extraction	1.14-6.53	0.98-3.77	33.3-64.2	10.9-19.4	2.49-12.6	6.89-40.9	Yellow brown	Pale yellow
Solvent extraction using hexane	12.2	3.55	40.1	12.3	6.7	14.9	Dark brown	Pale yellow
Hydro-distil-lation of groud seed	—	1.90	61.9	17.8	4.7	8.65	—	Pale yellow
*Steam distillation	—	—	65.6	16.7	3.3	5.19	—	Pale yellow

1. Limonene 2. Beta-selenene 3. 3-Butyl phthalide 4. Sedanoic anhydride

Table 5
Comparison of extracts of ajowan seed extracted by different methods

Method of extraction	Oleoresin extracted (wt% of seed)	Volatile oil separated (wt% of seed)	Composition of volatile oil				Color of oleo-resin	Color of vola-tile oil
			1	2	3	4		
Supercritical fluid extraction	1.42-9.15	1.39-4.12	3.49-6.87	17.9-30.1	21.4-35.4	25.6-55.9	Yellow brown	Pale yellow
Solvent extraction using hexane	13.8	3.65	3.03	16.1	20.2	58.8	Dark brown	Pale yellow
Hydro-distil-lation of groud seed	—	3.44	3.78	18.7	21.3	54.7	—	Pale yellow
*Steam distillation	—	—	3.66	21.7	28.8	43.8	—	Pale yellow

1. Beta-pinene 2. Para-cymene 3. Gamma-pinene 4. Thymol
* Commercial sample obtained from Synthite Chemicals Ltd., India.
Note: Odour of oleoresin and volatile oil obtained by supercritical extraction was like that of fresh crushed seed.

4.4.5. Hops extraction

The use of hops extract in the production of beer has increased to the point that almost 25% of the world's supply of hops is converted to hops extract.

The desirable constituents of hops are the soft resins which contain an alpha-acids fraction (mixture of several humulones). In the brewing process, the alpha-acids are isomerised to give beer its characteristic bitter taste. Thus, in the production of hops extract, it is necessary that the extraction conditions favour the extraction of the soft resins with minimal production of undesirable by-products. The process conditions used with supercritical carbon dioxide are in the temperature range of 35-80°C and pressure range of 80-300 bar. This gives an olive-green, pasty extract with an intense aroma of hops. The extent of extraction of alpha-acids is almost 99% and is above the required minimum of 95%. The extract is superior to the ones obtained by using either methylene dichloride or n-hexane as solvent.

The hops extraction may also be carried out by using liquid carbon dioxide at lower temperatures and pressures of 50-70 bar. The hard resins can be eliminated from the extract in this way.

5. AQUEOUS TWO-PHASE EXTRACTION

Biomaterials derived from or produced by the plants, animals or the micro-organism mainly consist of proteins, fats and carbohydrates. Production of biomaterials on a commercial scale involves cell disruption followed by separation of the desired products. The cost of the separation step may be as high as 90% of the total cost of the production. For the separation of enzymes and proteins, aqueous two-phase extraction process has become a versatile and efficient method. The method can be used for the removal of cell debris as well as further purification of the biomolecules. Although, not of current interest, the developments in the field of biotechnology for separation using aqueous two-phase extraction will be of importance for macromolecules of interest to the food industry.

Aqueous two-phase systems (ATPS) are known for a long time. Beijerinck (1896) observed the formation of two liquid phases upon mixing agar with soluble starch or gelatin. However, a breakthrough in the useful aqueous two-phase systems was made by Albertsson (1956) when he obtained aqueous two-phase systems in which both phases contained very high concentration of water. This solved the problem of denaturation and precipitation of proteins.

The advantages of the aqueous two-phase extraction process are: (1) biocompatibility, (2) easy processing, (3) high capacity, (4) easy and precise scale-up, (5) high product yields, (6) high potential for continuous processing, and (7) low investment cost. The aqueous two-phase systems have extreme physical properties compared to the conventional liquid-liquid extraction systems. The interfacial tension is in the range of 10^{-3}-10 mN/m; phase viscosities are usually 1-10^3 mPa.s; and density differences between phases are low, ranging from 20-100 kg/m^3. The performance of extraction equipment for the aqueous two-phase systems is markedly different from that of conventional equipment.

The ATPS are usually based on polymer-polymer, PEG/Dextran being the most common, and polymer-salt systems. The salt-based systems are more economical because of low cost of the phase-forming components and are also easy to handle unlike dextran-based systems which are characterized by high viscosity. The two phase polymer-polymer systems and polymer-salt systems have been studied by many workers. Review articles by Koningsveld (1963), Cebezas *et al* (1990), Forciniti and Hall (1990) and Diamond and Hsu (1992) summarize the work in this field. Figure 5 presents a typical phase diagram for PEG (4000)-sodium sulfate-buffer system. Sodium orthophosphate solution in required quantity was used as buffer.

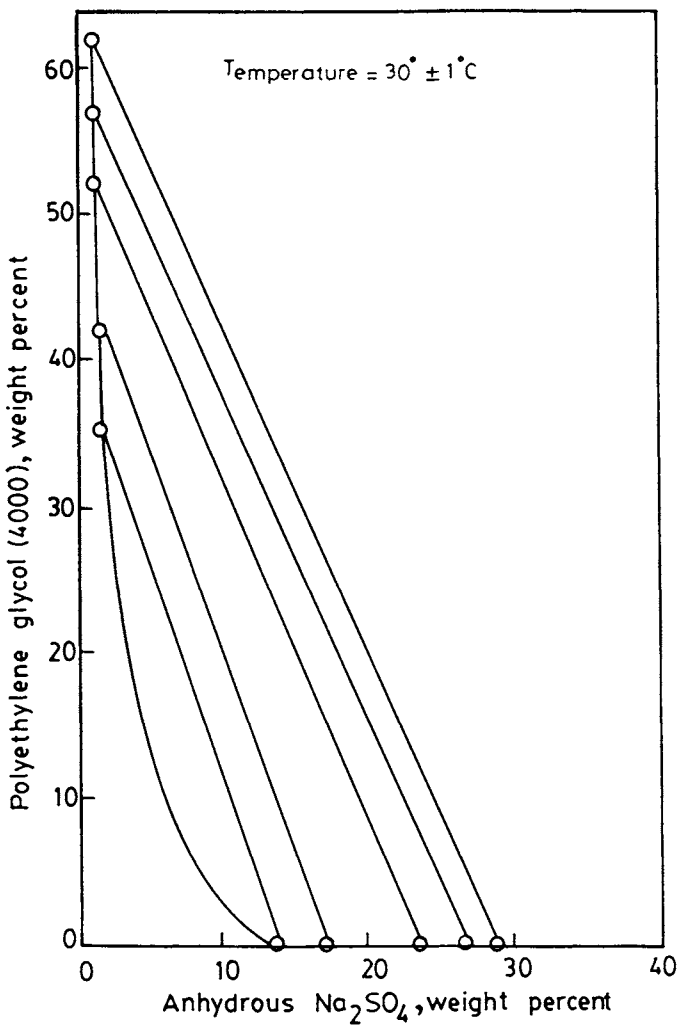

Figure 5. Phase diagram of the PEG(4000)-sodium sulphate-buffer system

5.1. Protein partitioning

Protein partitioning in ATPS depends on many factors such as phase polymers, the ionic composition and the partitioned substance. The type of polymer as well as their molecular weights and the presence of certain chemical groups influence the partitioning of proteins. The ionic composition is of vital importance as the sign and magnitude of the interfacial electric potential are determined by the ions present. The properties such as size, charge and biospecific surface properties, presence of receptors and biospecific ligands and chirality govern partitioning. The partitioning of a protein between the top and bottom phases is defined by the partition coefficient (m) and is the ratio of concentrations in the top and bottom phases. The overall partition coefficient is resolved in a number of factors as given below:

$$m = m^{o}.m_{el}.m_{hfob}.m_{biosp}.m_{size}.m_{conf} \quad (1)$$

where the suffices el, hfob, biosp, size and conf stand for electrochemical, hydrophobic, biospecific, size dependent and conformational contributions, respectively, to the partition coefficient. The m^{o} includes all other factors such as relative solvation of the solute molecule in the phases.

Increased polymer concentration shifts the phase system away from the critical point and the physical properties of the coexisting phases become more different. The partitioning of the protein becomes more favourable. However, cell organelles adsorb more strongly and selectively to the interface when polymer concentration increases.

Molecular weight of the polymer affects the partitioning of high molecular weight proteins. The higher the molecular weight of the polymer, the lower will be the partition coefficient.

Salts have a paramount effect in the partitioning of all kinds of molecules and cell particles. Salts with different ions have different affinities for the two phases. An electric potential is created between the phases. A salt with two ions that have different affinities for the two phases will generate larger potential difference.

The isoelectric point of proteins can be determined by a cross-partition and partition coefficient is determined as a function of pH with two different salts (Albertsson *et al,* 1970).

Presence of a charged polymer in one of the phases has a stronger effect on the partitioning of charged macromolecules than that of the salts (Johansson *et al,* 1973).

Hydrophobic groups such as palmitate bound to polyethylene glycol (PEG) show increased affinity for proteins with hydrophobic binding and cause protein to be partitioned in the PEG-rich phase.

Affinity ligands attached to one of the polymers can be used to extract ligand-binding proteins and nucleic acids into the corresponding phase.

The composition of the two phases changes with temperature. Proteins partition more equally between phases of a two-polymer system when temperature is increased. This effect may be counteracted by using higher concentrations of polymer.

5.2. Aqueous two-phase separation of protein mixtures

Partitioning in two aqueous phases can be done for the separation of proteins from cell debris and for purification from other proteins. Differences in partition coefficients between different proteins are high. Therefore, the number of stages for purification is

less. First stage of extraction in PEG-rich phase and a second stage for back-extraction in a salt phase may purify the enzyme (Hustedt, 1985). Partitioning may be done in a multistage unit or a continuous extraction unit such as spray tower or a rotating disc contactor. Albertsson (1986), Nguyen *et al* (1988), Stewart (1990) and Pathak *et al* (1991) have presented various aqueous systems used for extraction of biomolecules. Table 6 provides examples of partitioning of proteins in aqueous two-phase and three-phase systems. Table 7 gives examples of proteins purified by subsequent extraction steps. The table also includes information on the number of extraction steps, enrichment factor and the final yield.

A typical process for enzyme purification is shown in Figure 6. The cells are disrupted by wet milling and passed through a heat exchanger. The PEG and salts are added into this stream of broken cells. After mixing and attainment of equilibrium, the phases are separated. The product-rich top PEG phase is sent to a second mixer after addition of more salt. The bottom salt phase containing cell debris and proteins is discarded. After attaining equilibrium in the second back-extraction mixer, the process stream is sent to a separator. The top PEG phase is subjected to by-product recovery and recycled or goes to waste. The bottom salt phase is used for the product recovery. A large scale separation using this strategy was reported by Kula *et al* (1982).

Table 6
Examples of partitioning of proteins in aqueous two-phase and three-phase systems

Protein	Main System Components	Reference
Albumins	Dextran, PEG	Albertsson *et al* (1970); Johansson *et al* (1970a,b, 1978)
Haemoglobins	Dextran, PEG	Albertsson *et al* (1970); Silverman *et al* (1979);
Proteins from Baker's yeast (glycolytic enzymes)	Dextran, PEG	Johansson and Hartman (1974) Johansson *et al* (1973), Shanbhag *et al* (1972)
Histone protein	Salt, PEG Dextran, PEG	Bidney and Reeck (1977) Axelsson and Shanbhag (1976) Gineitis *et al* (1984)
Membrane proteins	Dextran, PEG Detergent	Albertsson and Andersson (1981); Svensson *et al* (1985)
Protein from micro-organisms	Salt, PEG Dextran, PEG	Hustedt *et al* (1983) Kroner *et al* (1982)
Cellulases	Dextran, PEG	Tjerneld *et al* (1985)
Fusion protein BetaGal	Salt, PEG	Strandberg *et al* (1991)

Table 7
Examples of proteins purified by subsequent extraction steps

Enzyme	Organism	Number of extraction steps	Enrichment factor	Final yield	Source
Formate dehydrogenase	*Candida boidinil*	3	4.4	78	Kroner *et al* (1982)
Aspartase	*E.Coli*	4	18.0	82	Hustedt *et al* (1983)
Fumarase	*Brovibacterium ammonigagenes*	2	22.0	75	ibid
Penicillin acylase	*E.Coli*	2	10.0	78	ibid
L-2 Hydroxy-isocaproate dehydrogenase	*Lactobacillus confusus*	2	21.0	90	Hummel *et al* (1984)
Interferon	Human fibroblasts	1	>350.0	75	Menge *et al* (1983)

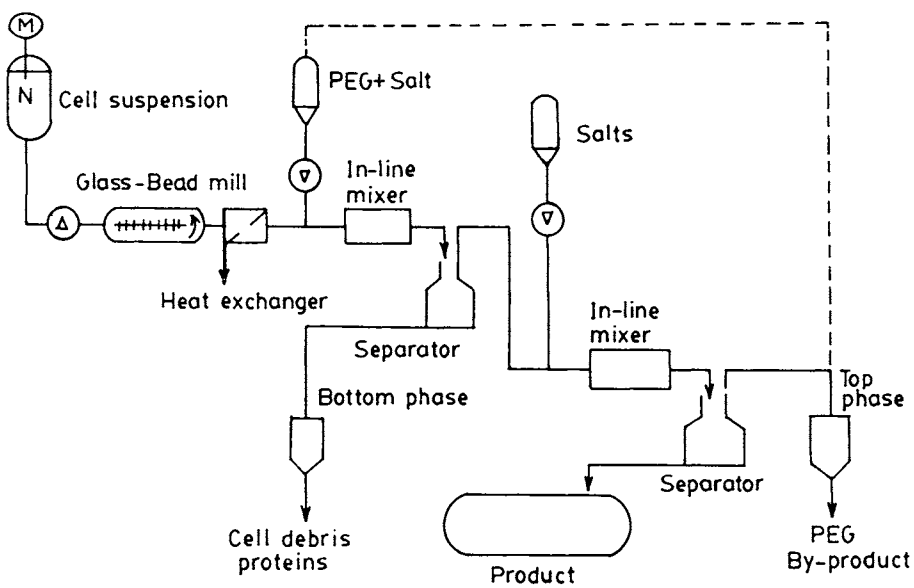

Figure 6. Scheme for enzyme purification by liquid-liquid extraction

Enzyme/protein mass transfer coefficients in polymer/salt systems in spray columns have been studied by Pawar *et al* (1993). The spray columns were modified to have larger cross sectional area of the coalescing zone. This resulted in a higher dispersed phase (PEG-rich) throughput (7 fold). Correlations for enzyme/protein hold-up and mass transfer coefficients have been presented. Hydrodynamics and mass transfer for enzyme extraction in polymer/salt systems have also been reported by Bhavsar *et al* (1994). The extractor used was a sieve plate column. Use of hydrodynamic cavitations for large scale microbial cell disruption has been studied by Save *et al* (1994). It was found that the cavitation does not have adverse effect on enzymes such as invertase and glucosidase. Also, the hydrodynamic cavitation was simpler and more energy-efficient as compared to the conventional mixer system. All the above studies help in the equipment design for continuous processing of enzyme/protein systems.

6. REVERSE MICELLE EXTRACTION

The extraction and purification of proteins using reversed phase micelle systems has been the subject of extensive study in recent years (Luisi, 1985; Martinek *et al,* 1986; Fletcher and Parrot, 1988; Krei and Hustedt, 1992; Matzke *et al,* 1992). Reverse micelles are aggregates of surfactant molecules in organic solvents. The polar head groups of the surfactant are directed towards the interior of the micelle and form a polar core which can solubilize water and hence the proteins, while the lipophilic chains are exposed to the organic solvent. Figure 7 shows a schematic diagram of protein solubilization using reverse micelles. Here an electrolyte/protein solution is contacted with an organic solvent containing oil-soluble surfactant. After the mixture is vigorously shaken and interphase equilibrium is achieved, the phases are separated by centrifugation. When a double tailed

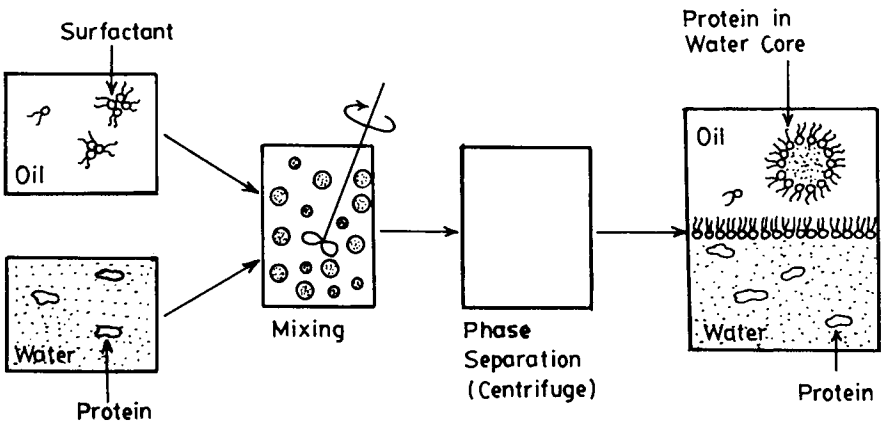

Figure 7. Schematic diagram of the phase transfer process for solubilization in reversed micelles

surfactant such as AOT (2-diethylhexyl sulfosuccinate or Aerosol-OT) is used with electrolyte in the aqueous phase, a water-in-oil (W/O) microemulsion is formed. This is also favoured by addition of long chain alcohols and increase in temperature.

The greatest advantage of using reverse micelles to solubilize proteins is that the proteins can be used in apolar solvents. Thus, enzymes have been encapsulated inside the water cores of reverse micelles with retention of catalytic activity (Barbaric and Luisi, 1981; Levashov *et al,* 1982; Fletcher *et al,* 1984). Table 8 illustrates some of the recently reported work on protein solubilization using reverse micelle extraction.

Table 8
Some recent studies on reverse micelle extraction of proteins

Protein/Enzyme	System	Reference
Horse liver alcohol dehydrogenase (HLADH)	SDS & Cetyltrimethyl-ammonium bromide (CTAB)	Samana *et al* (1984)
Lysozyme	Iso-octane/AOT	Steinmann *et al* (1986)
Lysozyme	Chloroform/CTAB	ibid
Lysozyme	Iso-octane/Tetraethylene glycol dodecyl ether	ibid
Beta-hydroxy butyrate dehydrogenase	Iso-octane-hexanol/CTAB	Giovenco *et al* (1987)
HLADH	Triton X-100	Lee and Biellmann (1987)
Protease from Bacillus sp. ATCC 21536	—	Woll *et al* (1987)
Lysozyme	Iso-octane/AOT	Fletcher and Parrot (1988)
Alpha-chymotrypsin	Isotridecanol in iso-octante/ Aliquat 336	Jolivalt *et al* (1990)
Beta-amylase	Iso-octane/TOMAC	Dekker *et al* (1991)
Horseradish peroxidase	Iso-octane/AOT	Paradkar and Dordick (1991)
Alpha-chymotrypsin	Hexane/Tween-85	Ayala *et al* (1992)
Haemoglobin	Hexane/Tween-85	ibid
Alpha-chymotrypsin	Iso-octane/AOT	Ichikawa *et al* (1992)
Alpha-amylase	Iso-octane-hexanol/CTAB	Krei & Hustedt (1992)
Yeast alcohol dehydrogenase (YADH)	Iso-octane/AOT	Sarcar *et al* (1992)

The solubilization of the proteins in the reverse micelles depends upon several factors. These include pH, ionic strength and ion type, choice of surfactant and solvent, water content of the micellar core and the physicochemical properties of the protein such as isoelectric point, hydrophobicity, size, charge density and charge distribution. Temperature has also been found to affect the solubilization process.

Leodidis and Hatton (1989) have reviewed the interphase transfer for selective solubilization of ions, aminoacids and proteins in reversed micelles. A phenomological model capable of predicting the selectivity of the water pools for the different cations in the AOT system has been developed by them. Solubilization of various amino acids in AOT reverse micelles has been analyzed and the partition coefficient has been correlated with a number of meaningful parameters. Similarly, the liquid-liquid extraction of proteins from an aqueous solution to a reversed micellar organic phase have been analyzed for the effect of pH, surfactant concentration and salt.

Hatton and his coworkers (Goklen and Hatton, 1987; Hatton 1989; Woll *et al,* 1989; Rahaman *et al,* 1988) have shown that the selective extraction of targetted protein and extra cellular alkaline protease can be achieved by manipulating several parameters such as the aqueous phase pH, ionic strength and the biospecific nature and concentration of ligand.

The denaturation of proteins has been reviewed by Sadana (1994). He has discussed the effects of concentration of surfactant, type of solvent, temperature, processing conditions and solubilization process on the protein inactivation by compiling data from different sources.

Reverse micelle extraction is of great importance as it can be made selective, is easily scalable, and can be operated continuously. The solubilized protein can be partitioned back into aqueous phase by suitable modification of the conditions such as pH, ionic strength, etc.

7. EMULSION LIQUID MEMBRANE EXTRACTION

Extraction using emulsions, often termed as emulsion liquid membrane (ELM) was first perceived by Li (1968). Since then, ELM processes have been developed for a diverse variety of applications. Oil-in-water (O/W) emulsions are used for extraction of solutes from non-aqueous solutions while water-in-oil (W/O) emulsions are used for the extraction of solutes from aqueous solutions. The potential of ELM as an effective separation technique in the area of biotechnology has not been fully explored. The promising applications are encapsulation of enzymes and separation of products from fermentation broth.

7.1. Extraction system

A typical scheme for ELM is shown in Figure 8. An emulsion liquid membrane is formed by creating a dispersion of the stripping phase within an organic solvent which forms nonporous film around the stripping phase droplets. The emulsion is stabilized by surfactants of proper HLB values. The organic solvent may be a kerosene-xylene

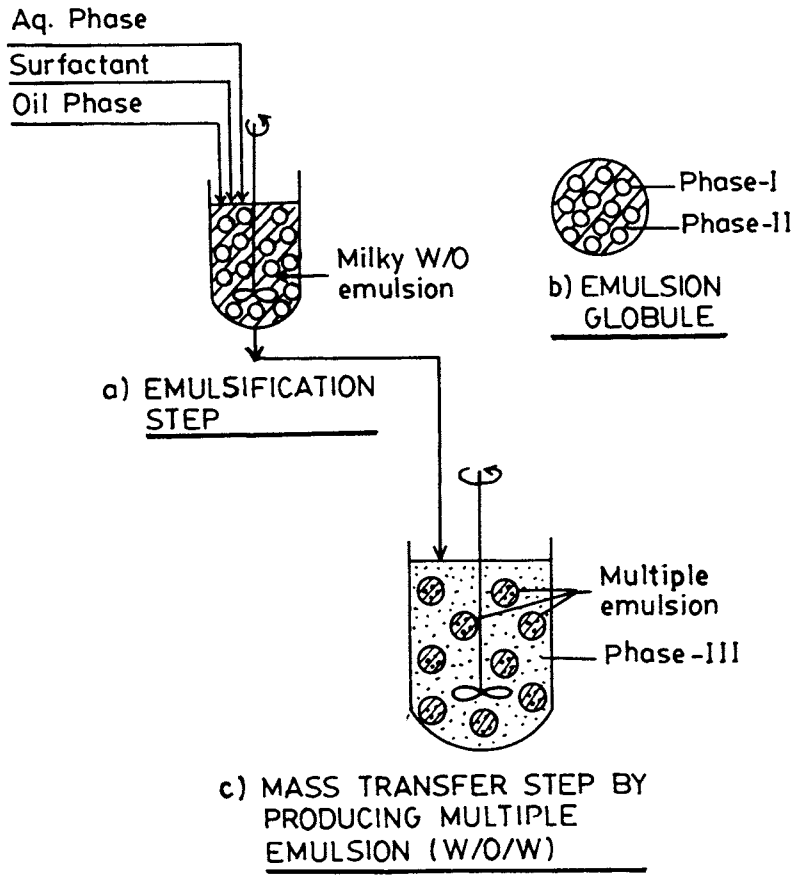

Figure 8. Schematic representation of an emulsion liquid membrane system

mixture into which the required concentration of carrier may be added to facilitate the solute transport. The stripping phase is a solution which forms the inner encapsulated phase (e.g., inner aqueous phase in W/O/W multiple emulsion). The inner aqueous phase is emulsified with the oil phase containing a lipophilic surfactant to obtain a W/O emulsion. The emulsion thus obtained is dispersed in the outer aqueous feed phase to get a W/O/W multiple emulsion system.

The two aqueous phases can not physically contact each other and the solute is transported from the outer feed phase to the inner stripping phase droplets through the oil membrane. The reaction in the inner phase prevents the solute from diffusing back across the membrane. The ELM extraction can be used to completly remove the solute from the feed to the stripping phase.

After the extraction is complete, the emulsion layer is separated from the outer aqueous layer. After drainage of the bottom aqueous layer, the emulsion is broken.

The demulsification may be accomplished chemically, electrostatically, thermally, acoustically or mechanically. The inner aqueous phase is utilized to obtain the solute. Selectivity is obtained by choosing the carrier in the membrane phase. Phosphorous containing compounds and secondary and tertiary amines have been used as carriers.

Emulsion swelling, because of the water transport, causes membrane rupture and this results in poorer extraction. Mukkolath *et al* (1990) have suggested ways to reduce emulsion swelling.

7.2. Extraction equipment

The extraction equipment for contact of emulsion phase and the feed phase can be a continuous contactor or a battery of mixer-settlers. A recycle arrangement for the oil phase is necessary to conserve the oil and the carrier after the oil phase is separated in a demulsification unit.

7.3. Encapsulation of enzymes

It is often necessary to protect enzymes from deactivating substances while maintaining free access to the desired substrate. In addition, it is frequently useful to "immobilize'' enzymes in order to enhance rates and gain additional control over enzymatic reactions. ELM systems have shown considerable potential for achieving some or all of these objectives (Mohan and Li, 1975). One of the first successful experiments in immobilizing enzymes by ELM encapsulation was performed by May and Li (1974). They encapsulated purified phenolase and used it for phenol oxidation. Frankenfeld and Li (1982) have reviewed the encapsulation of enzymes.

7.4. Product recovery from fermentation broth

Several important categories of biochemicals classified as zwitterions, namely, phospholipids, amino acids and beta-lactam antibiotics are potential candidates which could be separated from the fermentation broth by ELM. Due to the charge on these compounds, their solubility is greatly decreased in coventional organic solvents. As an alternative to the currently used techniques of derivatization followed by extraction or ion exchange, ELM has been examined for the economical recovery. A comprehensive examination of L-phenylalanine recovery by ELM has been reported by Thein *et al* (1986) and Itoh *et al* (1990).

Conventional recovery methods for carboxylic acids from fermentation broth require precipitation of calcium salt followed by dissolution in sulphuric acid. This is followed by treatment with active carbon, evaporation in multiple effect evaporator, and vacuum crystallization. ELM as an alternative strategy for recovery has been proposed by Wennersten (1983). Recovery of citric acid using ELM has been studied by Boey *et al* (1987). Alamine 336 was incorporated as a carrier in the membrane phase to obtain faster rate of extraction with the carrier-mediated transport. In the case of lactic acid, Boey *et al* (1987) used similar systems. Chaudhari and Pyle (1990) and Mukkolath *et al* (1990) have studied the recovery of lactic acid and the latter used trioctyl amine as carrier and sodium carbonate and bicarbonate as stripping agent. Itoh *et al* (1990) have studied the recovery of amino acids. Antony (1993) has exhaustively studied the extraction of penicillin-G using ELM with secondary and tertiary amines as carriers.

8. A PERSPECTIVE ON THE FUTURE

Liquid-liquid extraction process as a separation process is less energy intensive compared to other processes. By selecting a suitable solvent having high selectivity and distribution coefficient for the solute, the energy requirement in the separation step of the extraction process may be significantly reduced. This is of paramount importance in view of the ever increasing energy cost.

The complex nature of molecules in food materials and their thermolabile characteristics restrict the use of solvents and temperature of operation. In addition, presence of surface active constituents reduces the interfacial tension between the contacting phases. The reduced density difference between the phases also leads to entrainment. In view of the above, the extractors have to be designed on the basis of experimental data for specific systems. Multistage extractors with reduced backmixing will be of interest. The mixed solvents and specially designed solvents will find more use in future.

Supercritical fluid extraction is an excellent separation process for food materials. Mild conditions of extraction permit the separation of components in an unaltered state with no residual solvent. The extraction process is amenable to multistage separation; this permits fractionation of the various components of the food material. Carbon dioxide, the most commonly used supercritical fluid is nonpolar in nature. The solubilities of food materials can be increased by several orders of magnitude by using a cosolvent (entrainer). The phenomenon of crossover in the solubility with changes in temperature and pressure has promise of utilization for separation of some food materials (Chimowitz and Pennisi, 1986). One of the biggest disadvantages of supercritical fluid extraction is the high capital cost of the equipment. This restricts its use to the extraction of high-value low-volume materials. Future technology should permit reduced cost of equipment and lower pressure operations.

ATPS have the characteristics of high biocompatibility and low interfacial tension. Polymer-polymer systems exhibit better selectivity as compared to polymer-salt systems but high viscosity and higher cost make their use economically unviable. ATPS can be used for bulk separations, becoming complementary to other purification methods such as electrophoresis or chromatography. Polymer-salt systems have lower viscosities, demix rapidly and are also cheaper. Therfore large scale operations will be based on salt based ATPS. Flavouring substances, dipeptides and nucleotides from acid hydrolysis in food industry can be easily handled with ATPS. The use of ATPS in isolation/purification of such compounds will be of interest in future.

The presence of bio-specific ligands can improve the partitioning of various bio-products. Since the affinity ligands are expensive, other ligands such as dyes, mimicking the biospecificity, need to be investigated in detail. The loss of such ligands from bound state may contaminate the bioproduct. Biocompatible affinity ligands thus need to be developed in future.

In the coming years, the enzymatic reactions, either substrate or product inhibited, will be increasingly investigated in ATPS which are more suitable than organic solvent based extractive conversions.

The reverse micelles can act as compatible hosts for proteins and amino acids. Their transparency lends them to be used for biomimetic phososynthetic systems. Enzymes in reverse micelles, after extraction, can be directly used for reaction in organic phase, e.g., lipase, alpha-chymotripsin and dehydrogenase. The use of mixed surfactant reverse micelles in extraction of proteins shows promising results over other techniques such as those based on pH and ionic strength.

Emulsion liquid membrane systems are similar to reverse micelle systems except that the encapsulated drops are much larger in size. The ELM extraction has been used for separation of carboxylic acids and penicillin-G. The problem of emulsion swelling and demulsification are major hurdles in its commercialization. It has good scope because of its fast extraction rates and use of conventional extraction columns.

The important issues in the development of separation techniques are selectivity, simplicity of systems and equipment, speed of separation, ease of scale-up and possibility of continuous operation. Some of the present and future work in extraction of food materials will look into these issues. Alternative separation techniques using adsorption systems, perfluorocarbon affinity separations will compete with the separation processes presented in this article.

Processes where simultaneous bioreaction and product separation is achieved by an integrated process will be of paramount interest in the future.

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