

## Review

# Processing of Oilseeds to Recover Oil and Protein Using Combined Aqueous, Enzymatic and Membrane Separation Techniques

N.S. Krishna KUMAR, Mitsutoshi NAKAJIMA\* and Hiroshi NABETANI

National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, 2–1–2 Kannondai, Tsukuba, Ibaraki 305–8642, Japan

Received October 15, 1999; Accepted December 18, 1999

Extraction of oil and separation/concentration of proteins using aqueous, enzymatic and membrane separation processes have been described in this article. Aqueous extraction of oilseeds has significance because of its energy efficiency and the environmental safety of the process. Also, it has been observed that the various unit operations involved in this process like grinding, solid-liquid separation, centrifugation, and drying played a vital role in the efficiency of the oil yield. It has further been demonstrated that the low yield of oil in this aqueous extraction can be enhanced by using enzymes and, in general, the enzyme mixture in combination gives better results than a single enzyme. The soluble proteins from the aqueous phase were separated using ultrafiltration membranes, thereby avoiding the generation of whey formation which otherwise is a matter of environmental concern. Even reverse osmosis membranes can be employed to process the ultrafiltered permeate to recover the secondary products and render effluent water suitable for reuse. These considerations suggest that by using these processing techniques good quality oil and protein products with better yield can be obtained from the oilseeds along with a solution addressing the associated environmental problems.

Keywords: aqueous extraction, degassing, emulsion, oilseeds, ultrafiltration, reverse osmosis, proteins, trypsin inhibitor

The production of oils and fats has an important role to play in the global economy. World production of oilseeds, which is approximately 150 million metric tons (Bockish, 1998), of which 80% is accounted for by the major crops viz. soybean, cottonseed, sunflower and groundnuts (Salunke *et al.*, 1991). In addition to the importance of oils and fats for human nutrition there is a substantial market for technical fats. The importance of these oils and fats will increase considerably in the future, because they represent a vast potential of naturally regenerating raw materials in which the chemical and pharmaceutical industries have a special interest. If the oilseeds are properly processed and utilised, it would provide quite a large amount of proteins, which could be used by human beings both qualitatively and quantitatively. Conventionally, oilseeds are processed mainly to recover oil by pressing and solvent extraction, which depends normally on the oil content of the seed. A hexane based process has long been used, but the major concern with this process has been the safety implications in using hexane. Organic solvents such as hexane in particular can contribute to the emission of volatile organic compounds which is harmful as they can react in the atmosphere with other pollutants to produce ozone and other photochemical oxidants; these can be hazardous to human health, and some of these gases are also carcinogenic and have toxic properties (Rosenthal *et al.*, 1996). This has caused concern and the need to develop an alternative extraction technique has been recognized; from this recognition came the aqueous extraction technique we discuss in the ensuing paragraph.

### Aqueous extraction process and its merits

The aqueous extraction of oilseeds to recover oil and other value added products has significance because of its energy efficiency and the fact that the extraction process does not require any petroleum solvent. Oil can be obtained from oilseed materials by the process of an aqueous extraction step followed by a centrifugal separation which divides the aqueous extract into oil, solid and an aqueous phase. The various unit operations involved in this process are grinding, solid-liquid separation, centrifugation, demulsification and drying (Cater *et al.*, 1974). The advantages of the aqueous extraction process are the simultaneous recovery of oil and protein, the fact that no energy is required for organic solvent stripping, no high investment is required for a volatile organic compound emission monitor and control, there is less fire and explosion hazard and a high quality of oil can be obtained without degumming requirements. Further, less denatured protein is obtained with high biological value and the products obtained are usually without antinutritional factors. With the increase in population and wide prevalence of protein malnutrition, attempts are being made to utilise protein from several unconventional sources (Sosulski, 1962). Increasing concern about the environment and energy necessitates that technologies be modified or replaced with those which are cleaner and less energy intensive. Also, the oil cake obtained after the extraction of oil is a rich source of protein. Due to the presence of some antinutritional components, it is not always possible to use this protein for edible purposes (Nichols & Cheryan, 1981). If processed properly, the oil cake can be a major source of protein supply to a nutrition deficient population of a country. Further, the present technique of producing edible protein from oil cake

\*To whom the correspondence should be addressed.  
E-mail: mnaka@nfri.affrc.go.jp

gives rise to a whey solution, the disposal of which is another matter of environmental concern.

The aqueous extraction step is carried out by dispersing the ground seeds in water and then agitating the dispersion to enhance the extraction of seed constituents. For better extraction efficiency, factors like the solid to water ratio, pH of dispersion, extraction time and temperature are important. When the percentage of solids in the dispersion is high, it is important to remove undissolved solids, making it possible to efficiently recover oil by centrifugation. The main solids are fibrous materials, undissolved carbohydrates and proteins depending upon the pH employed in the extraction step. Centrifugation is also one of the key steps in the aqueous process in which the liquid containing both dissolved and undissolved matter is separated into oil, solid and aqueous phases. Depending on the material and the conditions selected, the oil can be recovered as free oil or as an oil-in-water emulsion. If an emulsion has been formed, it can be broken using the phase inversion technique or some other method. Oil extraction using the aqueous extraction process is based more on the insolubility of oil in water than the dissolution of oil (Johnson & Lusas, 1983). The process involves mixing ground and dehulled oilseeds in vats of hot water and skimming off the oil which rises to the surface (Lusas & Gividan, 1987); this process also uses the same principle as hot water flotation (Southwell & Harris, 1991). Nowadays in the aqueous extraction process centrifuges have been incorporated instead of gravity separation which results in highest oil and protein recovery as well less damage to the nutritional value of food proteins (Hagenmaier *et al.*, 1972, Embong & Jelen, 1977). The unit operations involved in this process depend on the various types of oilseeds (Lusas *et al.*, 1982). The oil recovered after breaking the emulsion and separating the phases is usually of a high quality. This process requires no degumming treatment of the oil before it is subjected to refining (Dominguez *et al.*, 1995).

The grinding operation determines the oilseed particle size. Sufficient grinding which breaks down the walls of the cells containing the oil is essential. The type of grinding (wet or dry) depends on the oilseed and is dependent based on several factors: initial moisture content, chemical composition and the structure of the oilseed. For coconut, wet grinding is more appropriate as this avoids the drying step prior to the grinding, whereas for materials with low initial moisture content like peanuts, rapeseed and soybean, dry grinding is considered more suitable. Excessive grinding induces cell ruptures and increases the efficiency of oil and protein extraction, however, it also produces smaller oil globules, which makes demulsification more difficult. With respect to power consumption level, simple stirring is sometimes sufficient to obtain high yields in the cases of peanuts and sunflower (Hagenmaier, 1974). High shear stirring also achieves further disintegration of the cells and thereby releases the oil. Increasing the blending time in general improves the yield of oil extracted, but there is also a chance that this will result in the formation of a stable emulsion, which will adversely affect the total yield (Embong & Jelen, 1977). pH plays a vital role in the extraction step and varies with the oil bearing materials. The temperature also seems to have a considerable effect on oil yield; in some oilseeds the extraction temperature was not critical for protein recovery but was critical to oil extraction yield (Lusas *et al.*, 1982). Also, to obtain a less stable emulsion, the solid:water ratio

should be high and to obtain the highest extraction rates and yields, it is usually necessary to use a large quantity of water. The solid : water ratio reported in the literature for peanut oil extraction is 1 : 5–12 (Subramanian *et al.*, 1959; Rhee *et al.*, 1972; Bhatia *et al.*, 1996), for sunflower 1:10 (Hagenmaier, 1974), for soybeans 1 : 12 (Lusas *et al.*, 1982) and for rapeseed 1 : 2.5–3.5 (Embong & Jelen, 1977).

#### **Application of aqueous extraction process to various oilseeds**

It is also believed that the application of aqueous processing to soybean might remove sugars and flavor which cause problems of "off-flavor" in this major plant protein source (Cater *et al.*, 1974). It has been reported that 62% oil has been extracted using aqueous extraction in soybean seeds and a period of 40 min was sufficient to extract both the oil and protein (Yoon *et al.*, 1991). Separation and recovery of protein and oil include a demulsification step followed by separation of the aqueous and oil phase by centrifugation. Two oilseeds that might be partially potential candidates for aqueous processing are sunflower and rapeseed. Sunflower seed contains chlorogenic acids which cause it to turn dark green or brown when wetted. It has been reported that these phenolic acids can be removed by aqueous extraction (Sosulski & McCleary, 1972). Rapeseed contains toxic sulfur compounds which must be removed before the protein can be considered edible, and this can be achieved by aqueous extraction (Sosulski *et al.*, 1972). Simultaneous recovery of oil and separation of toxic sulfur compounds from protein suggested that aqueous processing might be applied advantageously to rapeseed. Peanut is the world's third important oilseed crop in terms of production after soybean and cottonseed (F.A.O., 1988). Besides being rich in edible oil, peanut is also a good source of dietary proteins, vitamins and minerals. In the developing countries of Asia and Africa, peanuts are used mainly for their edible oil. Only a small proportion of peanuts produced is processed for direct consumption of salted peanuts, sweetened products and in a number of indigenous products such as peanut butter, candies, salads, cheese and yogurt-like products, protein concentrates and isolates and protein rich peanut meals (Woodroof, 1983; Kadam & Chavan 1988). It has been reported that by employing the aqueous extraction and membrane separation processes both good quality oil and protein can be obtained from peanut (Lawhon *et al.*, 1981).

Application of aqueous processing to cottonseed is also interesting. Since glanded cottonseed contains glands which rupture in aqueous media and react with the proteins, the application of the aqueous process seems impractical. However, new varieties of the glandless type which are free of gossypol might be processed successfully by this method. Very recently a process has been developed for recovering oil and protein from canola seed without the use of organic solvent. The process consists of pre-pressing to remove most of the oil, followed by extraction with aqueous alkali. The resulting emulsion is separated into an oil and aqueous phase (Diosady, 1999). The time required to reach a desired extraction level depends on the oilseed as well the process variables. Coconut cake contains a substantial amount of nutritionally balanced protein. However, due to its high fiber content its potential use in the food industry is limited (Chakraborty, 1985). Coconut would be a valuable source of high grade protein if a suitable method of oil extraction could be devised. The tradi-

tional method is to dry the kernel of the coconut to give copra, which is then pressed in an expeller at high temperature. The residue containing the protein is not fit for human consumption and the oil is of poor quality. Coconut protein represents a substantial source of potentially available food protein in a number of tropical countries, but the protein is currently not usable as food because of the unsanitary manner of copra processing. Consequently, efforts have been made to process fresh coconuts in a way that food grade protein can be recovered. Another major problem is the handling of large amounts of fibrous material and low protein content. The coconut meat must be thoroughly ground to achieve efficient oil extraction from the fiber. Further, the meat initially contains a high amount of moisture so that in the grinding process a rather stable emulsion is formed, and this is broken by inversion or agitation. Recovery of proteins from coconut for use as human food is possible by aqueous processing. The high temperatures inside the copra expeller normally lower the nutritional value of coconut protein, and consequently this protein is generally not highly regarded for its nutritional value. However, if the coconut oil is removed without exposing the coconut to excessive heat, the protein retains its nutritive value. Because the nutrition value of this protein is considered important, aqueous processing has as one of its primary goals the preparation of coconut protein food products (Hagenmaier, 1997). More income can be realized if the available proteins can be recovered for human consumption.

### Disadvantages

Although it is accepted that wet processing is by far the superior process, it has its own drawbacks. Wet processing generates a substantial quantity of water that has a high BOD value. The aqueous extraction process, however, is somewhat less efficient in oil extraction and demulsification is necessary to recover clear oil when an emulsion is formed; some oil is also invariably lost in the aqueous emulsion phase. Further, increased care is necessary to prevent microbial contamination.

### Extraction using enzymes

It has been reported that the lower yield of oil obtained using aqueous extraction can be increased by using enzymes as well better quality meal can be obtained (Rosenthal *et al.*, 1996). Further, the enzyme complexes fit the processing requirements because of the mild conditions that avoid drastic operating conditions. Enzymes have been used in oils and fats in several areas like oil extraction, mono- and diglyceride production, steroid and fatty acid transformation (Caragay, 1983; Poroske, 1984; Ratledge, 1984; Hollo, 1987; Graille *et al.*, 1990). Table 1 gives the different types of enzymes used and the yield of oil obtained from some oilseeds. Because of the enzymatic action, the cell wall of the oilseed is ruptured, thereby releasing the oil from the seed. This has already been practised for the extraction of olive oil and has also been tried for other oilseeds (Christensen, 1989). The different types of enzymes used in the oil extraction process are amylase, glucanase, protease, pectinase, cellulolytic and hemicellulolytic enzymes. Because of the mild conditions employed during extractions good quality valuable components are obtained (Olsen, 1988). It has further been reported that an enzyme mixture in combination gives better results in general than a single enzyme. Mixtures of enzymes yield more oil due to

their combined effect on colloidal and lipoproteic structures (Dominguez *et al.*, 1994). Proteolytic enzymes enhance the yield of oil and protein by hydrolysing the structural fibrous proteins in which fat globules are bound (McGlone *et al.*, 1986). Thus it has been reported that using proteolytic enzymes in soybean oil extraction resulted in 86% yield compared to 62% without enzymes, while protein increased from 62 to 89% (Dominguez *et al.*, 1993). Most of the enzyme extraction studies employ different extraction conditions: different pH, particle size and temperature to improve the efficiency of oil extraction.

Using the aqueous enzymatic extraction employing protease, cellulase and  $\alpha$ -1,4 galacturonide glycano hydrolase either separately or in combination 74–78% of peanut oil has been extracted (Lanzani *et al.*, 1975), and employing a combination of cellulase and pectinase for sunflower seed approximately 30% more oil yield was obtained (Dominguez *et al.*, 1995). For coconut oil extraction using enzymes McGlone *et al.* (1986) reported obtaining an oil yield of 80% with a combination of polygalacturonase,  $\alpha$ -amylase and protease. In this process enzymes were used to hydrolyse the cellular material and oil was recovered by centrifugation. This technique for recovering oil from fresh coconut meat with enzymes was a significant improvement in both oil yield and quality over the conventional processes (Che Man *et al.*, 1996). Also, in shea fat extraction an increase of 20% was possible when a mixture of enzymes (protease and an enzyme with both cellulase and hemicellulase) was used while extracting the fat from the shea kernel (Debrah & Ohta, 1994). In corn germ pre-treatments were made to the germ to inactivate the native enzyme present and thereby the structure was loosened. It was then ground and treated with enzymes, the oil was separated by centrifugation and 84% of the oil was recovered (Bocevskva & Karlovic, 1993).

Oil accumulates in seeds in the intracellular vacuoles from which its extraction could be enhanced by the hydrolytic action

**Table 1.** Aqueous enzymatic extraction for some oil bearing materials in comparison to control.

Oilseed	Enzyme	Concentration (%)	Oil yield (%)	Reference
Coconut	Control (without enzyme)		19.3	
	Cellulase+ $\alpha$ -amylase+Polygalacturonase+protease	0.1	41.6	Che Man <i>et al.</i> , 1996
	Cellulase+ $\alpha$ -amylase+Polygalacturonase+protease	0.5	49.7	
	Cellulase+ $\alpha$ -amylase+Polygalacturonase	1.0	73.8	
Control (without enzyme)		40.0	Debrah & Ohta, 1995	
Protease+cellulase&hemicellulase+glucanase	0.5	74.1		
Avocado	Control (without enzyme)		2.0	Buenrostro & Lopez-Munguia, 1986
	$\alpha$ -amylase	1.0	70.0	
	$\alpha$ -amylase+protease	1.0	67.0	
	$\alpha$ -amylase+cellulase	1.0	67.0	
	$\alpha$ -amylase+protease+cellulase	1.0	62.0	
Peanut	Control (without enzyme)		72.0	Lanzani <i>et al.</i> , 1975
	Protease+cellulose+ $\alpha$ -1,4 galacturonide glucano hydrolase	1.0	78.0	
Corn germ	Control (without enzyme)		20.0	Bocevskva & Karlovic, 1993
	Pectinex (ultra SP-L)	2.0	80.0	
Soybean	Control (without enzyme)		62.08	Dominguez <i>et al.</i> , 1993
	Cellulase+pectinase	0.1	6.0	

of carbohydrates, which act on the glucans of the cell wall and thereby release the oil. The main parameters affecting the hydrolytic process are particle size, moisture and enzyme concentration. The nature of the enzymatic formulation and the enzyme/seed ratio determines the optimum hydrolysis time. Apart from the concentration of enzymes and the type, there are other factors which play a role in the oil yield like degree of grinding, pH, temperature, time and centrifugation conditions. Another factor to be considered is the difference in the roles of enzymes. Carbohydrases act only on the cell wall which allows the release of oil whereas proteolytic enzymes act not only on the membrane surrounding the lipid bodies and the cytoplasmic protein, but also have a better emulsifying capacity. Thus, in comparison with the hydrolytic enzymes used in extraction, proteolytic enzymes can potentially have a better effect depending on the degree of hydrolysis of a protein.

### Membrane process

Membrane separation technology, although still remaining unconventional, has attained a level of maturity as a common chemical engineering unit operation. It has several advantages over conventional separation techniques:

- Flexibility in equipment design and operation, as the systems are modular in nature
- Brings separation without any phase change (except pervaporation) and hence a number of other conventional and more costly separation technologies like distillation, cryogenic separation and chromatographic separation.
- Minimum space requirement in relation to throughput
- Membrane process is gentle and mild and hence the chemical identity of the feed component remains intact
- Wide range of molecular weight components in the process stream can be taken care of
- Offers lower costs and less maintenance
- Improves final product quality
- Performs difficult separations not possible by other separation technologies
- Effective in processing dilute solutions
- Eco-friendly process

Ultrafiltration (UF) was considered a concentration and purification technique in laboratory preparations in the last century. The breakthrough came from the development of asymmetric membranes, which were composed of an ultra-thin microporous layer supported by a microporous structure. In comparison to symmetrically structured membranes, asymmetric ones exhibited a much higher permeability owing to a reduced effective pore length and the avoidance of deep bed filtration. The asymmetric UF membranes are now standard and are manufactured in numerous geometric configurations and modules, so as to achieve high surface areas per unit volume (packing density). The modules range from flat membranes to plate and frame systems, sandwich modules, tubular system, capillary devices and hollow fibre cartridges (Koseoglu & Engelgau, 1990). Commercial UF membranes are now available which offer good selectivity, a high permeability and considerable chemical stability. Inorganic membranes have recently been developed which are capable of withstanding high temperatures and pressures (Kawakatsu & Nakajima, 1995).

Extensive reviews on different membrane processes, mem-

brane materials, membrane preparation, transport mechanisms and models, potential applications etc. have been reported in the literature giving a vivid picture of this separation technology (Meares, 1976; Lancey & Loes, 1979; Belfort, 1984; Kesting, 1985; Sourirajan & Matsura, 1985; Drioli & Nakagaki, 1986; Parekh, 1988; Sidhoum *et al.*, 1988; Cecille & Toussaint, 1989; Rautenbach & Albrecht, 1989; Porter, 1979). With the advent of the pressure-driven membrane processes like reverse osmosis (RO) and UF and also the high degree of technical maturity of these separation techniques, it is now possible to handle the aqueous effluents both for by-product recovery and pollution abatement. UF is increasingly being used as a concentration and separation technique in a variety of industries, mainly due to its low energy requirements, non-thermal nature and simplicity. The membrane separation technique, which has already been applied to oilseed protein processing, enables the removal of antinutritional factors and other indigestible components such as sugar and ash by the addition of water soluble chemicals. Transition from conventional iso-electric precipitation to UF in the preparation of protein isolates resulted mainly from the advantage of UF in terms of yield (due to recovery of whey protein), energy efficiency and the enhanced functional properties of the isolates owing to mild processing conditions. In addition to pollution abatement, the membrane isolation process offers products with enhanced nitrogen solubility, greatly reduced process water requirements and products with more desirable functional properties.

Increasing concern about the environment and energy necessitates technologies to be modified or replaced with cleaner and less energy intensive ones. Also, the oil cake obtained after oil extraction is a rich source of protein. Because of the presence of some antinutritional components, it is not always possible to use this protein for edible purposes. If processed properly, however, this can be a major source of protein supply to a nutrition deficient population. The present technique of producing edible protein from an oil cake results in a whey solution, disposal of which is a matter of environmental concern. Considering this rapid growth of membrane technology in the pharmaceutical field and those of food processing, medical and biotechnology as a separation technique, its potential application in the area of oilseed processing and utilisation deserves considerable merit. Current membrane separation research has been applied to miscella distillation, vapour recovery, condensate return, wastewater treatment, degumming, refining and bleaching, hydrogenation catalyst recovery, oilseed protein processing and nitrogen production (Koseoglu & Engelgau, 1990; Snape & Nakajima, 1996). In the membrane isolation process, UF membranes were used to recover protein directly from oilseed flour extracts, thereby avoiding the generation of whey, which resulted from the acid precipitation procedure. RO membranes were employed to process the UF permeate to recover a secondary product and to render the effluent water suitable for reuse. These considerations motivated investigators to merge two significant processing techniques i.e., the aqueous extraction process and the membrane isolation process into a single procedure having the significant advantages of each method. The new technique was applied to the production of oil and protein food products from the oil-bearing materials.

A great deal of information about vegetable oilseed proteins, their chemistry and processing using UF as a separation process

has been published in review papers and books. Many authors (Okubo *et al.*, 1975; Lawhon *et al.*, 1978; Hensley & Lawhon, 1979; Manak *et al.*, 1980; Culioli & Maubois, 1975; Knuckles *et al.*, 1980; Eakin *et al.*, 1978; Olsen, 1978; Goldberg & Chevrier, 1979; Tsai *et al.*, 1977; Payne *et al.*, 1973; Melling, 1974) have summarised the literature related to work on vegetable proteins by UF. With increasing stress on the quality of meal and protein for human food uses, several innovations in oil milling technology such as selection of raw materials, pre-treatments, use of special solvent systems and a selective extraction procedure have contributed in a large measure to the production of oilseed meals of superior quality. But to meet the standards specified by national and international agencies for edible meals, the post harvest technologies need to be developed to obtain a cake/meal low in pigments and fibre by suitably modifying the conventional practices. These include primarily the removal of phenolics, bitter-tasting materials and other toxic compounds from the meal to convert it into light-colored edible grade product (Vix & Decosas, 1969). By leaching out the soluble constituents and coarse fibrous components, it is possible to obtain protein concentrates containing approximately 70% protein on a moisture-free basis. The protein concentrates are claimed to have improved flavor characteristics and superior functional properties when incorporated in processed foods (Bondi, 1959). The preparation of protein isolates containing not less than 90% protein provides another important avenue for the better utilisation of oilseed proteins in processed foods. Elimination of the insoluble and partly indigestible carbohydrate fractions as well as removal of odoriferous, toxic and anti-nutritional factors, serve to improve the quality of protein.

### Vegetable protein processing

Table 2 gives the summary of work done on vegetable proteins. Vegetable protein products have been consumed for centuries in Asia and Middle Eastern countries with a growing interest in health and fitness. There are signs that vegetable proteins will constitute a much higher proportion of the human diet in the future. Soybeans are by far the most important source of these proteins. The desirable components of soybeans are protein and fat, but there are also some undesirable components that must be removed or reduced to increase the usefulness and functionality of soybean products. Lipid-lipoxygenase interactions must be avoided to prevent painty off-flavors from developing. Phytic acid forms insoluble chelates with minerals and can form complexes with proteins that reduce bioavailability of the minerals and proteins. Trypsin inhibitors are proteinaceous compounds that affect the efficiency of protein concentrates and isolates and partially overcome these problems. These methods involve extraction, heat treatment and centrifugation to separate the protein and fat from other components. These conventional methods are time consuming, sometimes result in products with less than desirable functional properties and generate a whey-like waste stream which constitutes a water pollution threat.

UF has been investigated as a replacement for the conventional isolation methods (Frazier & Huston, 1971; Iacobucci *et al.*, 1973). Since the undesirable oligosaccharides, phytic acid and some of the trypsin inhibitors are smaller in molecular size than proteins, it should be possible by careful selection of the membrane and operating parameters to selectively remove these unde-

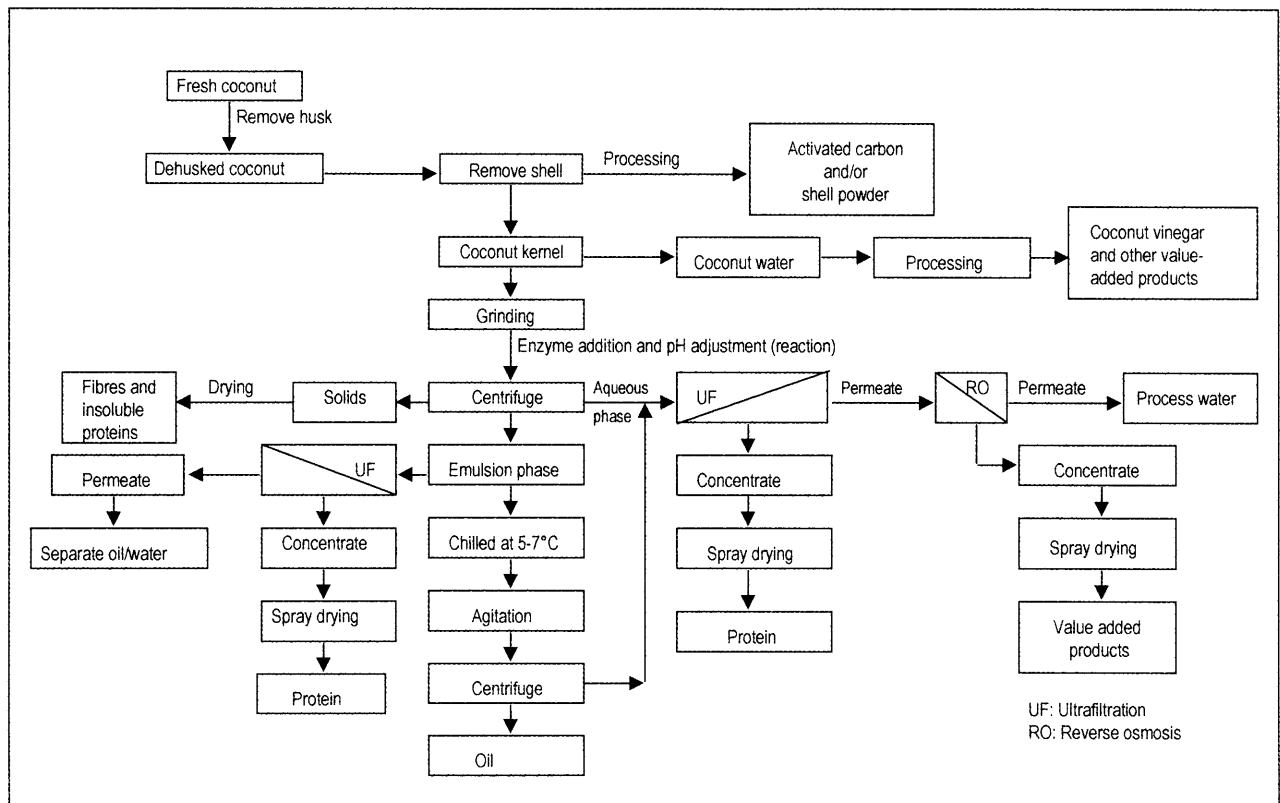
**Table 2.** Summary of work on vegetable proteins.

Material	Description of work	Reference
Soybean	Dissociation of phytate from protein, followed by removal of dissolved phytate by UF	Okubo <i>et al.</i> , 1975
	UF aqueous extracts of whole soybeans	Omosaiye & Cheryan, 1979
	Process optimisation	Lawhon <i>et al.</i> , 1978
Glandless cottonseed	Stable products from glandless cottonseed using membranes	Lawhon <i>et al.</i> , 1979
	Processing of cottonseed protein extracts using UF	Hensley <i>et al.</i> , 1977
	Combination of UF and isoelectric precipitation	Lawhon <i>et al.</i> , 1980
Sunflower	Alkaline extraction, UF and texturisation of the product	Culioli & Maubois, 1975
	Alkaline extraction, UF, diafiltration and spray drying	Culioli <i>et al.</i> , 1975
Alfalfa leaf protein	Processing of pasture herbage using membranes	Ostrowski, 1979
	UF pilot scale studies of clarified alfalfa juice	Knuckles <i>et al.</i> , 1980
	Fractionation of alfalfa protein by UF	Eakin <i>et al.</i> , 1978
Beans	Pilot plant production of bean protein by UF	Olsen, 1978
Potato	Use of tubular UF system to concentrate protein in potato processing waste. Effects of processing variables on the permeate rate	Eriksson & Sivik, 1976
Jojoba	Optimal separation of Jojoba protein using membrane process	Nabetani <i>et al.</i> , 1995

sirable components and produce a purified protein isolate or lipid protein concentrate (depending on the starting material) with superior functional properties. The manufacture of soy products by UF usually results in higher yields because of the inclusion of whey proteins that are normally lost in conventional manufacturing methods. These whey proteins also contribute to the superior functional properties of the UF soy products, in addition to the benefits of the non-thermal and chemical nature of the UF process. The application of UF in several other vegetable protein systems has been studied, among them alfalfa, cottonseed, fava beans, lupinus albus, sunflower seeds, rapeseed, pasture herbage and leaf protein (Lah & Cheryan, 1980; Lah *et al.*, 1980; Knuckles *et al.*, 1975; Lawhon *et al.*, 1977; Diosady *et al.*, 1984; Ostrowski, 1979; Tragardh, 1974; Lawhon *et al.*, 1981; Cheryan, 1986; Krishna Kumar & Bhowmick, 1995a; b; Nabetani *et al.*, 1995). Figure 1 shows a conceptual flow diagram for the recovery of oil and proteins from coconut using aqueous, enzymatic and membrane separation processes. As shown, by using aqueous enzymatic extraction and membrane separation process, some value added products like oligosaccharides, fibres are obtained in addition to the oil and protein. Further, the process water obtained after the UF permeate will be treated with RO membrane and can be reused, thereby avoiding the generation of whey which otherwise is an environmental concern.

### Research and development needed for the future

Aqueous enzymatic extraction of oilseeds to recover oil offers better advantages than the normal solvent extraction process. However, development of a suitable enzyme for a particular oil-



**Fig. 1.** A conceptual flow diagram for the recovery of oil and protein from fresh coconut using aqueous enzymatic extraction and membrane separation processes.

seed processing has to be explored; for the process to be economically viable, reuse of the enzyme must be considered along with water. Although the membrane separation process may overcome this aspect, it is so far restricted to lab scale studies, barring a few industrial applications like in the case of soybeans where oligosaccharides are purified by UF and  $\beta$ -amylase production from defatted soybean using UF membrane (Nakajima *et al.*, 1996). Protein processing by UF is attractive, because it recovers all the soluble proteins through proper membrane selection, removal of low molecular weight solutes by diafiltration and recycling of UF permeate by RO. However, a pre-requisite for the successful application of UF in oilseed protein processing appears to be the conditions and characteristics of the starting materials.

Consequently, it is realized that there is still much to be learned about the UF process in general and about applications in particular. Except for the dairy industry, work on the factors influencing membrane performance is limited, especially to oilseed proteins. The main aspects which need attention for successful operation in the UF processing of a given protein solution are the proper selection of the membrane which gives a balance of selectivity and permeation rate, methods of feed pre-treatment for UF to reduce fouling and enhancement of the membrane performance. Studies on aqueous extraction of oilseed proteins have not given any useful information regarding the system design, process development etc. for the maximum recovery of oil and protein. Further process development and economic viability studies are necessary before the scale of the entire system can be raised.

**Acknowledgements** This research was partly funded by the Program for Promotion of Basic Research Activities for Innovative Biosciences. The first author would like to express sincere thanks to the Japan Science and Technology (JST) Corporation and the Japan International Science and Technology Exchange Center (JISTEC) for their award of a Science and Technology Agency (STA) fellowship.

## References

- Belfort, G. (1984). "Synthetic Membrane Processes." Academic Press, New York.
- Bhatia, D.S., Pappia, M.A.B. and Baliga, B.P. (1996). Peanut proteins isolate production and properties. *J. Food Sci. Technol.*, **3**, 2-7.
- Bocevska, M. and Karlovic, D. (1993). Quality of corn germ oil obtained by aqueous enzymatic extraction. *J. Am. Oil Chem. Soc.*, **70**, 1273-1277.
- Bockish, M. (1998). The importance of fats. In "Fats and Oils Handbook," AOCS, Champaign, Illinois, pp. 1-52.
- Bondi, A. (1959). "Processed Plant Protection Foodstuffs," ed. by A.M. Altschul, Academic Press, New York, pp. 469.
- Buenrostro, A. and Lopez-Munguia, C. (1986). Enzymatic extraction of avocado oil. *Biotechnol. Lett.*, **8**, 505-506.
- Caragay, A. (1983). Pacing technologies in the fats and oil industry. *J. Am. Oil Chem. Soc.*, **60**, 1641-1644.
- Cater, C.M., Rhee, K.C., Hagenmaier, R.D. and Mattil, K.F. (1974). Aqueous extraction — An alternative oilseed milling process. *J. Am. Oil Chem. Soc.*, **51**, 137-141.
- Cecille, L. and Toussaint, J. (1989). "Future Industrial Prospects of Membrane Processes." Elsevier Publishing Co., New York.
- Chakraborty, P. (1985). Functional properties of coconut protein isolate by ultrafiltration in food engineering and process applications. ed. by M. Lemaguer and P. Jelen. Elsevier Applied Science Publication, pp. 305-315.
- Che Man, Y.B., Suhardiyono, Asbi, A.B., Azudin, M.N. and Wei, L.S. (1996). Aqueous enzymatic extraction of coconut oil. *J. Am. Oil Chem. Soc.*, **73**, 683-686.

- Cheryan, M. (1986). "Ultrafiltration Handbook." Technomic Publishing Company, Lancaster Inc., pp. 260.
- Christensen, F.M. (1989). Enzyme technology versus engineering technology in the food industry. *Biotechnol. Appl. Biochem.*, **11**, 249–265.
- Culioli, J. and Maubois, J.L. (1975). Purification des protéines de tourteau de tournesol par ultrafiltration. *Rev. Fr. Corps Gras*, **22**, 521–525.
- Culioli, J., Maubois, J.L., Chopin, A. (1975). L'ultrafiltration appliquée à la préparation d'un isolat de protéines de tourteau de tournesol. *Ind. Aliment. Agric.*, **92**, 1029–1034.
- Debrah, K.T. and Ohta, Y. (1994). Enzyme assisted aqueous extraction of fat from kernels of the shea tree, *Butyrospermum parkii*. *J. Am. Oil Chem. Soc.*, **71**, 979–983.
- Debrah, K.T. and Ohta, Y. (1995). Enzyme assisted aqueous extraction of shea fat: A rural approach. *J. Am. Oil Chem. Soc.*, **72**, 251–256.
- Diosady, L.L. (1999). Aqueous extraction of canola. Paper presented at the 90th AOCS annual meeting and expo., Orlando, Florida, May 9–12.
- Diosady, L.L., Tzeng, Y.M. and Rubin, L.J. (1984). Preparation of rapeseed protein concentrates and isolates using ultrafiltration. *J. Food Sci.*, **49**, 768–770, 776.
- Dominguez, H., Nunez, M.J. and Lema, J.M. (1993). Oil extractability from enzymatically treated soybean and sunflower: Range of operational variables. *Food Chem.*, **46**, 277–284.
- Dominguez, H., Nunez, M.J. and Lema, J.M. (1994). Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: A review. *Food Chem.*, **49**, 271–286.
- Dominguez, H., Nunez, M.J. and Lema, J.M. (1995). Enzyme assisted hexane extraction of soybean oil. *Food Chem.*, **54**, 223–231.
- Drili, E. and Nakagaki, M. (1986). "Membrane and Membrane Processes." Plenum Press, New York.
- Eakin, D.E., Singh, R.P., Kohler, G.O. and Knuckles, B.E. (1978). Alfalfa protein fractionation by ultrafiltration. *J. Food Sci.*, **43**, 544–547, 552.
- Embong, M.B. and Jelen, P. (1977). Technical feasibility of aqueous extraction of rapeseed oil, a laboratory study. *J. Inst. Can. Sci. Technol. Aliment.*, **10**, 239–243.
- Eriksson, G. and Sivik, B. (1976). Use of tubular system to concentrate protein in potato processing waste: Effects of processing variables on the permeate rate. *Potato Res.*, **19**, 279.
- F.A.O. (1988). "Production Yearbook." Food and Agricultural Organisation, Rome.
- Frazeur, D.R. and Huston, R.B. (1971). Protein and method of extracting same from soybeans employing reverse osmosis. U.S. Patent, 3,728,327.
- Goldberg, M. and Chevrier, D. (1979). Traitement du plasma de bovins par ultrafiltration estimation des surfaces nécessaires. *Ind. Aliment. Agric.*, **96**, 951–956.
- Graille, J., Pira, M. and Montet, D. (1990). Biotechnology of lipids: Some possible applications. *Oleagineux*, **67**, 29–34.
- Hagenmaier, R., Cater, C.M. and Mattil, K.F. (1972). Critical unit operations of the aqueous processing of fresh coconuts. *J. Am. Oil Chem. Soc.*, **49**, 178–181.
- Hagenmaier, R.D. (1974). Aqueous processing of full fat sunflower seeds: Yields of oil and protein. *J. Am. Oil Chem. Soc.*, **36**, 66–70.
- Hagenmaier, R.D. (1997). "Characteristics of Fresh Coconut as Raw Material in Coconut Aqueous Processing." San Carlos, Cebu City, Philippines, pp. 113–123.
- Hensley, D.W. and Lawhon, J.T. (1979). Economic evaluation of soy isolates production by a membrane isolation process. *Food Technol.*, **33**, 46–50.
- Hensley, D.W., Lawhon, J.T., Cater, C.M. and Mattil, K.F. (1977). A study of factors affecting membrane performance during processing of cottonseed protein extracts by ultrafiltration. *J. Food Sci.*, **42**, 812–815, 820.
- Hollo, J. (1987). Trends in the Afarbeitung van oslamer. *Fette Wiis. Technol.*, **89**, 2–7.
- Iacobucci, G.A., Meyers, D.V. and Okubo, K. (1973). Process for preparing protein products. U.S. Patent, 3,736,147.
- Johnson, L.A. and Lusas, E.W. (1983). Comparison of alternative solvents for oil extraction. *J. Am. Oil Chem. Soc.*, **60**, 229–242.
- Kadam, S.S. and Chavan, J.K. (1988). Utilisation of groundnut in India and scope for novel and alternative uses, in process consultants meet on uses of food legumes, 27–30 March, 1988, Patancheru, India, pp. 242–248, ICRISAT.
- Kawakatsu, T. and Nakajima, M. (1995). Concentration and separation of protein using ceramic membranes. *Chem. Eng. Sym. Ser.*, **46**, 6–9.
- Kesting, R.E. (1985). "Synthetic Polymeric Membranes: A Structural Perspective" 2nd ed, John Wiley and Sons, New York.
- Knuckles, B.E., Defremey, D., Bickoff, E.M. and Kohler, G.O. (1975). Soluble protein from alfalfa juice by membrane filtration. *J. Agric. Food Chem.*, **23**, 209–212.
- Knuckles, B.E., Edwards, R.H., Miller, R.E. and Kohler, G.O. (1980). Pilot scale ultrafiltration of clarified alfalfa juice. *J. Food Sci.*, **45**, 730–734, 739.
- Koseoglu, S.S. and Engalgau, D.E. (1990). Membrane application and research in the edible oil industry: An assessment. *J. Am. Oil Chem. Soc.*, **67**, 239–249.
- Krishna Kumar, N.S. and Bhowmick, D.N. (1995). A fresh look at coconut and its processing. *INFORM*, **6**, 1217–1222.
- Lah, C.L. and Cheryan, M. (1980). Emulsifying properties of full fat soy protein product produced by ultrafiltration. *Lebensm. Wiss. U-Technol.*, **13**, 259–263.
- Lah, C.L. and Cheryan, M. (1980). Protein solubility characteristics of an ultrafiltered full fat soybean product. *J. Agric. Food Chem.*, **28**, 911–916.
- Lah, C.L., Cheryan, M. and Deror, R.E. (1980). A response surface methodology approach to the optimisation of whipping properties of an ultrafiltered soy product. *J. Food Sci.*, **45**, 1720–1726.
- Lancey, R.E. and Loes, S. (1979). "Industrial Processing with Membranes." Robert E. Krieger Publishing Co., Huntington, New York.
- Lanzani, A., Petrini, M.C., Cozzoli, O., Gallavresi, P., Carola, C. and Jacini, G. (1975). On the use of enzyme for vegetable oil extraction. A preliminary report. *Riv. Ital. Sostanze Grasse*, **L11**, 226–229.
- Lawhon, J.T., Hensley, D.W., Mulsow, D. and Mattil, K.F. (1978). Optimisation of protein isolate production from soyflour using industrial membrane systems. *J. Food Sci.*, **43**, 361–364, 369.
- Lawhon, J.T., Kajs, T.M. and Ki Soon Rhee (1979). Tamucurd: A high protein ingredient stable product from glandless cottonseed has many potential food applications. *Food Technol.*, **33**, 82–86.
- Lawhon, J.T., Manak, L.J. and Lusas, E.W. (1980). An improved process for the isolation of glandless cottonseed protein using industrial membrane system. *J. Food Sci.*, **45**, 197–199.
- Lawhon, J.T., Manak, L.J., Rhee, K.C. and Lusas, E.W. (1981). Production of oil and protein food products from raw peanuts by aqueous extraction and ultrafiltration. *J. Food Sci.*, **46**, 391–395.
- Lawhon, J.T., Manak, L.J., Rhee, K.C., Rhee, K.S. and Lusas, E.W. (1981). Combining aqueous extraction and membrane isolation techniques to recover protein and oil from soybeans. *J. Food Sci.*, **46**, 912–916, 919.
- Lawhon, J.T., Mulsow, D., Cater, C.M. and Mattil, K.E. (1977). Production of protein isolates and concentrates from oilseed flour extracts using industrial ultrafiltration and reverse osmosis system. *J. Food sci.*, **42**, 389–394.
- Lusas, E.W. and Gividan, G.M. (1987). Glandless cottonseed: A review of the first 25 years of processing and utilisation research. *J. Am. Oil Chem. Soc.*, **64**, 839–854.
- Lusas, E.W., Lawhon, J.T. and Rhee, K.C. (1982). Producing edible oil and protein from oilseeds by aqueous processing. *Oil Mill Gazet.*, **4**, 28–34.
- Manak, L.J., Lawhon, J.T., Lusas, E.W. (1980). Functioning potential of soy, cottonseed and peanut protein isolates produced by industrial membrane systems. *J. Food Sci.*, **45**, 236–238, 245.
- McGlone, O.C., Lopez-Munguia, C.A. and Cater, C.V. (1986). Coconut oil extraction by a new enzymatic process. *J. Food Sci.*, **51**, 695–697.
- Meares, P. (1976). "Membrane Separation Processes." Elsevier Scientific Publishing Co., United Kingdom.
- Melling, J. (1974). Application of ultrafiltration—Modifying factors. *Proc. Biochem.*, **9**, 7–10.

- Nabetani, H., Abbott, T.P. and Kleiman, R.E. (1995). Optimal separation of Jojoba protein using membrane processes. *Ind. Eng. Chem. Res.*, **34**, 1779–1788.
- Nakajima, M., Snape, J.B., Reddy, K.K. and Nabetani, H. (1996). Application of membrane technology to soybean processing. Proceedings of the Second International Soybean Processing and Utilization Conference, 8–13 January, Bangkok, Thailand, pp. 508–514.
- Nichols, D.J. and Cheryan, M. (1981). Production of soy isolates by ultrafiltration: Factors affecting yield and composition. *J. Food Sci.*, **46**, 361–367.
- Okubo, K., Waldrop, A.B., Iacobucci, G.A. and Meyers, D.V. (1975). Preparation of low phytate soybean protein isolates and concentrate by ultrafiltration. *Cereal Chem.*, **52**, 263–270.
- Omoseyiye, O. and Cheryan, M. (1979). Low phytate, full fat soy protein product by ultrafiltration of aqueous extracts of whole soybeans. *Cereal Chem.*, **56**, 58–62.
- Olsen, H.S. (1978). Continuous pilot plant production of bean protein by extraction, centrifugation, ultrafiltration and spray drying. *Lebensm.-Wiss. U-Technol.*, **11**, 57–64.
- Olsen, H.S. (1988). Aqueous enzymatic extraction of oil from seeds. Presented at ASEAN Food Conference, 24–26 October, Bangkok, Thailand, NOVO A-06041a/HSO.
- Ostrowski, H.T. (1979). Membrane filtration for isolation, fractionation and purification of food and feed grade proteins from pasture herbage. *J. Food Proc. Preserv.*, **3**, 59–84.
- Parekh, B. (1988). "Reverse Osmosis Technology." Marcel Dekker Publication, New York.
- Payne, R.E., Hill, C.G. and Amundson, C.H. (1973). Concentration of egg white by ultrafiltration. *J. Milk Food Technol.*, **36**, 359–363.
- Poroske, L.H. (1984). Industrial scale applications of enzymes to the fats and oil industry. *J. Am. Oil Chem. Soc.*, **61**, 1758–1760.
- Porter, M.C. (1979). "Handbook of Separation Techniques for Chemical Engineers." ed. by P.A. Schweitzer, McGraw-Hill Book Company, New York, pp.114–117.
- Ratledge, C. (1984). Biotechnology as applied to the oils and fats industry. *Fette Seifen Anstrichm.*, **10**, 379–389.
- Rautenbach, R. and Albrecht, R. (1989). "Membrane Processes." John Wiley and Sons, New York.
- Rhee, K.C., Cater, C.M. and Mattil, K.F. (1972). Simultaneous recovery of protein and oil from raw peanuts in an aqueous system. *J. Food Sci.*, **37**, 90–93.
- Rosenthal, A., Pyle, D.L. and Niranjana, K. (1996). Aqueous enzymatic processes for edible oil extraction. *Enzyme Microb. Technol.*, **19**, 402–420.
- Salunke, D.K., Chavan, J.K., Adsule, R.M. and Kadam, S.S. (1991). "World Oilseeds Chemistry, Technology and Utilisation." Von Nostrand Reinhold, New York, pp. 247.
- Sidhoum, M., Majumdar, S., Bhave, R.R., Sengupta, A. and Sirkar, K.K. (1988). Experimental behavior of asymmetric cellulose acetate membranes and its use in novel separation schemes. AIChE Symposium Series No. 261, ed. by K.K. Sirkar and D. Lloyd, New York, pp.102–112.
- Snape, J.B. and Nakajima, M. (1996). Processing of agricultural fats and oils using membrane technology. *J. Food Eng.*, **30**, 1–41.
- Sosulski, F.W. (1962). The centrifuge method for determining flour absorption in hard red spring wheats. *Cereal Chem.*, **39**, 344–350.
- Sosulski, F.W. and McCleary, C.W. (1972). Diffusion extraction of chlorogenic acid from sunflower kernels. *J. Food Sci.*, **37**, 253–256.
- Sosulski, F.W., Soliman, F.S. and Bhatta, R.S. (1972). Diffusion extraction of glucosinolates from rapeseed. *Can. Inst. Food Sci. Technol. J.*, **5**, 101–104.
- Sourirajan, S. and Matsuura, T. (1985). "Reverse Osmosis/Ultrafiltration Process Principles." National Research Council Publication, Ottawa, Canada.
- Southwell, K.H. and Harris, R.V. (1991). Extraction of oil from oilseeds using the hot water flotation method. *Trop. Sci.*, **32**, 217–221.
- Subramanian, J., Bhatia, D.S., Kalbag, S.S. and Subramanian, N. (1959). Integrated processing of peanut for the separation of major constituents. *J. Am. Oil Chem. Soc.*, **36**, 66–70.
- Tragardh, C. (1974). Production of leaf protein concentrates for human consumption by isopropanol treatment. A comparison between untreated raw juice and raw juice concentrated by evaporation and ultrafiltration. *Lebensm. Wiss. U-Technol.*, **7**, 199–201.
- Tsai, L.S., Ijicij, K. and Harris, M.W. (1977). Concentration of egg white by ultrafiltration. *J. Food Protec.*, **40**, 449–455.
- Vix, H.L.E. and Decosas, K.M. (1969). Processing oilseeds into edible products. *Chem. Eng. Progr. Symp. Series*, **65**, 49–56.
- Woodroof, J.G. (1983). "Peanut: Production, Processing and Products." CT-AVI Publishers, Wessport.
- Yoon, S.H., Kim, I.H., Kim, S.H. and Kwon, T.W. (1991). Effects of enzyme treatments and ultrafiltration on extraction yields of lipids of proteins from soybeans by aqueous process. *Korean J. Food Sci. Technol.*, **23**, 673–676.