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Optimized extraction of soluble defatted rice bran fiber and its application for microencapsulation of fish oil

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OPTIMIZED EXTRACTION OF SOLUBLE DEFATTED RICE BRAN FIBER AND ITS APPLICATION FOR MICROENCAPSULATION OF FISH OIL

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ABSTRACT

Defatted rice bran (DRB) is a byproduct of rice milling and rice bran oil extraction. Soluble rice bran fiber (SRBF) extracted from defatted rice bran is known for its antioxidant activity and hypocholesterolemic effects in human, while purified menhaden oil (PMO) is a good source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The goal of the study was to estimate optimum extraction conditions to extract SRBF from DRB, develop a cost effective method to purify SRBF and produce microencapsulated PMO with SRBF. The response surface methodology showed that an estimated optimum yield of SRBF (7.89%) could be extracted from DRB with 3% $Ca(OH)_2$ solution to DRB ratio 29.75:1 and stirred for 1 hr at 84^{o}C and also $Ca(OH)_2$ solution concentration was the most effective factor among the conditions used to extract SRBF. Our study showed that conventional processing steps, such as dialysis and alcohol precipitation, for removing mineral and monosaccharides and other small molecules from SRBF, could be replaced with the ultrafiltration technology. The ultrafiltration for purifying SRBF solution at 100 kPa with 10 kDa MWCO membrane required less time than filtering the solution at the same pressure with 1 and 5 kDa MWCO membranes. The estimated microencapsulated PMO with SRBF powder (MFMO) production rate using spray dryer was 3.45×10^{-5} kg dry solids/s and was higher than the actual production rate 2.31×10^{-5} kg dry solids/s. The energy required to increase the inlet ambient air temperature from 27.1 to 180 °C and evaporation rate for spray drying the emulsion was 2.78 kJ/s and 7.8×10^{-3} kg water/s, respectively. EPA and DHA contents of MFMO were 11.52% and 4.51%, respectively. The particle size of 90% MFMO ranged from 8 to 62 μm, and the volume-length diameter of MFMO was 28.5 μm. The study demonstrated that optimum extraction conditions for extracting SRBF from DRB could be achieved through the response surface methodology, conventional

purification steps of SRBF including dialysis and alcohol precipitation could be replaced with ultrafiltration technology, and the MFMO could be provided with potential health benefits for humans.

CHAPTER 1. LITERATURE REVIEW

1.1 Dietary Fiber

1.1.1 Definition and Composition

The definition of dietary fiber is controversial through time and countries. Big events in history for the definition of the dietary fiber and various versions of definition generated or adopted by the scientists in different organizations and countries were summarized by several scientists (De Vries and others 1999, Tungland and Meyer 2002). In 1953, Hipsley was the first to generate 'dietary fiber' as the shorthand term for the non-digestible constituents that make up the plant cell wall (Hipsley 1953), in which only cellulose, hemicelluloses and lignin were included. In 1972, Trowell developed the definition into the residue of plant foods that resisted digestion by alimentary enzymes of humans (Trowell 1972a, Trowell 1972b), and through the efforts of several scientists between 1971 and 1974 (Painter and Burkitt 1971, Burkitt and others 1972, Trowell 1974), the component definition of dietary fiber were enlarged to include associated minor substances, such a waxes, cutin, and suberin. However, polysaccharides such as plant gum, algal polysaccharides, pectin and modified cellulose were not included. In 1976, the dietary fiber definition was extended to include all indigestible polysaccharides, such as gums, modified celluloses, mucilages, oligosaccharides, and pectins, and the definition was primarily physiological and based on characters of edibility and resistance to digestion (De Vries and others 1999). However, alternative definitions of dietary fiber did not stop being proposed (Asp and Johansson 1984, Cummings and Englyst 1991, Selvendran and Robertson 1994). In 1992 and 1993, two international surveys were conducted to reaffirm consensus on the physiological dietary fiber definition and inclusion of nondigestible oligosaccharides and resistant starch (Lee and Prosky 1995, Cho and Prosky 1999). Currently, the mostly widely

accepted definition of dietary fiber is a statement of their physiological and metabolic significance proposed by the American Association of Cereal Chemists in 2000: 'the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine'.

The reason for considerable debate worldwide on the definition of dietary fiber and its constituents was that dietary fiber does not constitute a single defined chemical group but a combination of complex chemically heterogeneous substances (Thebaudin and others 1997). Dietary fiber consists of cellulose, hemicelluloses, lignin, gums, pectins, modified celluloses, mucilages, oligosaccharides and associated minor substances, such as waxes, cutin, and suberin according to the definition. The main compositions of dietary fiber are briefly introduced below.

Cellulose is the most abundant compound on earth since it is the principal component of plants cell wall. Sequential β-D-glucopyranosyl units are joined by (1→4) glycosidic linkages to form the linear homopolymer with high molecular weight (BeMiller and Whistler 1996). It is insoluble in water and most organic solvents. A purified cellulose powder is colorless, odorless and tasteless and used as a food ingredient to provide non-caloric bulk. Its derivatives are believed to keep bakery goods moist and fresh for longer time (Collar and others 1998).

Hemicellulose is not chemically homogeneous but a complex material containing several hexoses, pentoses, hexuronic acids and amino acids with different functional groups (Gírio and others 2010). It consists of shorter chains compared with cellulose, and its random, highly branched structure makes some hemicelluloses soluble in water and easily hydrolyzed by dilute acid or alkali solution.

Lignin is the second most abundant natural polymer present in nature after cellulose (Boudet and Grima-Pettenati 1996). It is a highly complex non-polysaccharide polymer

consisting of phenylpropane units derived from aromatic alcohols such as coumaryl, coniferyl and sinapyl alcohols (Chakar and Ragauskas 2004). Lignin is highly insoluble in water.

Gums are important thickening and/ or gelling agents when they are dissolved or dispersed in water. Gums come from seaweed extracts (carrageenan, alginates), plant extracts (gum acacia, gum karaya, and gum tragacanth), leguminous seed plants (guar, locust bean), or microbial biosynthesis (xanthan, gellan) (Tungland and Meyer 2002). Gum molecules are comprised of long polysaccharide chains with plentiful branches of oligosaccharides and monosaccharides to provide high solution viscosity.

Structures of pectins are different between commercial and native sources. Commercial pectins are based on a polymer of D-galacturonic acid linked by α-1, 4 linkages with various contents of methyl ester groups; native pectins are more complex, which are simplified into commercial product by acid extraction (BeMiller and Whistler 1996). Pectins are primarily soluble in water and can form spreadable gels.

1.1.2 Classifications

Based on the type of polysaccharide, their simulated gastrointestinal solubility, the site of digestion, products of digestion and physiological classification, the components of dietary fiber can be classified into several different classification systems (Tungland and Meyer 2002). The most popularly used classification for dietary fiber has been to differentiate dietary components on (1) their role in the plant (Fig.1.1), (2) their fermentability in an in vitro system using an aqueous enzyme solution representative of human alimentary enzymes (Table 1.1), and / or (3) their solubility in water (Table 1.2).

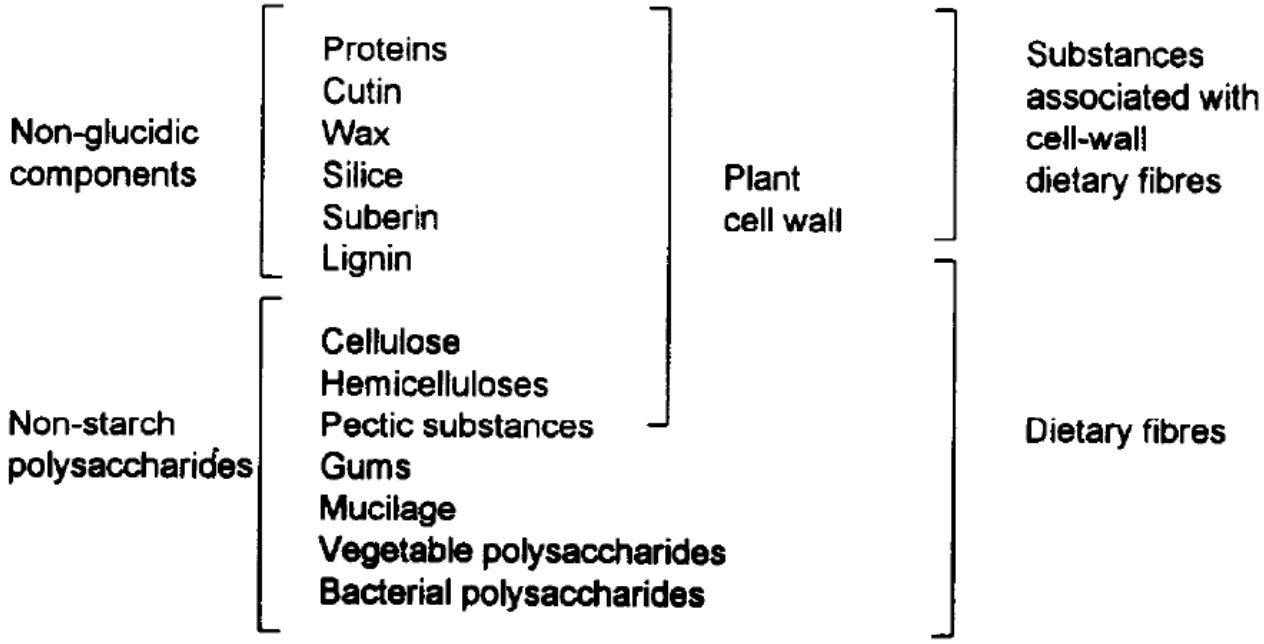

Fig. 1.1 Composition of Dietary Fibers and Associated Substances (Schweizer 1986)

Table 1.1 Classification of Dietary Fibers Based on Fermentability (Tungland and Meyer 2002)

Characteristic	Fiber component	Main food source
Partial or low fermentation	Cellulose	Plants (vegetables, sugar beet, various brans)
	Hemicellulose	Cereal grains
	Lignin	Woody plants
	Cutin/suberin/other plant waxes	Plant fibers
	Chitin and chitosan, collagen	Fungi, yeasts, invertebrates
	Resistant starches	Plants(corn, potatoes, grains, legumes, bananas)
	Curdlan	Bacterial fermentation
Well fermented	beta-glucans	Grains(oat, barley, rye)
	Pectins	Fruits, vegetables, legumes, sugar beet, potato
	Gums	Leguminous seed plants, seaweed extracts, plant extracts, microbial gums
	Inulin	Chicory, Jerusalem artichoke, onions wheat
	Oligosaccharides/analogues	Various plants and synthetically produced
	Animal origin	Chondrotin

Table 1.2 Classification of Dietary Fibers Based on Water Solubility (Wardlaw and Kessel 2002)

Type	Components	Physiological effects	Major food sources
Insoluble			
Noncarbohydrate	Lignin	increase fecal bulk; estrogen like effects	Whole grains, flax seeds
Carbohydrate	Cellulose	increase fecal bulk	All plants
	Hemicellulose	Decrease intestinal transit time	Wheat, rye, rice, vegetables
Soluble			
Carbohydrate	Pectins, gums, mucilages, some hemicellulose	Delay gastric emptying; slow glucose absorption; lower blood cholesterol	Citrus fruits, oat products (β-glucan inparticular), beans, thickeners added to foods

Since none of the classification systems can entirely distinguish the difference in dietary fiber compositions, the debate on the most appropriate means to classify dietary fibers is still on –going. Dietary fibers that are soluble in water are the focus in the study, and therefore the classification based on solubility is discussed in detail. According to their water solubility, dietary fiber constituents can be classified in two categories: insoluble dietary fibers and soluble dietary fibers. The insoluble dietary fibers include cellulose, hemicelluloses, lignin, cutin and plant waxes; while the soluble dietary fibers include some hemicelluloses, gums, pectins, mucilages and others. The solubility depends on the nature of the glucidic components, the structural characteristics and the electric charge of the dietary fiber (Thebaudin and others 1997). Polysaccharides with linear chain structure, which increases the strength of the links and stabilizes the ordered conformation, are poorly solubilized; on the other hand, chemically irregular or highly branched polysaccharides are more easily dissociated and solubilized. Also, polysaccharides with charges are more soluble in water.

1.1.3 Physiological Properties

Although dietary fibers cannot be digested by alimentary enzymes of humans, they affect carbohydrate metabolism, lipid metabolism, mineral bioavailability, bacterial fermentation in the

colon, and fecal bulk formation and transition. The physiological properties of dietary fibers make them functional ingredients in food product.

1.1.3.1 Effects on Carbohydrate Metabolism

Little effect of insoluble dietary fibers on carbohydrate metabolism is known, but meals containing soluble dietary fibers and food rich in viscous fibers have resulted in reductions in postprandial increases in blood glucose and insulin (LeBlanc and others 1991, Ohta and others 1997, Hallfrisch and Behall 2000). The viscous soluble dietary fibers increase the viscosity of intestinal contents and decrease the glycemic response (Dikeman and Fahey 2006).

1.1.3.2 Effects on Lipid Metabolism

The effect of dietary fibers on lipid metabolism is similar as that on glucose metabolism. Glore and others (1994) summarized studies on soluble dietary fibers and serum lipids and found that soluble dietary fibers have the potential to decrease the levels of total cholesterol and low-density lipoprotein cholesterol in the serum. Guillon and Champ (2000) believed that the viscosity and the ion-change capacity of the dietary fibers are important in lipid absorption. Thebaudin and others (1997) emphasized that the absorption of bile salts by dietary fibers and the products of bacterial fermentation of dietary fibers play key roles in the changes in lipid and cholesterol metabolism.

1.1.3.3 Effects on Mineral Bioavailability

It is difficult to predict the effect of dietary fibers on bioavailability of minerals due to the complex interactions exist among minerals and dietary fibers in varying conditions (Thebaudin and others 1997). Studies concerning interactions of minerals with dietary fibers found that phytate (polyphenols) was likely to be responsible for the inhibition of mineral absorption in both animals and humans (Morris and Ellis 1989, Torre and others 1991, Wisker and others 1991). Removal of phytate, dietary fibers can improve the bioavailability of minerals, such as

iron, zinc, and calcium (Torre and others 1991, Wisker and others 1991, Thebaudin and others 1997). Certain highly fermentable fibers, such as pectin, gums and oligosaccharides, were reported to improve metabolic absorption of certain minerals even when phytate was present (Baba and others 1996, Lopez and others 1998).

1.1.3.4 Fermentation in Colon

Guillon and Champ (2000) believed that dietary fibers are major sources of energy for bacteria in the large intestine during fermentation to short chain fatty acids. Short chain fatty acids are produced in the colon from the bacterial breakdown of dietary fibers and proved to have benefits to the human body, including inhibition of growth of harmful bacteria and yeasts, improvement of mineral absorption, and reduction of liver toxin (Hague and others 1993, Scheppach 1998, Tungland and Meyer 2002). Soluble dietary fibers are generally more rapidly fermented than the others (Guillon and Champ 2000).

1.1.3.5 Promoting Laxation

Increasing faecal bulk, increasing stool weight, softening faeces, and reducing intestinal transit time are widely known physiological properties of dietary fibers. These effects are usually related to the insoluble dietary fibers, which cannot be digested in the small intestine by alimentary enzymes and/ or fermented in the large intestine by bacteria. The increased fecal weight is attributed to the undigested dietary fibers and water that is bound to the dietary fibers. Soluble dietary fibers do not markedly result in fecal-bulking and intestinal transit time decrease (Thebaudin and others 1997).

1.1.4 Physicochemical Properties

Dietary fibers are used as a functional ingredient in food products since they can modify the consistency, texture, rheological properties and sensory characteristics of the fiber

supplemented food. These functions are due to their physicochemical properties (Collar and others 2009). Moreover, the physiological functions of the dietary fibers, such as hydration properties, viscosity and ion/ organic molecule binding properties, are often attributed to their physicochemical properties (Dikeman and Fahey 2006).

1.1.4.1 Hydration Properties

The hydration properties are not only related to the fate of dietary fiber in the digestive tract and the physiological effects of dietary fiber, but also determine the optimal usage level of dietary fiber in food products (Thebaudin and others 1997, Guillon and Champ 2000). Four different parameters are used to evaluate the hydration properties, among which water-holding capacity, water-binding capacity, and swelling are used to judge insoluble dietary fiber, and solubility is relevant for soluble dietary fiber. The hydration properties are different among dietary fibers from various sources and are related to the particle sizes and surface area characteristics of dietary fiber (Thebaudin and others 1997, Guillon and camp 2004; Rosell and others 2009). The environmental conditions, such as pH, ionic strength, and temperature can also influence the hydration values (Fleury and Lahaye 1991).

1.1.4.2 Viscosity

Viscosity describes a fluid's resistance to flow, and it is the relationship between the shear rate and shear stress. The chemical composition and concentration of polysaccharide as well as the environmental conditions, such as pH, temperature, and processing condition are related to alterations in the viscosity of dietary fibers in solutions (Dikeman and Fathey 2006). Dietary fibers that form viscous solutions are usually used as thickening agents to increase the viscosity in food systems (Nelson 2001). The physiological properties of dietary fibers, such as

lowering cholesterol, decreasing the rate of glucose absorption and decreasing post-prandial plasma glucose concentrations are due to their viscosity (Dikeman and Fathey 2006).

1.1.4.3 Adsorption of Ions and/ or Organic Molecules

The capacity of binding ions depends largely on the type of dietary fibers and attributes of the charge group and associated substances such as phytates in the dietary fibers. Guillon and Champ (2000) found that charged dietary fibers did not have nutritional effects on mineral and trace element absorption, but associated substances had a negative effect.

Dietary fibers that are rich in uronic acids and phenolic compounds exhibit ability to sequester and even chemically bind bile acids (Guillon and Champ 2000). In addition, cholesterol, drugs, and toxic compounds can also be adsorbed by dietary fibers. The adsorption capacity of dietary fiber may be influenced by physical and chemical forms of themselves and/ or environmental conditions (Thibault and others 1992, Dongowski and Ehwald 1998)

1.1.5 Sources

The amount and composition of fibers differ from food to food (Desmedt and Jacobs 2001). Dietary fibers are usually found in plants, such as cereals, legumes, fruits, vegetables, nuts, and seeds, and also can be obtained from seaweeds. Among the different fiber - rich foods, cereals contribute about 50% of the dietary fiber intake in western countries (Lambo and others 2005); vegetables and fruits provide more than 40 % dietary fiber in the diet, and the remaining is from other minor sources (Gregory and others 1990, Cummings 1996).

1.2 Defatted Rice Bran

Rice, a staple food in many countries, is usually consumed polished (Zha and others 2009). Rice bran is derived from the outer layer of brown rice as a by-product of milling, and includes the pericarp, seed coat, aleurone layer, germ and a small portion of starchy endosperm

(Shibuya and Iwasaki 1984). Rice bran makes up about 10% of brown rice weight (Li and others 2008), but it is underutilized as feed for animals or discarded directly (Harada and others 2008). However, since rice bran oil was promoted for its health benefits (Seetharamaiah and Chandrasekhara 1989, Ausman and others 2005, Zigoneanu and others 2008), the demand for milled rice bran is increasing in the worldwide market and the latent demand estimated in the US in 2011 is 99.18 million dollars (Parker 2005). Defatted rice bran is a by-product of rice bran oil extraction from whole rice bran. As a by-product, defatted rice bran is actually an excellent source of protein, minerals, dietary fiber and other components (Abdul-Hamid and Luan 2000).

1.2.1 Soluble Rice Bran Fiber from Defatted Rice Bran

Rice bran is reported to contain 25.5-40.0% total dietary fibers and 2.3-4.3% soluble dietary fibers (Aoe and others 1993, Abdul-Hamid and Luan 2000, Sudha and others 2007). Soluble dietary fibers extracted from defatted rice bran using alkali solution are mainly composed of hemicelluloses (Mod and others 1978, 1979, Aoe and others 1993). Previous studies found that water-soluble and alkali-soluble rice bran hemicelluloses could be used as a dietary fiber source (Normand and others 1981, Mod and others 1981, Normand and others 1984). Glucose, arabinose, xylose and galactose were the main monosaccharides found in rice hemicelluloses (Bevenue and Williams 1956, Gremli and Juliano 1970, Cartaño and Juliano 1970). Xylan provides as the backbone of rice bran hemicelluloses and other sugars act as side chains (Harada and others 2005).

1.2.2 Functional Properties

Dietary fibers from defatted rice bran have great application potential in food due to their functional properties. Abdual-Hamid and Luan (2000) prepared dietary fibers from defatted rice bran and found rice bran fiber exhibited comparable water binding capacity, fat binding capacity,

and emulsifying capacity, but was less viscous than a commercial fiber from sugar beet. Moreover, soluble dietary fibers extracted from defatted rice bran show benefits to human health, such as antioxidant activity, cholesterol reduction activity and other properties.

1.2.2.1 Antioxidant Activity

Polysaccharides from different resources were shown to have antioxidant properties (Chen and others 2008, Tseng and others 2008, Sun and others 2009, Lai and others 2010, Wang and others 2010). Zha and others (2009) found that polysaccharides extracted from rice bran showed good antioxidant activities, including capability of scavenging radical, anti-lipid peroxidation, potential for reducing power, chelating ability and so on. A modified arabinoxylane from rice bran, named MGN-3 was reported to possess antioxidant and free radical scavenging property in an *in vivo* study dealing with female mice injected with ascites carcinoma cells and MGN-3 (Noaman and others 2008).

1.2.2.2 Hypocholesterolemic Activity

The water soluble polysaccharide fraction in rice bran was found to be an active component having a hypocholesterolemic effect (Vijayagopalan and Kurup 1972, Saunders 1990). Aoe and others (1993) reported that rats fed cholesterol enriched diets with alkaline extracted soluble rice bran fiber for 9 days showed lower serum cholesterol level with the same body weight gain as controls. Rouanet and others (1993) reported that the high soluble fiber content of rice bran had a more obvious hypocholesterolemic activity than that of wheat bran when comparing of the plasma and liver cholesterol concentrations measured in rats. Rats fed high-cholesterol diets containing parboiled and crude rice bran fiber for 21 days had lower plasma LDL cholesterol concentrations and less liver cholesterol than rat fed a wheat bran diet. There was a greater impact of the parboiled rice bran than crude rice bran which might be due to

its higher soluble fiber content. It was reported that feeding a rice bran diet increased fecal bile acid and sterol excretion so that the plasma LDL cholesterol was lowered by compensation through the uptake of LDL cholesterol by the liver (Illman and Topping 1985, Topping and others 1990).

1.2.2.3 Other Properties

Water soluble polysaccharides from defatted rice bran also have other physiological properties, such as immune-modulation and anti-tumor properties. Wang and others (2008) reported that non-starch polysaccharides extracted and purified from defatted rice bran exhibited anti-complementary activities. Non-starch polysaccharides isolated from rice bran also showed antitumor properties in gastrointestinal carcinoma and colon cancer (Cummings and others 1992, Takeshita and others 1992). Other effects were found from biologically active hemicelluloses from rice bran, including raising the amounts of the peripheral blood lymphocytes, reducing thymus atrophy in rats, enhancing the immune function, increasing T and B cell mitogen response, and aiding human health and preventing disease (Hikino and others 1988, Takenaka 1992, Takenaka and Itoyama 1993, Ghoneum 1998, Tzianabos 2000, Ghoneum and Gollapudi 2003).

1.3 Soluble Dietary Fiber Extraction

There are mainly two methods of soluble dietary fiber extraction. One is enzymatic-gravimetric method, and the other one is alkali/acid treatment method.

1.3.1 Enzymatic-Gravimetric Method

The enzymatic-gravimetric method is actually generated from the analytical method for dietary fibers. In 1979, Prosky led an international cooperative study to pursue a methodology to quantify dietary fibers. It was supported by 43 laboratories in 29 countries (De Vries and others

1999), and finally an accurate enzymatic-gravimetric methodology was obtained and adopted by AOAC as the official method of analysis for total dietary fibers (Official Method 985.29). The method protocol related to soluble dietary fiber extraction is described as follows: duplicate samples of dried foods (fat content < 10%, if not, extracted by solvent) are hydrolyzed with Termamyl (heat-stable α-amylase), and digested with amyloglucosidase and protease to remove protein and starch; 4 volumes of ethanol are added to precipitate soluble dietary fiber (AOAC 2000). Based on the benchmark method, scientists improved the method by using phosphate buffer (Official Method 993.16) and/or MES-TRIS buffer (Official Method 991.43) to reduce the complex procedure for adjusting pH and limit extraction solution volume (AOAC 2000).

Specifications on enzyme purity and precise handling of the digestion steps of the method are believed to be critical keys to success in obtaining the soluble dietary fiber (De Vries and others 1999). Abdul-Hamid and Luan (2000) extracted dietary fibers from defatted rice bran using a modified enzymatic-gravimetric method, and obtained 27.04% total dietary fibers and 2.25% soluble dietary fibers. The enzymatic-gravimetric method is too complex and expensive to be applied in the industry, because by using several enzymes, the pH of the extraction solution has to be adjusted several times and therefore a large volume of ethanol is needed to precipitate soluble dietary fiber since the volume of extraction solution is increased during the pH adjustment. Moreover, the sample size processed each time is small, which is not suitable for the industry.

1.3.2 Alkali/Acid Treatment Method

The alkali/acid treatment method employs alkali or acid, and water as the solvent to extract hemicelluloses from defatted rice bran. Hot water is considered to catalyze acetyl groups liberation during the breakdown of bonds in the material and the hydronium ions of water

(Buranov and Mazza 2010). When distilled water was used to extract hemicelluloses but only 1.64% soluble non-starch polysaccharides was obtained from rice bran (Zha and others 2009). Acid in low concentration increased the hemicelluloses extraction, but induced the degradation of hemicelluloses into monomers (Grohmann and others 1985, Torget and others 1991, Teramoto and others 2008). Aoe and others (1993) used 3% CH_3COOH and/or 4% HCl solution to extract hemicelluloses from defatted rice bran, and lower yield or hydrolyzed products were obtained. Alkaline solution helps to hydrolyze the ester linkages to liberate hemicelluloses into aqueous media (Buranov and Mazza 2010). Cartaňo and Juliano (1970) extracted hemicelluloses from rice using 0.5 mol/L NaOH repeated three times and obtained arabinoxylans containing glucose and galactose. Shibuya and Iwasaki (1984) treated defatted rice bran with 4 mol/L NaOH and got 4.6% crude hemicelluloses. Aoe and others (1993) compared the extraction of soluble dietary fibers from defatted rice bran with 2% NaOH and/or 2% $Ca(OH)_2$ with acid solution extraction. Higher yield and non-hydrolyzed hemicelluloses retaining bioactive activity were obtained from the alkali extractions. This method is simpler than the enzymatic gravimetric method and the sample size to be processed can be controlled accordingly.

1.4 Ultrafiltration

Ultrafiltration is a kind of membrane filtration process in which hydrostatic pressure difference is used as the driving force to separate solutes according to molecular weight against a semi permeable membrane (De Bruijn and others 2005). High molecular weight solutes and suspended solids are retained; low molecular weight solutes and water permeate the membrane.

Ultrafiltration has already been used in food industry. Since the late 70's, ultrafiltration, as a cost, time and labor- saving method, has been applied commercially to clarify pectins, cellulose, hemicelluloses, starch and proteins from juices, such as apple, grape, pear, pineapple,

cranberry and citrus juices (Jőnsson and Trägårdh 1990). It also replaced centrifugation to clarify wine and remove the nutrients and bacteria from the vinegar (De La Garza and Boulton 1984, Joshi and Sharma 2009). It is used in the dairy industry for milk dehydration and whey concentration (Kazemimoghadam and Mohammadi 2007). In egg processing, ultrafiltration operations could be used for concentrated egg-white or whole egg before spray-drying (Jőnsson and Trägårdh 1990). Chackravorty and Singh (1990) reported that ultrafiltration improved product quality and reduced emissions and water usage to concentrate and purify weak gelatin liquor when compared with a conventional evaporation processes.

Ultrafiltration can be used to purify soluble dietary fiber. Sheng and others (2007) used ultrafiltration to separate the main fraction of polysaccharides extracted from alga. Nabarlatz and others (2007) used an ultrafitration technique with thin-film polymeric membrane to purify the xylo-oligosacchardes obtained from almond shells by autohydrolysis, and lignin-related impurities were separated and discarded. Gabrielii and others (2000) used ultrafiltration to purify hemicelluloses from aspen after extraction by an alkali method and hydrogen peroxide treatment. Low– molecular hemicelluloses extracted from flax shives with ethanol precipitation were separated through ultrafitration (Buranov and Mazza 2010).

1.5 Microencapsulation

1.5.1 Microencapsulation Techniques

Microencapsulation is a technology of packaging solid, liquid, or gaseous materials in miniature sealed capsules for release at controlled rates using desired release triggers (Klaypradit and Huang 2008). There are a large number of different processes to produce microcapsules, such as spray drying, spray cooling/ chilling, spinning disk and centrifugal coextrusion, extrusion, fluidized bed, coacervation, alginate beads, liposome entrapment, freeze drying and so

on (Gouin 2004, Gharsallaoui and others 2007), but spray drying is the most common technology used in the food industry. Spray drying encapsulation is considered low cost and straightforward; however, since the spray drying processes are carried from aqueous feed formulations, the solubility of the material limits the utilization of spray drying technology: large amounts of water to be evaporated from the feed makes the process time consuming and expensive (Gouin 2004). Therefore, the ideal shell materials for use in spray drying encapsulation should be highly water soluble. Soluble dietary fibers can also be used as a functional ingredient to develop a stable microcapsule. Doleyres and others (2002, 2004), Lee and others (2004), Saénz and others (2009), Choi and others (2010) have reported that soluble dietary fibers, such as cyclodextrin, inulin, κ-carrageenan and chitosan are good functional ingredients for microencapsules.

1.5.2 Microencapsulated Fish Oil with Soluble Dietary Fiber

Long chain polyunsaturated fatty acids, particularly omega-3 fatty acids, are reported to exhibit physiological benefits to humans, such as reducing the risk of cardiovascular disease, autoimmune disorders and other inflammations (Weaver and Holub 1988). With the growing awareness of nutritional benefits of omega-3 fatty acids, more physiological effects have been explored, including lowering plasma triglycerides, increasing aggregation time for platelets, decreasing viscosity of blood, decreasing blood pressure, reduction in atherosclerosis, reduction of inflammation, and reduction in tumors (Mishra and others 1993). Fish oil is a favorable source of omega-3 fatty acids, such as docosahexaenoic acid (DHA, C22:6n-3) and EPA (eicosapentaenoic acid, C20:5n-3). Fish oil usually contains 4.1-37% DHA and 5-24% EPA (Kinsella 1986), due to the variation in fish species, seasons, locations of the catch, food habitats and parts of the fish body (Mishra and others 1993).

Development of food fortified with omega-3 fatty acids has drawn a lot of attention from academic and industrial aspects. However, attempts to incorporate fish oil directly into food formulations have had limited success because of 'fishy' flavors in the finished products and oxidation of polyunsaturated fatty acids. The main problem of food fortification with omega-3 polyunsaturated fatty acids is the unpleasant fishy flavor that has a negative influence on food acceptability (Kolanowski and others 1999). The chemical structures of the polyunsaturated fatty acids show abundant double bonds which are easily oxidized. One of the technologies used for overcoming these problems is microencapsulation of fish oil. Among the reasons that Shahidi and Han (1993) proposed for applying microencapsulation in the food industry, the critical points for microencapsulation of fish oil were to reduce the core (fish oil) reactivity with environmental factors (oxygen) and to mask the core taste (unpleasant fishy flavor). The microencapsulation process makes it possible to transform the fish oil into a powder, where the small droplets of the fish oil are surrounded by a shell coating of proteins and/or carbohydrate resulting in small dry granules that have powder like flow characteristics. Oxidative stability of fish oil is improved by the microencapsulation process and its shelf life then can be extended when stored in a dry cool environment. The microencapsulation of fish oil also provides a taste profile barrier eliminating fish oil taste and odor.

Compared with simply converting food from liquid to solid form and masking the unpleasant flavor of ingredients in the past, more complex properties from several food ingredients entrapped in a homogeneous or heterogeneous food grade matrix are expected for microencapsulation techniques applied in the food industry (Gouin 2004). A microcapsule containing fish oil and soluble dietary fibers from defatted rice bran are hypothesized to provide omega-3 polyunsaturated fatty acids combined with physiological properties from dietary fibers.

Moreover, hemicelluloses showed low oxygen permeability (Grőndahl and others 2004, Hartman and others 2006), and therefore had potential as oxygen barrier films (Krawczyk and others 2008), to prevent oxidation of the fish oil.

CHAPTER 2. DETERMINATION AND OPTIMIZATION OF SOLUBLE DIETARY FIBER EXTRACTION FROM DEFATTED RICE BRAN USING RESPONSE SURFACE METHODOLOGY

2.1 Introduction

Defatted rice bran (DRB) is a by-product of rice bran oil processing and contains both soluble and insoluble dietary fibers. Soluble rice bran fiber (SRBF) is more applicable than insoluble rice bran fiber (IRBF) for use in food products. Developing a DRB or IRBF fortified food may not be practical and acceptable to many consumers because the color of fiber fortified food may turn darker and the fiber may produce unpleasant mouth feel. SRBF is more applicable for use in food products than DRB or IRBF. Therefore, Aoe and others (1993) have attempted to extract water soluble dietary fibers by an alkali treatment method. Soluble dietary fibers extracted from defatted rice bran using an alkali solution are mainly composed of hemicelluloses (Mod and others 1978, Mod and others 1979, Aoe and others 1993), which is believed to be one of the active components with hypocholesterolemic effect (Vijayagopalan and Kurup 1972, Aoe and others 1989, Saunders 1990). Soluble rice bran fiber is highly soluble in water and is reported to have beneficial functional properties including hypocholestrolemic, antioxidant, immune-modulation and anti-tumor activities (Aoe and others 1989, Saunders 1990, Cummings and others 1992, Wang and others 2008, Zha and others 2009).

Different conditions for extracting SRBF from rice bran were previously studied by Cartaňo and Juliano (1970), Mod and others (1978) and Aoe and others (1993) and reported the yield and the quality of soluble dietary fibers for the relevant conditions used to extract the fiber. Numerous other studies have also shown that the extraction of non-starch polysaccharides is significantly affected by the extraction conditions such as temperature, time, ratio of water to raw material, and solvent concentration (Wu and others 2007, Yin and Dang 2008, Liu and others

2009, Li and others 2009, Qiao and others 2009, Zhao and others 2009, Guo and others 2010, Sun and others 2010, Zhu and others 2010).

It is evident that the optimum SRBF yield cannot be estimated based on just the one-factor-at-a-time (single factor) approach because it is not only time consuming, but also the interaction between extraction conditions may not be included in the determination of the yield and/or quality. A study is needed not only to determine the optimum extraction conditions to obtain a desirable yield or quality of SRBF from defatted rice bran but is also required to understand the degree of interactions between the extraction conditions such as temperature, time, ratio of solvent to raw material, and solvent concentration. A statistical optimization procedure such as response surface methodology (RSM) includes the interaction of the extraction factors into computation (Haaland 1989).

RSM is widely applied in the food industry to determine the effects of multiple processing variables and their interaction on response variables (Senanayake and Shahidi 2002, Téllez-Luis and others 2003, Vasquez and Martin 1998). Myers and Montogomery (2002) have expressed that RSM can be used to develop, improve, and optimize a process because the methodology includes both statistical and mathematical techniques. The RSM reduces the number of experimental trials and is considered to be less laborious and time consuming to optimize a process (Glovanni 1983). The RSM can also be applied to an experimental design such as Box-Behnken (BBD) with three factors and three levels variables to fit a second-order polynomial by least squares (Liu and others 2009).

The objectives of this study were to 1) determine the effects of single factors such as temperature, time, calcium hydroxide [$Ca(OH)_2$] concentration, and the ratio of $Ca(OH)_2$ solution to DRB on SRBF yield and 2) optimize the effects of the ratio of extraction solution to

defatted rice bran, concentration of extraction solution, and extraction temperature on the SRBF yield using response surface methodology with a three level and three independent variables Box-Behnken factorial design.

2.2 Materials and Methods

2.2.1 Proximate Analysis of Defatted Rice Bran

Defatted rice bran powdered (DRB) provided by Riceland Foods, Inc., Stuttgart, AK was analyzed for moisture, fat, protein, ash, total dietary fiber (TDF), insoluble dietary fiber (ISDF), and soluble dietary fiber (SDF) contents. The moisture content was determined according to AOAC procedure 985.14 (AOAC 1995) using a SMART System 5 microwave moisture/solids analyzer (CEM Corporation, Matthews, NC) and then the dehydrated DRB was used to extract the fat using methylene chloride with a FAS-9001-3 fat analyzer (CEM Corporation, Matthews, NC). The protein content was determined according to AOAC procedure 992.15 (AOAC 1995) using a Perkin Elmer Model 2410 Nitrogen Analyzer (Perkin Elmer Instruments, Norwalk, CT). The ash content was determined by a Thermolyne Type 6000 muffle furnace (Thermo Scientific, Lawrence, KS) at 549 ºC as described in AOAC method 920.153 (AOAC 1995). The TDF, ISDF, and SDF contents were determined according to AACC method 32-07 (AACC 2000) using an enzymatic-gravimetric method with MES-TRIS buffer. A 1 g sample of DRB was placed in a 500 mL beaker with 40 mL MES-TRIS buffer and then heat-stable α-amylase (Sigma Chem. Co., St.Louis, MO) was added and heated in a water bath at 95-100 ^{o}C for 35 min. The mixture was cooled to 60 ^{o}C, 100 µL protease (Sigma Chem. Co., St.Louis, MO) was added and kept for 30 min. After 30 min, a 5 mL of HCl and 300 µL amyloglucosidase (Sigma Chem. Co., St.Louis, MO) were added and the beaker was placed in the water bath at 60 ^{o}C for 30 min. After hydrolyzing the starch and protein, the DRB was washed with distilled water. The

insoluble and soluble portions were separated by Buchner funnel. Determined protein and ash contents were subtracted from the insoluble portion for estimating the ISDF content. The separated soluble portion was precipitated with 4 volumes of 95 % ethanol and the precipitated soluble portion was collected, dried in oven at 105 ^{o}C for 24 hr and then protein and ash contents were determined.

The SDF content was estimated by subtracting protein and ash content from the dried soluble portion. The TDF content and carbohydrate content were estimated using equations 2.1 and 2.2, respectively.

$$TDF = ISDF + SDF \quad \text{(Eq.2.1)}$$

$$\%\ \text{digestible carbohydrate} = 100-(\%\ \text{moisture} + \%\ \text{fat} + \%\ \text{protein} + \%\ \text{ash} + \%\ \text{TDF}) \quad \text{(Eq.2.2)}$$

2.2.2 Extraction of SRBF from Defatted Rice Bran

SRBF from defatted rice bran was extracted (Fig. 2.1) by a modified procedure of Aoe and others (1993). The starch in the defatted rice bran (50 g) was digested with 0.5 mL amylase solution (13083 units/ mL, Sigma-Al, St Louis, MO) at pH 6.9 for 15 min at 90 °C, and the starch-free defatted rice bran was separated by Buchner funnel with the aid of filter paper. The separated starch-free defatted rice bran was washed with deionized water four times and mixed by Talboys laboratory 134-1 stirrer (Troemner LLC, Thorofare, NJ) in a 2 L beaker with $Ca(OH)_2$ solution for extraction.

Four single factor experimental extraction conditions (Table 2.1) were used to produce soluble fractions from the starch-free defatted rice bran by: 1) 2 % (w/v) $Ca(OH)_2$ solution added to defatted rice bran in a 20:1 ratio (v/w) and stirred at 20, 40, 60, 80, and 100 $^{\circ}$C for 4 hr; 2) 2 % $Ca(OH)_2$ solution added to defatted rice bran in a 20:1 ratio and stirred at 60 $^{\circ}$C for 1, 2, 3, 4, and/or 5 hr; 3) 2 % $Ca(OH)_2$ solution added to defatted rice bran in 10, 12.5, 15, 17.5, 20, 22.5,

25, 27.5 and/or 30:1 ratios (v/w) at 60 °C for 4 hr; and 4) 0.5, 0.8, 1.0, 1.2, 1.5, 1.8, 2, 2.2, and/or 2.5 % (w/v) $Ca(OH)_2$ solution added to defatted rice bran in a 20:1 ratio and stirred at 60 °C for 4 hr. The extracted soluble fractions were neutralized with concentrated acetic acid and centrifuged at 4 °C and 15,300 x *g* for 30 min to separate soluble fractions. The supernatants were dialyzed at 23 °C for 24 hr under running deionized water at a flow rate of 0.15 m^3/s. After dialysis, 4 volumes of 95% ethanol were used to obtain a final concentration of 80% ethanol solution to precipitate the SRBF. The SRBF precipitate was stored overnight at room temperature and freeze-dried.

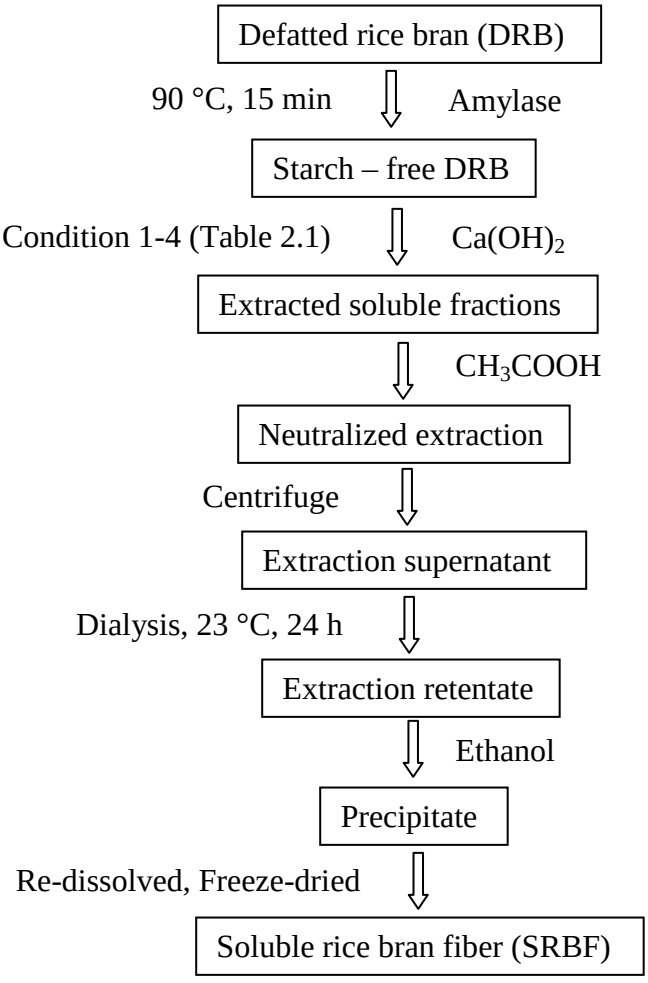

Fig. 2.1 SRBF Extraction Flow Diagram

Table 2.1 Single-Factor Extraction Parameters

Conditions	Extraction Temperature (°C)	Extraction Time (h)	Ratio of $Ca(OH)_2$ solution to defatted rice bran	Concentration of $Ca(OH)_2$ solution (%)
1	20-100	4	20:1	2
2	60	1-5	20:1	2
3	60	4	10:1-30:1	2
4	60	4	20:1	0.5-2.5

2.2.3 Optimization of Extraction of SRBF from Defatted Rice Bran

The best combination of extraction variables for extracting SRBF was determined using a three levels and three independent variables Box-Behnken factorial Design (BBD) (Sun and others 2010). The independent extraction variables (Table 2.2) including ratio of $Ca(OH)_2$ solution to defatted rice bran (X_1), concentration of $Ca(OH)_2$ solution (X_2), and extraction temperature (X_3) were used for this study. The suitable ranges of X_1, X_2, and X_3 were determined based on the single factor experiment (Table 2.2) for extraction of SRBF. The variables (X_1, X_2 and X_3) were coded according to Equation 2.3 for statistical calculation.

$$x_i = \frac{X_i - X_0}{\Delta X} \qquad \text{(Eq. 2.3)}$$

where x_i was the coded value of the variable; X_i was the actual value of the independent variable; X_0 was the actual value of the independent variable at the center point; and ΔX was the step change value of the independent variable.

Table 2.2 Independent Variables and Levels Used in the Response Surface Design

Independent variables	Factor level		
	-1	0	1
Ratio of $Ca(OH)_2$ solution to defatted rice bran (X_1)	25	27.5	30
Concentration of $Ca(OH)_2$ solution (X_2)	2	2.5	3
Extraction temperature (°C) (X_3)	20	60	100

The dependent variable was the yield (%, w/w) of SRBF in this experimental design. The complete experimental design consisted of 15 experimental points is shown in Table 2.3.

All of the 15 treatments were taken in random order. The conditions at the center of the BBD (13-15) were repeated three times to estimate the pure error sum of squares (Sun and others 2010).

2.2.4 Data Analysis

Mean values from the three separate experiments or replicate analyses are reported with standard deviation. The statistical significance of observed differences among treatment means was evaluated by analysis of variance (ANOVA) (SAS Version 9.2, SAS Institute Inc., Cary, NC, U.S.A).

Data from the BBD were analyzed by multiple regressions to fit the quadratic polynomial model (Equation 2.4):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \qquad \text{(Eq. 2.4)}$$

where, Y is the dependent variable (yield of SRBF from defatted rice bran). β_0, β_i, β_{ii}, and β_{ij} are the coefficient regression for constant, linear, quadratic and interactive terms, respectively. x_i and x_j are the coded independent variables. The regression coefficients of linear, quadratic and interaction terms were determined based on ANOVA, and the P-value (<0.05) of each coefficient was considered to be statistically significant. The 3-D response surface plots and contour plots were generated from the fitted polynomial equation to predict the relationships between the independent and dependent variables. The 3-D response surface plot and contour plot were obtained using the response value along with two independent variables, while the other variable was a fixed constant at their respective 0 level (center value of the testing ranges).

Table 2.3 Box-Behnken Design Matrix of Three Variables

Treatments	x_1: ratio of $Ca(OH)_2$ solution to defatted rice bran	x_2: concentration of $Ca(OH)_2$ solution	x_3: extraction temperature (°C)	Yield of SRBF (%)
1	-1(25)	-1(2)	0(60)	6.41
2	-1(25)	1(3)	0(60)	6.77
3	1(30)	-1(2)	0(60)	6.53
4	1(30)	1(3)	0(60)	7.90
5	0(27.5)	-1(2)	-1(20)	6.65
6	0(27.5)	-1(3)	1(100)	6.47
7	0(27.5)	1(3)	-1(20)	6.84
8	0(27.5)	1(3)	1(100)	7.60
9	-1(25)	0(2.5)	-1(20)	6.37
10	1(30)	0(2.5)	-1(20)	6.60
11	-1(25)	0(2.5)	1(100)	6.30
12	1(30)	0(2.5)	1(100)	6.74
13	0(27.5)	0(2.5)	0(60)	7.10
14	0(27.5)	0(2.5)	0(60)	7.11
15	0(27.5)	0(2.5)	0(60)	7.10

2.3 Results and Discussion

2.3.1 Proximate Analysis

The proximate analysis based on the wet weight of defatted rice bran is shown in Table 2.4. The moisture content of defatted rice bran was similar to the value (11.66%) provided by Riceland Foods, Inc. and reported value (11.19%) by Abdul-Hamid and Luan (2000). The protein content (12.75%) was less than the reported values 17.5 % by Aoe and others (1993) and 14.6% by Abdul-Hamid and Luan (2000), but it was similar to reported value (13.0%) by Sudha and others (2007), which might be due to the difference in protein content of the rice varieties (Oszvald and others 2008). For example, the protein content of *Oryza sativa* ranged from 4.5 to 15.9 %, while it ranged from 10.2 to 15.9% for *Oryza glaberrima* (Juliano and Villarea 1993). Houston (1972) has also reported that rice bran obtained from different rice varieties exhibit different chemical compositions. The ash content of defatted rice bran was 14.80%, which demonstrated that defatted rice bran was a good source of minerals. The defatted rice bran had

higher ash content than that reported by Abdul-Hamid and others (2007) for rice obtained from Oryza sativa L. (4.2 to7.7 %) in Malaysia. Babcock (1987) reported an ash content of rice bran that ranged from 9 to 12%, while Saunders (1990) reported that ash content ranged from 7 to 14% for paddy harvested in the USA. Krishnarao and others (1991) reported that ash in rice husks was related to the variety of rice, climate and geographic location where the paddy was grown. The defatted rice bran contained 30.98% TDF, which was higher than the value 25.5 % reported by Aoe and others (1993) and 27.0% reported by Abdul-Hamid and Luan (2000), but lower than 40.0% reported by Sudha and others (2007). The defatted rice bran sample used in this study showed a slightly higher SDF content compared with a reported SDF value of 2.8 % by Aoe and others (1993), 2.25 % by Abdul-Hamid and Luan (2000) and 4.33 % by Sudha and others (2007). This variation might be due to the different rice milling processing equipment and conditions, for example, a certain processing condition and equipment may remove rice bran from rice with more or less endosperm. Lia and others (2006) reported the higher degree of milling rice had lower hemicelluloses content; therefore, the process could produce rice bran containing a greater percentage of soluble dietary fiber with removed endosperm from brown rice.

Table 2.4 Proximate Analysis of Defatted Rice Bran

Parameters	Percentage (%)
Moisture	11.67±0.25
Fat	2.97±0.13
Protein	12.75±0.52
Ash	14.80±0.66
TDF	30.98±0.22
ISDF	25.53±0.90
SDF	5.45±0.68
Digestible Carbohydrates	26.83±0.94

TDF = Total dietary fiber; ISDF = Insoluble dietary fiber; SDF = Soluble dietary fiber.

2.3.2 Effect of Single Factor Conditions on the Yield of SRBF from Defatted Rice Bran

The yield of SRBF ranged from 5.00 to 5.29 % and was not significantly affected by increasing the temperature from 20 °C to 100 °C (Fig. 2.2a). Polysaccharides extraction was temperature dependent (Cai and others 2007, Wang and others 2007, Sun and others 2010), but Qiu and others (2010) reported that the linear effect of extraction temperature on the yield of pectin from banana peel was insignificant and a number of other factors affected the extraction of soluble dietary fibers.

The yield of SRBF from defatted rice bran was 5.17-5.55% during the different extraction times (Fig. 2.2b), which indicated that both short time and longer extraction times had a similar SRBF yields. This study also showed that a one hour extraction time was sufficient to extract SRBF from defatted rice bran. Therefore, a 1 h extraction time was adopted for determining the optimum extraction conditions of SRBF.

The yield of SRBF increased as the ratio of $Ca(OH)_2$ solution to defatted rice bran increased from 10:1 to 27.5:1 (Fig. 2.2c). The yield of SRBF reached a maximum percentage of 6.36 ± 0.05 when the ratio of $Ca(OH)_2$ solution to defatted rice bran increased to 27.5:1. Both $Ca(OH)_2$ solution to defatted rice bran ratio 27.5: 1 and 30:1 had a similar SRBF yield.

The yield of SRBF increased significantly from 2.49 ± 0.05 % to 6.33 ± 0.04 % when the concentration of $Ca(OH)_2$ solution increased from 0.5 to 2.5% (Fig. 2.2d). In this study, a maximum yield of SRBF was obtained at 2.5% $Ca(OH)_2$ solution concentration. However, another study showed that 2% $Ca(OH)_2$ solution was sufficient to extract SRBF (Aoe and others 1993). The 2%, 2.5% and 3% concentrations of $Ca(OH)_2$ solution were selected for lower, center, and upper levels to optimize the SRBF extraction conditions experiment, respectively.

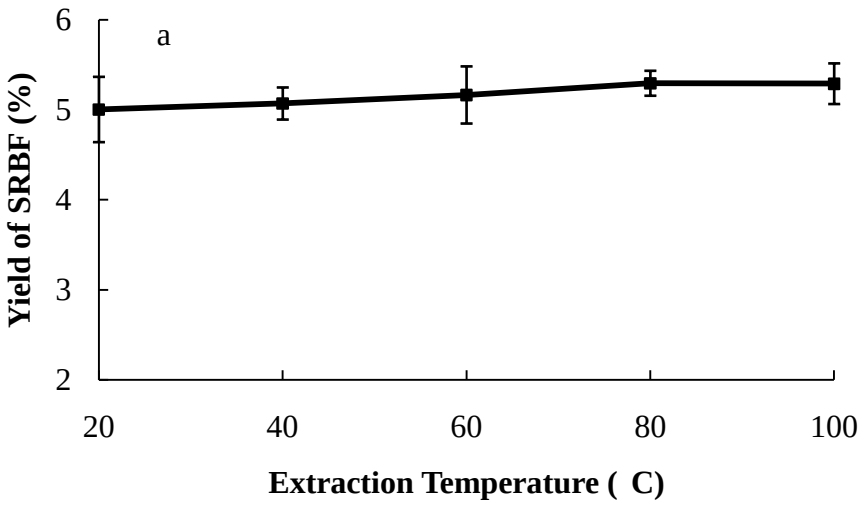

Fig. 2.2a Effect of Extraction Temperature on the Yield of SRBF from Defatted Rice Bran

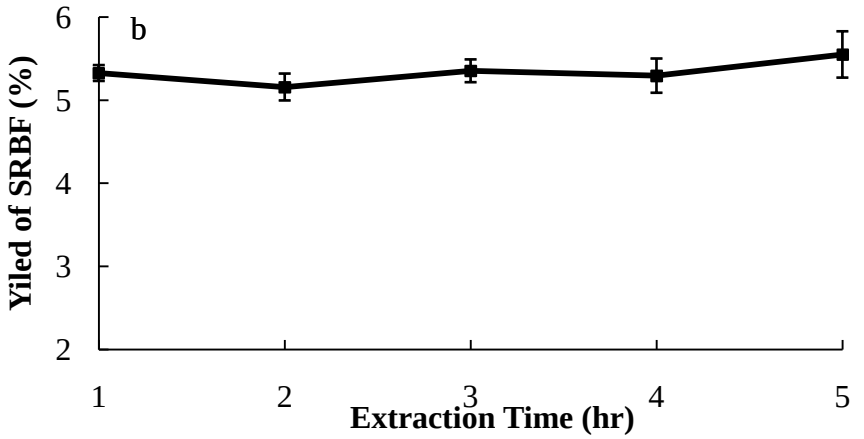

Fig. 2.2b Effect of Extraction Time on the Yield of SRBF from Defatted Rice Bran

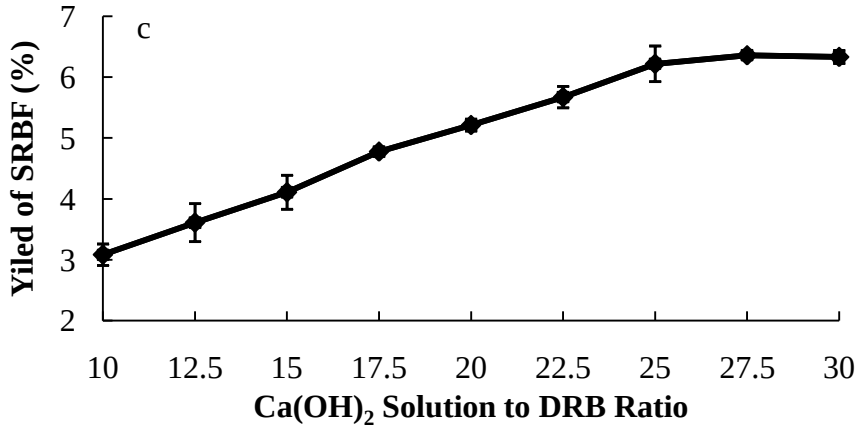

Fig. 2.2c Effect of Ratio of $Ca(OH)_2$ Solution to Defatted Rice Bran on the Yield of SRBF from Defatted Rice Bran

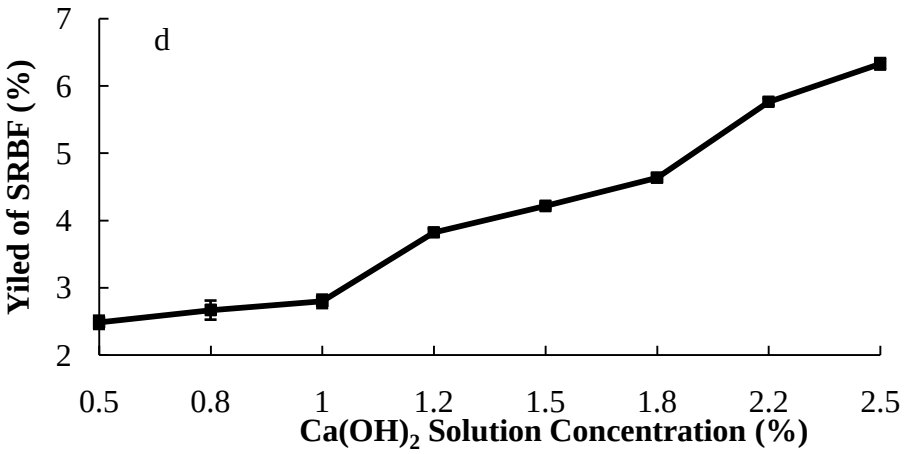

Fig. 2.2d Effect of $Ca(OH)_2$ Solution Concentration on the Yield of SRBF from Defatted Rice Bran

2.3.3 Optimization of Extraction of SRBF from Defatted Rice Bran

The extraction conditions were optimized by a second order polynomial equation using a 15-run BBD with three factors and three levels including three replicates at the center point. The experimental conditions according to the factorial design in coded units along with the uncoded experimental values are shown in Table 2.2. Maximum yield of SRBF (7.9%) was obtained under the extraction conditions (treatment 4) of a ratio of $Ca(OH)_2$ solution to defatted rice bran of 30:1, a concentration of $Ca(OH)_2$ solution of 3%, and an extraction temperature of 60 ° C for 1 hr. By analyzing the experimental data through multiple regressions, a second-order polynomial equation was generated in terms of coded values as following:

$$Y = 7.1 + 0.24x_1 + 0.38x_2 + 0.08x_3 - 0.29x_1x_1 + 0.25x_1x_2 + 0.05x_1x_3 + 0.09x_2x_2 + 0.23x_2x_3 - 0.31x_3x_3$$

The ANOVA of the regression parameters of the predicted response surface quadratic models for yield of SRBF from defatted rice bran is shown in Table 2.5. The results indicated that all linear, quadratic and interaction parameters were highly significant ($p<0.05$).

Table 2.5 Analysis of Variance for the Fitted Quadratic Polynomial Model of Extraction of SRBF

Source	DF	SS	MS	F value	Prob. > F
Model	9	2.85	0.32	16.55	0.0033
Linear	3	1.68	0.56	29.23	0.0013
Quadratic	3	0.68	0.23	11.91	0.0103
Cross Product	3	0.49	0.16	8.49	0.0209
Residual	5	0.10	0.02	—	—
Lack of fit	3	0.10	0.03	99.99	<.0001
Pure Error	2	-2.01E-13	0	—	—
Total	14	2.94	—	—	—

DF= Degree of freedom; SS = Sum of square; MS= Mean Square

The analysis of variance, goodness-of-fit and the adequacy of the models are summarized in Table 2.6 and the model was found to be adequate for prediction with the range of selected experimental variables. The determination coefficient (R^2=0.9675) obtained by ANOVA of the quadratic regression model indicated that only 3.25% of the total variation was not explained by the model. The value of the adjusted determination coefficient (Adjusted R^2=0.9090) indicated a high degree of correlation between the observed and predicted values, while a lower coefficient of variation value (2.02) showed the experimental values were reliable (Liyana-Pathirana and Shahidi 2005).

Table 2.6 Fit Statistics for Response Variable Y (Yield of SRBF)

	Master Model	Predictive Model
Mean	6.83	6.83
R-square	96.75%	96.75%
Adjusted R-square	90.90%	90.90%
Coefficient of Variation	2.02	2.02

The regression coefficient values of the quadratic polynomial equation are listed in Table 2.7. The P-value is used to verify the significance of each coefficient and to describe the degree of interactions strength between the variables (Muralidhar and others 2001). A smaller P-value means the corresponding coefficient is more significant (Muralidhar and others 2001). The concentration of $Ca(OH)_2$ solution was the most significant (P-value = 0.0006) factor affecting

the yield of SRBF and the concentration of $Ca(OH)_2$ solution on the effect of SRBF yield analysis exhibited a largest positive linear regression coefficient (0.38, Table 2.7). This indicated that a greater yield of SRBF could be significantly extracted at 3 % concentration of $Ca(OH)_2$ solution. Park and others (1998) have also reported that the solvent concentration was critical in the extraction of soluble solids from natural products. The extraction temperature alone had no significant effect on yield of SRBF (P-value =0.1574). The interaction coefficients of $x_1 \times x_2$ and $x_2 \times x_3$ were significant, which indicated the interaction of the ratio of $Ca(OH)_2$ solution to defatted rice bran, the concentration of $Ca(OH)_2$ solution, and the interaction between the concentration of $Ca(OH)_2$ solution and extraction temperature affected the yield of SRBF.

The response surface and the contour plots of yield of SRBF from defatted rice bran affected by ratio of $Ca(OH)_2$ solution to defatted rice bran, concentration of $Ca(OH)_2$ solution, and extraction temperature are shown in Fig. 2.3.

Table 2.7 Regression Coefficients of the Predicted Quadratic Polynomial Model

Parameters	Estimate	Standard Error	t ratio	P-value
x_1	0.24	0.049	4.91	0.0044
x_2	0.38	0.049	7.80	0.0006
x_3	0.08	0.049	1.66	0.1574
x_1*x_1	-0.29	0.072	-4.07	0.0097
x_1*x_2	0.25	0.069	3.65	0.0147
x_1*x_3	0.05	0.069	0.76	0.4818
x_2*x_2	0.09	0.072	1.32	0.2439
x_2*x_3	0.23	0.069	3.40	0.0193
x_3*x_3	-0.31	0.072	-4.24	0.0082

x_1: ratio of $Ca(OH)_2$ solution to defatted rice bran; x_2: concentration of $Ca(OH)_2$ solution; x_3: extraction temperature.

The ratio of $Ca(OH)_2$ solution to defatted rice bran demonstrated quadratic effects on the response; therefore, the yield of SRBF increased up to a critical value as the ratio of $Ca(OH)_2$ solution to defatted rice bran increased followed by a decline in SRBF with its further increase.

Meanwhile, the concentration of $Ca(OH)_2$ solution demonstrated a linear effect on the yield of SRBF (Fig. 2.3a and Fig. 2.3b).

The effects of the ratio of $Ca(OH)_2$ solution to defatted rice bran and extraction temperature on yield of SRBF are shown in Fig. 2.3c and Fig. 2.3d. Zhong and Wang (2009) have reported that the response surface and contour plots provide the information on the relationship between responses and experimental levels of each independent variable and the type of interactions between two variables, while the shapes of the contour plots can be used to illustrate whether the interactions between the independent variables are significant. In addition, Muralidhar and others (2001) have reported that a circular contour plot can be used to demonstrate insignificant interactions between the corresponding variables and an elliptical contour plot can be used to demonstrate significant interactions between the corresponding variables. Therefore, Fig. 2.3d illustrates the interactions between the ratio of $Ca(OH)_2$ solution to defatted rice bran and extraction temperature were insignificant, which is also in accordance with the P-value of coefficient value 0.4818 (Table 2.7).

The 3-D response surface plot and the contour plot based on the concentration of $Ca(OH)_2$ solution and the extraction temperature are shown in Fig. 2.3e and Fig. 2.3f. Although, our study showed the linear effect of extraction temperature was not significant on the yield of SRBF, the plots showed that the maximum yield of SRBF was achieved when extraction temperature was at 84 °C (coded value=0.6) and the concentration of $Ca(OH)_2$ solution was 3%. This indicated that there was an interaction between temperature and the concentration of $Ca(OH)_2$ solution.

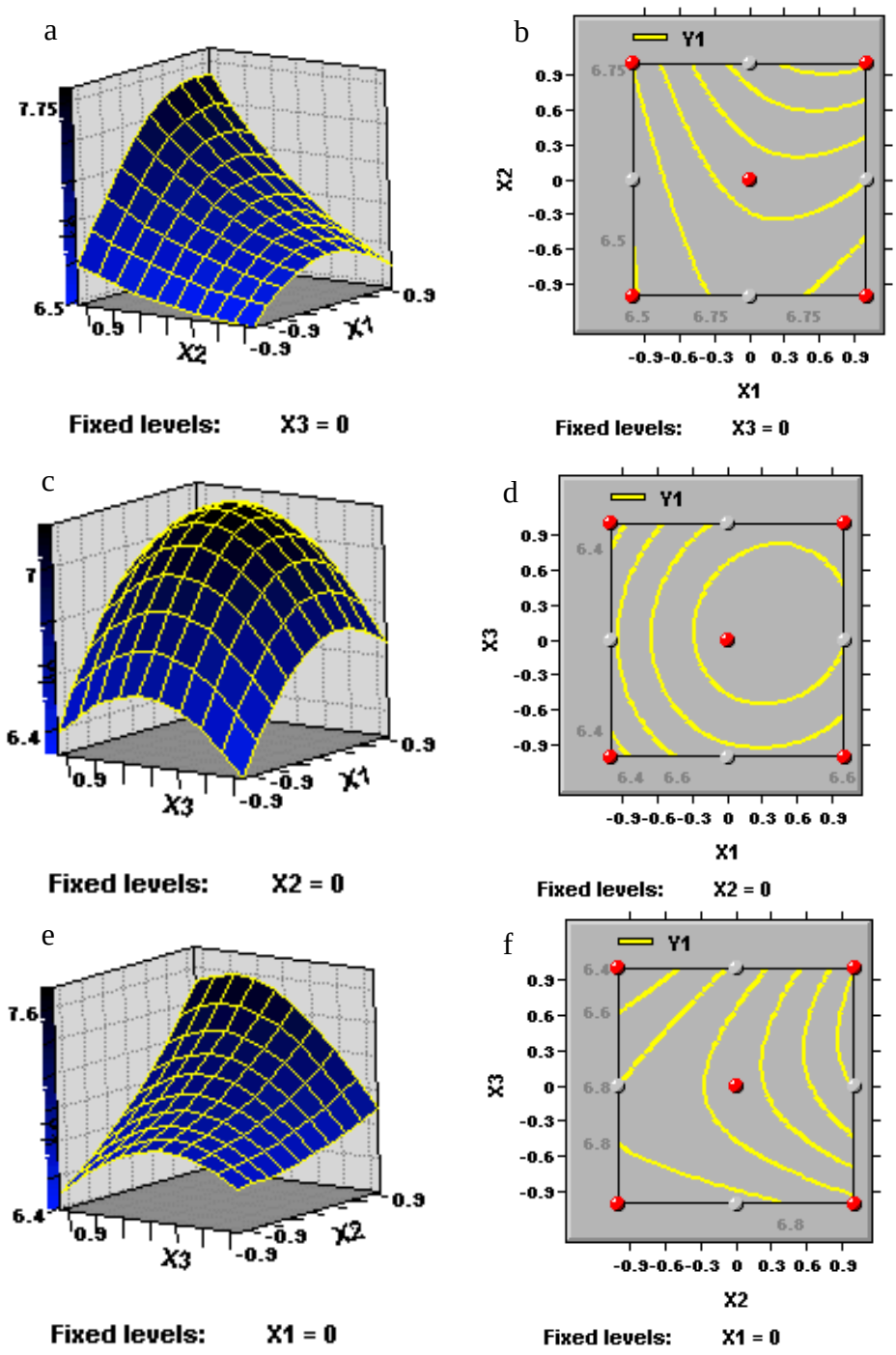

Fig. 2.3 Response Surface Plots (3-D) and Contour Plots (2-D) Showing Effects of Variables on the Response Y (X1, ratio of $Ca(OH)_2$ solution to defatted rice bran; X2, concentration of $Ca(OH)_2$ solution; X3, extraction temperature; Y, yield of SRBF from defatted rice bran).

Based on this study, an estimated optimum yield of SRBF (7.89%) could be obtained at the optimum extraction condition with a 3% $Ca(OH)_2$ solution to defatted rice bran (29.75:1) ratio and stirred for 1 hr at 84^oC.

2.4 Conclusions

Both single factor and optimization studies demonstrated that concentration of $Ca(OH)_2$ solution and ratio of $Ca(OH)_2$ solution to defatted rice bran significantly affected the yield of SRBF from defatted rice bran. This study also indicated the concentration of $Ca(OH)_2$ solution was the most effective factor among the factors including extraction temperature and the ratio of $Ca(OH)_2$ solution to defatted rice bran. The single factor study showed that extraction temperature and extraction time had no significant effect on the yield of SRBF. However, a highly correlated second-order polynomial model used to optimize the extraction conditions showed that interactions between temperature and other independent variables were evidence to extract the SRBF from the defatted rice bran. The study demonstrated that an optimum yield of SRBF (7.89%) could be obtained at the extraction condition with 3% $Ca(OH)_2$ solution to defatted rice bran (29.75:1) ratio and stirred for 1 hr at 84^oC.

CHAPTER 3. PURIFICATION OF SOLUBLE RICE BRAN FIBER USING ULTRAFILTRATION TECHNIQUE

3.1 Introduction

Defatted rice bran (DRB) is a by-product of rice bran oil processing and the former is also a good source of insoluble rice bran fiber (IRBF) and soluble rice bran fiber (SRBF). Both soluble and insoluble dietary fibers showed good physical and bioactive properties. The insoluble dietary fibers are not proper for use as food ingredients because they produce unpleasant mouth feel even if they are finely powdered (Thebaudin and others 1997). In addition, the colors of fiber fortified foods always turn darker, which may not be acceptable to many consumers. SRBF is more applicable than IRBF to use in food products. For these reasons, extraction of water soluble dietary fibers by an alkali treatment has been attempted (Aoe and others 1993). The extraction of SRBF from defatted rice bran using the alkali solution is a complex process and includes five processing steps. The first step is, an enzymatic digestion process, designed to remove starch from the defatted rice bran using amylase and the second step is to solubilize the starch-free defatted rice bran with the alkali solution. The third step is to neutralize the solution containing SBRF with acetic acid and separate the soluble fraction. The separated soluble fraction is considered as unpurified SBRF which contains minerals, monosaccharides, and other small molecules. Removal of impurities and non-fiber components from SBRF is necessary to produce pure SBRF with desirable properties. The conventional SRBF purification is achieved through the fourth and fifth steps, respectively, dialysis and alcohol precipitation. Minerals are removed by dialysis, while SRBF is precipitated from rest of the solution containing monosaccharides and other small molecules by adding 4 volumes of 95% ethanol solution to one volume of the soluble fraction. The separated SRBF precipitate is dried and produces SRBF powder. The main disadvantages of conventional purification methods

(dialysis and alcohol precipitation) are time consuming, requiring a large volume of alcohol, and high processing costs. In addition, recovery of large volumes of alcohol from the byproduct solution is a difficult task. Distillation is a good solution to recover the alcohol, but the process will add additional cost to production (Lo and others 1996). Ultrafiltration technology can potentially simplify the current purifying process of the soluble fraction containing SRBF. Studies have shown that an ultrafiltration process used for soluble dietary fiber purification not only removes minerals and monosaccharides, but is also a cost effective process (Gabrielii and others 2000, Nabarlatz and others 2007, Sheng and others 2007, Buranov and Mazza 2010). Ultrafiltration technology is gaining popularity as a non-thermal membrane separation process in many areas of liquid food processing.

The membrane separation requires a hydrostatic pressure (driving force) to filter a solution through the membrane and to separate solutes from a solution based on the molecular weight cut off of the semipermeable membrane. No phase or state change occurs during the ultrafiltration process and all the solutes separate at room temperature, therefore, requiring less energy for the process (Cheryan 1998). The separation of SRBF from soluble fraction containing small molecular weight sugars and minerals has not yet been investigated using an ultrafiltration process. The objectives of this study were to: (1) evaluate the effects of molecular weight cut off and transmembrane pressure of ultrafiltration membranes on the permeability of the solution containing SRBF and (2) determine the performance of ultafiltration for separating the small molecular weight sugars and minerals from solution containing SRBF.

3.2 Materials and Methods

3.2.1 Extraction of SRBF from Defatted Rice Bran

Soluble fraction containing SRBF from defatted rice bran was produced (Fig. 3.1) by a modified procedure of Aoe and others (1993). The starch in the defatted rice bran (50 g) was digested with 0.5 mL amylase solution (13083 units/ mL, Sigma-Al, St Louis, MO) at pH 6.9 for 15 min at 90 °C, and the starch-free defatted rice bran was separated with a Buchner funnel with the aid of filter paper. The separated starch-free defatted rice bran was washed with deionized water four times and mixed with $Ca(OH)_2$ solution in a 2 L beaker using Talboys laboratory 134-1 stirrer (Troemner LLC, Thorofare, NJ). The extracted soluble fraction containing SRBF was neutralized with concentrated acetic acid and centrifuged at 4 °C and 15,300 x *g* for 30 min to separate soluble fractions.

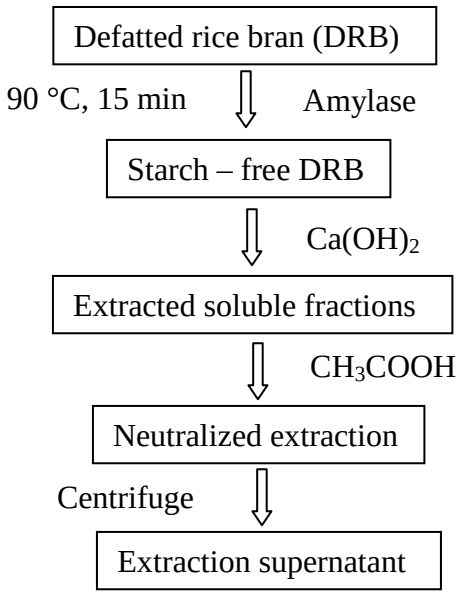

Fig. 3.1 Soluble Fraction Containing SRBF Extraction Flow Diagram

3.2.2 Permeate Flux Model for Purifying SRBF

The extracted soluble fraction containing SRBF was purified using a Millipore stirred ultrafiltration cell unit (Model 8400, Millipore Co., Bedford, MA) system. The unpurified SRBF

was separately filtered through the ultrafiltration using 1, 5, and 10 kDa molecular weight cut-off (MWCO) membranes. The 1 kDa MWCO membrane (Millipore Corp, Bedford, USA) was made of regenerated cellulose, while the 5 kDa and 10 kDa MWCO membranes (Millipore Corp, Bedford, USA) were made of polyethersulfone. A new membrane was used for each set of experiment. The unpurified SRBF extract was transferred to the unit. The ultrafiltration unit was placed on a S46725 Barnstead/ Thermolyne Cimarec 2 magnetic stirring table (Barnstead/ Thermolyne, Dueuque, IA) and was connected to a nitrogen pressurization system as shown in Fig. 3.2. The speed of stir was adjusted to create the vortex at approximately one-third the depth of the liquid volume according to the Millipore operating instructions.

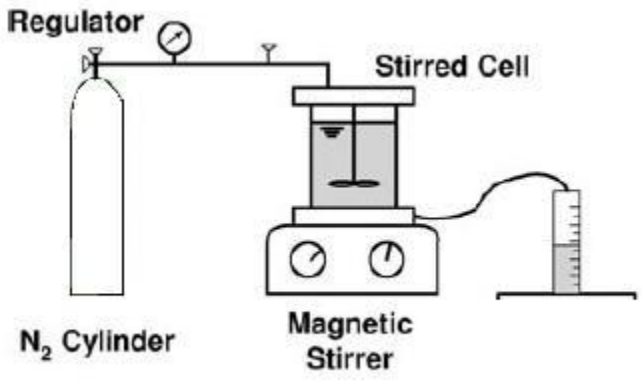

Fig. 3.2 Ultrafiltration System

Ultrafiltration was performed for permeability of soluble fraction containing SRBF at room temperature with pressures (ΔP) of 1×10^5, 2×10^5 and 3×10^5 Pa according to Gökmen and Çetinkaya (2007) with slight modification. New membranes were evaluated by measuring the deionized water flux before purifying the SRBF. The filtration unit was filled with 200 mL of distilled water or SRBF and time for collecting 10 mL of permeate at 1×10^5, 2×10^5 and/or 3×10^5 Pa was recorded for 1, 5, and/or 10 kDa MWCO membranes until the total volume of SRBF in

the membrane unit reached 70 mL. The total filtration resistance for filtering distilled water and purifying SRBF was described by Equation 3.1 (De La Garza and Boulton1984):

$$R_{tot} = R_m \exp(kV/A) \quad \text{(Eq.3.1)}$$

where R_{tot} is the total resistance (m^{-1}), R_m is the membrane resistance (m^{-1}), k is exponential fouling coefficient (m^{-1}), V is the filtrate volume (m^3) and A is the filtration area (m^2).

The general filtration equation (Gökmen and Çetinkaya 2007) is:

$$J = \frac{1}{A}\frac{dV}{dt} = \frac{\Delta P}{\mu \cdot R_{tot}} \quad \text{(Eq.3.2)}$$

where, J is flux (m^3/m^2•s), ΔP is the pressure (Pa) and μ is the viscosity (Pa•s).

Equation 3.1 was substituted with Equation. 3.2 and Equation 3.3 was obtained as:

$$\frac{1}{A}\frac{dV}{dt} = \frac{\Delta P}{\mu R_m \exp(kV/A)} \quad \text{(Eq.3.3)}$$

Equation 3.3 was rearranged as Equation 3.4, and integrated to obtain Equation 3.5 when $V = 0$ at $t = 0$ and $V = V$ at $t = t$.

$$\int_0^t dt = \frac{\mu R_m}{\Delta P A}\int_0^V \exp(kV/A)dV \quad \text{(Eq.3.4)}$$

$$t = \frac{\mu R_m}{\Delta P k}[\exp(kV/A) - 1] \quad \text{(Eq.3.5)}$$

where, R_m is the membrane resistance (m^{-1}), k is the exponential fouling coefficient (m^{-1}), V is the filtrate volume (m^3), A is the filtration area (m^2), ΔP is the pressure (Pa) and μ is the viscosity (Pa•s).

The experiment data for purifying SRBF were fitted with Equation 3.5 and a (s) and b (m^{-3}) were obtained using Curve Expert version 1.3 software.

$$a = \frac{\mu R_m}{\Delta P k} \quad \text{(Eq.3.6)}$$

$$b = \frac{k}{A} \quad \text{(Eq.3.7)}$$

The filtration parameters k and R_m were estimated using Equation 3.7 and 3.6, respectively.

3.2.3 Rheological Properties, °Brix, Mineral Content and Monosaccharides Composition of Unpurified and Purified SRBF

The retentates collected in the utrafiltration unit with the 1, 5, and 10 kDa MWCO membrane at 100 kPa were analyzed for rheological properties, °brix, mineral, and monosaccharides. Purified SRBF solution refers to retentates obtained by ultrafiltration and unpurified SRBF means soluble fraction containing SRBF before ultrafiltration. A pressure of 100 kPa was selected for driving force to filter the SRBF soluble fraction based on the permeability study as mentioned above.

Rheological properties of unpurified and purified SRBF solutions were measured using an AR 2000 ex Rheometer (TA Instruments, New Castle, DE) fitted with a plate geometry (steel plates with a 40-mm diameter and a gap of 100 μm). The sample was placed in the temperature-controlled parallel plate and allowed to equilibrate to 20 °C. The shear stress was measured at shear rates from 0 to 100 s^{-1}. The power law (Eq. 3.8) was used to analyze the flow properties of the samples.

$$\sigma = K\gamma^n \quad \text{(Eq.3.8)}$$

where σ = shear stress (Pa), γ = shear rate (s^{-1}), K = consistency index (Pa.s^n), and n = flow behavior index. Logarithms were taken on both sides of Equation 3.8, and a plot of log σ versus log γ was constructed, and the magnitude of K and n were determined from the resulting straight

line intercepts for log *K* and the slope for *n* values. The mean values of *n*, *K*, and apparent viscosity (at 100 s^{-1}) of triplicate samples were reported.

The °Brix of unpurified and purified SRBF solutions was measured using an AR200 Digital Hand-Held Refractometer (Reicher, Depew, NY). The mean values of triplicate samples were reported.

Unpurified and purified SRBF solutions were filtered through a 0.2 μm syringe filter and analyzed in Soil Testing and Plant Analysis Lab, School of Plant, Environmental, and Soil Sciences, LSU for calcium, magnesium, potassium, and sodium contents by ARCOS EOP-ICP (Spectro, Germany). Nitric Acid (2%, CSP Science, USA) was used to make up standards to quantify the minerals.

Unpurified and purified SRBF solutions were analyzed for monosaccharides as described below. Unpurified and purified SRBF solutions were hydrolyzed by 1.0 M H_2SO_4 in a SM-22/32/52 autoclave (Yamato Seientific America Inc, South San Francisco, CA) at 120 ºC for 1hr to convert polysaccharides into their constitutive monomers (Roos and others 2009). The hydrolyzed solutions were filtered through a 0.2 μm syringe filter and monosaccharides were quantified by high performance liquid chromatography (HPLC) (Waters high performance liquid chromatography, Milford, MA) at 30^{o}C with a 4 ×250 mm CarboPac PA 10 Analytical column (Dionex Corp., Sunnyvale, CA) according to a slightly modified method of Swallow and Low (1990). A 5.0 μL monosaccharides sample was injected and solvent (18 mmol/L NaOH) was supplied at a flow rate of 0.8 mL/min. A Waters 2465 electrochemical detector (Milford, MA) was connected in series. Conditioning for the column was performed every three sample injections with 200 mmol/L NaOH for 20 min at a flow rate of 1.0 mL/min to minimize the column contamination. Monosaccharides standard solutions including arabinose, galactose,

glucose, and xylose were purchased from Sigma-Aldrich (St Louis, MO). Standard curves were plotted between areas and the standard solution concentrations from 0.25 to 1 mg/ml to quantify monosaccharides composition.

3.2.4 Statistics Analysis

Mean values from the three separate experiments or replicate analyses are reported with standard deviation. The statistical significance of observed differences among treatment means was evaluated by analysis of variance (ANOVA) (SAS Version 9.2, SAS Institute Inc., Cary, NC, U.S.A), followed by post hoc Tukey's studentized range test.

3.3 Results and Discussion

3.3.1 Water Flux

The fluxes of water as a function of pressure for membranes with different MWCO are shown in Fig. 3.3. Water flux was linear with the pressure for all membranes, which was similar to what Nabarlatz and others (2007) found in their study for membranes with MWCO ranging from 1 kDa to 8 kDa at pressures of 2.6 to 9 bar. An idealized water flux versus pressure curve exhibited linear change for a membrane with a uniform pore size (Mulder 1996). Water fluxes for all three MWCO of the utrafiltration increased with increased pressure (Fig. 3.3). The regenerated cellulose membrane (MWCO 1 kDa) was less affected by transmembrane pressure than the polyethersulfone membranes (MWCO 5 kDa and 10 kDa), which might be due to narrower pore size distributions and higher rejection (Kim and others 1994). Water flux can also represent cleaning efficiency (Gökmen and Çetinkaya 2007). A higher water flux generally can be obtained for membrane filtration and the water flux can also be used to evaluate the degree of fouling (Cheryan 1998). It is important to evaluate the cleaned membrane performance before it

will be used for filtration. The performance of a cleaned membrane could be evaluated by determining the flux of water (Cheryan 1998).

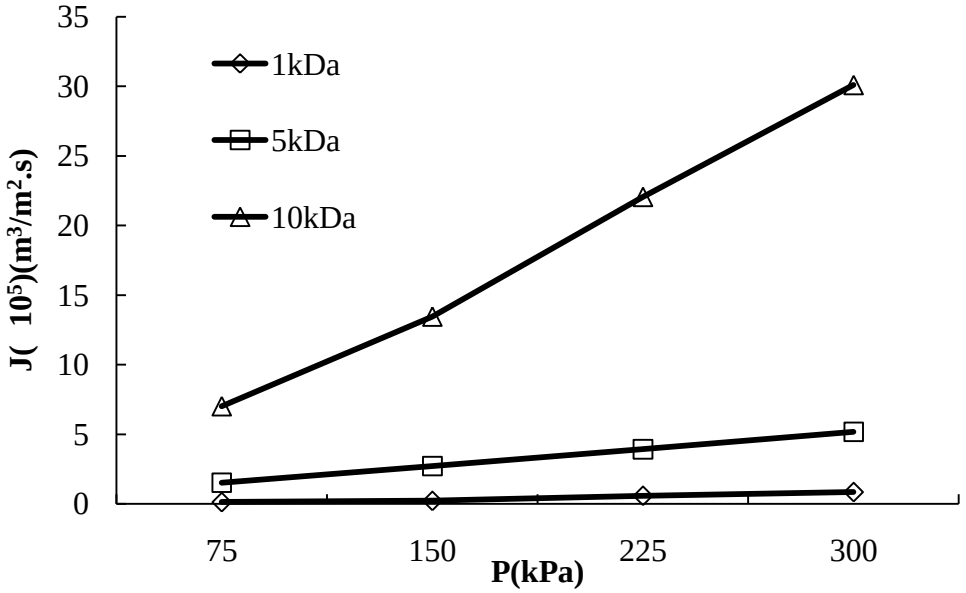

Fig. 3.3 Influence of Pressure on the Fluxes of Water for the Ultrafiltration Membrane

3.3.2 Effect of ΔP on Ultrafiltration Performance

A transmembrane pressure is needed in an ultrafiltration system to overcome the membrane resistance and alleviate the pressure drop of the fluid on the membrane surface (Hu and others 2004). Fig. 3.4 shows the plot of permeate volume versus time at different ΔP for SRBF solution ultrafiltration using membranes of 1 kDa MWCO, 5 kDa MWCO and 10 kDa MWCO. The increase of flux for purifying the SRBF was lower at 200 kPa to 300 kPa than that of 100 kPa. Hu and others (2004) reported that the permeate flux proportionally increased at a low transmembrane pressure condition when operating pressure for the filtration reached a critical value, the flux was decreased (Rao and others 1987). Lo and others (1996) reported the membrane permeation rate was reduced by the formation of a polarization layer on the membrane during the membrane filtration.

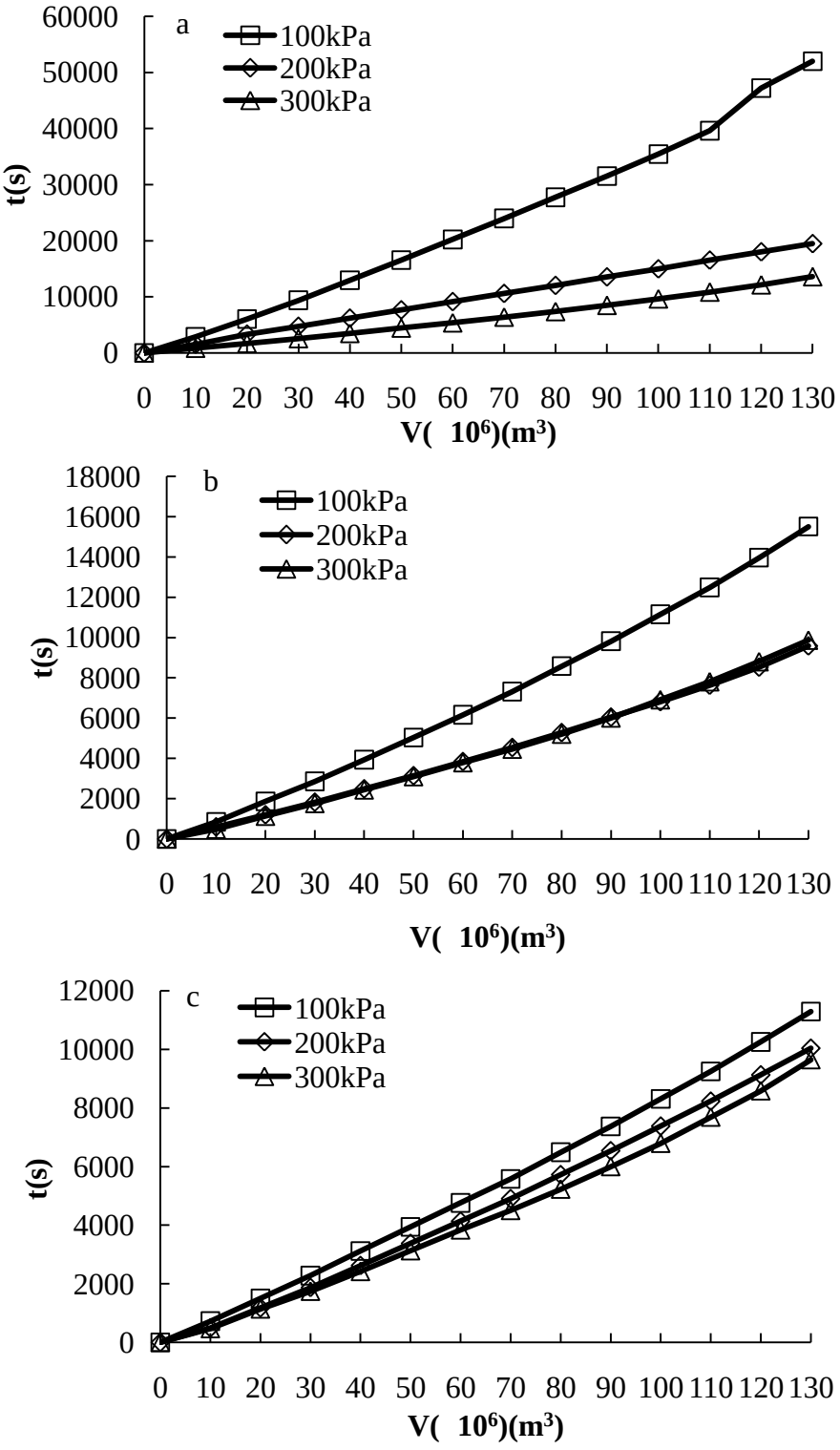

Fig. 3.4 Permeate Flux Volume versus Ultrafiltration Time Plots for Ultrafiltration of SRBF at Different ΔP. a) Membrane 1 kDa MWCO; b) Membrane 5 kDa MWCO; c) Membrane 10 kDa MWCO.

Membranes MWCO 1, 5, and 10 kDa had affected the flux for purifying SRBF (Fig. 3.4). Membrane materials, pore size distributions, and the chemical natures pore size can affect the solute-membrane interaction and affect the flux (Cheryan, 1998).

The R_m and k increased for 1 kDa , 5 kDa and 10 kDa MWCO membrane filtration as the applied pressure increased (Table 3.1). Gökmen and Çetinkaya (2007) reported that both R_m and k values increased with the increase in ΔP. The same researchers also reported that the effect of ΔP on the flux was greater than that of total resistance; therefore the flux also increased with increased pressure. In this study, the flux was higher at 100 kPa than those at 200 kPa and 300 kPa regardless of the MWCO, which might be due to more deposition and accumulation of SRBF residuals on the membrane at 200 and 300 kPa filtration conditions. The fouling layer on the membrane that resulted from deposition and accumulation of feed components became compacted at a higher pressure condition than at a lower pressure condition (Cheryan 1998, Jönsson 1993). The fouling layer formed at the higher pressure condition could reduce the SRBF permeability and increased fouling resistance (De Bruijn and others 2002, Gökmen and Çetinkaya 2007, Jönsson 1993).

3.3.3 Rheological Properties, °Brix, Mineral Content and Monosaccharides Composition of Unpurified and Purified SRBF

Based on the mechanism of ultrafiltration, as expected SRBF with molecular weights greater than membrane MWCO remained on the membrance as retentate, while minerals, monosaccharides, and other small molecules passed through the membrane.

The rheological properties and °Brix of unpurified and purified SRBF solutions are listed in Table 3.2. The apparent viscosity and °Brix of SRBF solution increased after ultrafiltration, which demonstrated that the SRBF solution was concentrated during the ultrafiltration process. The apparent viscosity of the retentate collected in utrafltration unit with the 1 kDa MWCO

membrane had a higher (P<0.05) viscosity than those collected in the 5 and 10 kDa MWCO membrane unit, which indicated that retentate obtained with the membrane with a MWCO of 1 kDa contained more poly molecules than the other two MWCO membranes. However, the soluble solids (°Brix) in purified SRBF solutions were the same (P< 0.05), which indicated that the majority of SRBF molecules were greater than 10 kDa.

Table 3.1 Model Parameters and R_m and k Values for Different ΔP

Membrane	ΔP (kPa)	*a* (s)	*b* (m^{-3})	*Rm* ($\times 10^{13} m^{-1}$)	*k* (m^{-1})
1kDa MWCO	100	58874.77 ± 1626.84^{aA}	4818.28 ± 108.57^{abA}	8.40 ± 0.04^{cA}	21.85 ± 0.49^{abA}
	200	33951.02 ± 416.91^{bA}	4830.26 ± 49.53^{bA}	9.72 ± 0.02^{bA}	21.90 ± 0.22^{bA}
	300	22436.42 ± 273.79^{cA}	4961.38 ± 42.31^{aA}	9.90 ± 0.04^{aA}	22.50 ± 0.19^{aA}
5kDa MWCO	100	21362.21 ± 723.94^{aC}	4195.77 ± 112.05^{bB}	3.38 ± 0.02^{cB}	19.02 ± 0.51^{bB}
	200	16014.16 ± 648.55^{bC}	4576.80 ± 114.71^{aB}	5.53 ± 0.09^{bB}	20.75 ± 0.52^{aB}
	300	11030.99 ± 763.49^{cC}	4897.34 ± 255.41^{aA}	6.10 ± 0.09^{aB}	22.21 ± 1.16^{aA}
10kDa MWCO	100	28513.42 ± 1127.83^{aB}	2561.95 ± 90.25^{cC}	2.47 ± 0.01^{cC}	11.62 ± 0.41^{cC}
	200	18017.63 ± 296.80^{bB}	3421.03 ± 48.66^{bC}	4.17 ± 0.01^{bC}	15.51 ± 0.22^{bC}
	300	12821.46 ± 19.51^{cB}	4282.24 ± 0.68^{aB}	5.57 ± 0.01^{aC}	19.42 ± 0.00^{aB}

Values are means and SD of triplicate determinations. ^{abc}means with different letters the parameters using same MWCO membrane with different transmembrane pressure are significantly different (P< 0.05). ^{ABC}means with different letters the parameters using different MWCO membrane with same transmembrane pressure are significantly different (P < 0.05). *a* and *b* are the model parameters determined by Curve Expert software. R_m is the membrane resistance (m^{-1}), *k* is exponential fouling coefficient (m^{-1}).

Table 3.2 Rheology Properties and °Brix of Unpurified and Purified SRBF

	n	*K*($\times 10^{-3}$ Pa.s^n)	Apparent Viscosity ($\times 10^{-3}$ Pa s)	°Brix
Unpurified SRBF	0.76 ± 0.02^{c}	4.54 ± 0.14^{c}	1.52 ± 0.13^{d}	6.13 ± 0.12^{b}
Purified SRBF (MWCO 1kDa)	0.82 ± 0.01^{b}	9.08 ± 0.56^{a}	3.93 ± 0.13^{a}	7.60 ± 0.20^{a}
Purified SRBF (MWCO 5kDa)	0.88 ± 0.01^{a}	5.42 ± 0.27^{b}	3.17 ± 0.00^{b}	7.87 ± 0.64^{a}
Purified SRBF (MWCO 10kDa)	0.90 ± 0.02^{a}	5.19 ± 0.86^{bc}	3.08 ± 0.07^{c}	7.30 ± 0.50^{a}

^{abcd}means with different letters in each column are significantly different (P< 0.05).
n = flow behavior index; *K* = consistency index (Pa.s^n).

The mineral contents of unpurified and purified SRBF solutions are listed in Table 3.3. The minerals in SRBF solution were mainly calcium, magnesium, potassium and sodium. The unpurified SRBF solution had higher amounts of calcium, magnesium, potassium and sodium than that of purified SRBF solutions. Calcium content was highest among minerals found in the unpurified solution, which might be due to Ca^{2+} that remained in SRBF solution from the extraction process. All mineral contents in the purified SRBF solution significantly ($P<0.05$) decreased after ultrafiltration regardless of MWCO of membrane. The calcium content and potassium content of purified SRBF using membranes of 5 kDa and 10 kDa MWCO were lower than those in the retentate from the 1 kDa MWCO membrane, while the retentates obtained from all three MWCO membranes had similar magnesium and sodium contents.

The HPLC analysis results of hydrolyzed SRBF monosaccharides composition are shown in Fig. 3.5. Glucose, arabinose, xylose and galactose were found to be the main monomers in rice hemicelluloses (Bevenue and Williams 1956, Gremli and Juliano 1970, Cartaño and Juliano 1970). Rice bran hemicelluloses are reported to consist of xylan as a backbone and other sugars as side chains (Harada and others 2005). The retained solutions of SRBF were concentrated hemicelluloses by the ultrafiltration process; therefore, the purified SRBF solution had greater levels of arabinose and xylose, but not galactose and glucose, than unpurified SRBF when they were hydrolyzed with sulfuric acid (Table 3.4). However, purified SRBF solution obtained from 1 kDa MWCO had similar galactose content as unpurified SRBF solution, but higher glucose content than unpurified SRBF solution; purified SRBF solution obtained from 5 kDa and 10 kDa MWCO had similar glucose content as unpurified SRBF solution. Glucose was the main monomer among the monosaccharides found in the SRBF solutions. All three purified SRBF solutions had similar concentrations of arabinose, galactose, and glucose, while the retentate of

SRBF obtained from 10 kDa MWCO membrane had slightly lower xylose concentration than that resulting from 1 kDa MWCO.

Table 3.3 Mineral Content of Unpurified and Purified SRBF Solution

Mineral content (ppm)	Ca	Mg	K	Na
Unpurified SRBF	1145.25±27.78[a]	13.56±2.86[a]	149.45±21.45[a]	6.13±1.82[a]
Purified SRBF (MWCO 1kDa)	344.27±11.82[b]	3.32±1.11[b]	31.07±6.94[b]	1.74±0.53[b]
Purified SRBF (MWCO 5kDa)	256.14±2.50[c]	2.55±0.65[b]	21.31±2.68[c]	1.07±0.44[b]
Purified SRBF (MWCO 10kDa)	249.33±5.72[c]	2.63±0.35[b]	21.50±2.32[c]	1.08±0.30[b]

[abc]means with different letters in each column are significantly different (P< 0.05).

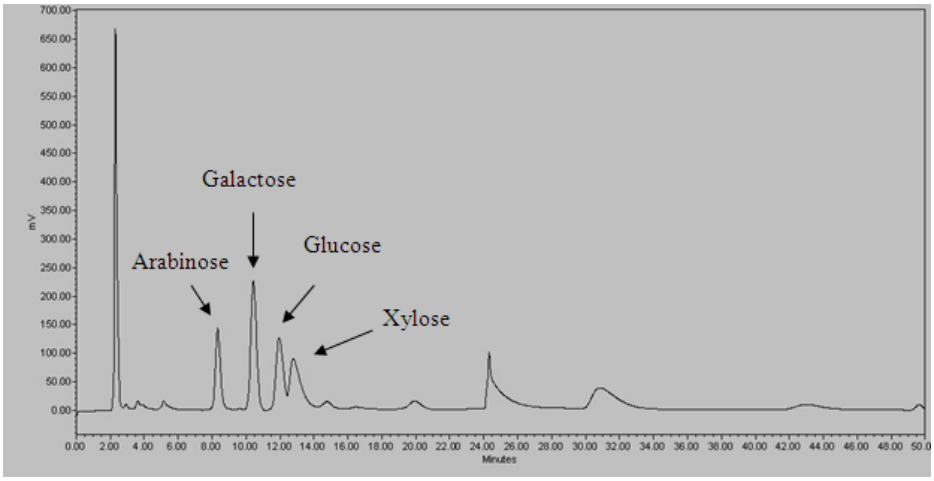

Fig. 3.5 HPLC Analysis of Hydrolyzed SRBF Monosaccharides Composition

Table 3.4 Monosaccharides Concentration of Unpurified and Purified SRBF

Monosaccharide composition (mg/g)	Arabinose	Galactose	Glucose	Xylose
Unpurified SRBF	1.48±0.02[b]	19.33±0.88[b]	39.97±2.08[b]	11.42±0.44[c]
Purified SRBF (MWCO 1kDa)	6.02±0.56[a]	27.89±6.56[ab]	47.26±2.99[a]	19.29±0.18[a]
Purified SRBF (MWCO 5kDa)	4.60±0.93[a]	29.32±3.83[a]	45.23±2.80[ab]	17.99±4.06[ab]
Purified SRBF (MWCO 10kDa)	6.33±2.33[a]	32.15±2.51[a]	43.98±3.58[ab]	17.81±0.55[b]

[ab]means with different letters in each column are significantly different (P< 0.05).

3.4 Conclusions

The permeate flux for the SRBF showed higher at 100 kPa than at 200 and 300 kPa conditions. The membrane resistance and fouling coefficient increased with an increased pressure, which resulted in a decline in permeate flux. The purified SRBF solution had higher viscosity and soluble solids content than unpurified SRBF soluble fraction, which resulted in an

increase in concentration soluble fraction of SRBF during the ultrafiltration. The monosaccharide concentrations in the hydrolyzed purified SRBF solution were increased. The mineral contents of SRBF solution were significantly decreased by ultrafiltration. The membranes with different MWCO exhibit similar purification efficiency when comparing viscosity, soluble solids, mineral content and monosaccharide composition. The 10 kDa MWCO membrane could be employed in the ultrafiltration process at a pressure of 100 kPa, which provided effective purification with an acceptable working time. The study demonstration showed that utrafiltration could be used to purify the SRBF without adopting the dialysis and alcohol precipitation process steps.

CHAPTER 4. MICROENCAPSULATION OF MENHADEN FISH OIL WITH SOLUBLE RICE BRAN FIBER USING SPRAY DRYING TECHNOLOGY

4.1 Introduction

Spray drying is one of the most popular microencapsulation techniques used to produce microencapsulated food ingredients. Spray drying is carried from aqueous feed formulations and the solubility of the feed material limits the utilization of spray drying technology (Gouin 2004). The microencapsulation process makes it possible to transform a core ingredient into a powder, where the small droplets of core ingredient are surrounded by a shell coating of proteins and/or carbohydrate resulting in small dry granules that have powder like flow characteristics.

A water soluble shell material is required for drying encapsulated core ingredient by spray dryer. The core material, shell material and water are initially homogenized well to form a liquid feed, such as emulsion and suspension. And the liquid feed is atomized into small droplets by pressure or centrifugal energy using pressure nozzle, spinning disk configurations, pneumatic atomizer and other nozzles (Masters 1968). Then the atomized droplets are dried in the hot air to obtain the powder encapsulating core material with shell material (Gharsallaoui and others 2007).

Soluble dietary fibers are widely used in developing microcapsules because they are water soluble and also have beneficial functional properties (Doleyres and others 2002, 2004, Lee and others 2004, Saénz and others 2009, Choi and others 2010). Soluble rice bran fiber is highly soluble in water and is reported to have beneficial functional properties including hypocholestrolemic, antioxidant, immune-modulation and anti-tumor activities (Aoe and others 1989, Saunders 1990, Cummings and others 1992, Wang and others 2008, Zha and others 2009). The main component of soluble rice bran is hemicelluloses, which exhibit low oxygen permeability (Grőndahl and others 2004, Hartman and others 2006). Therefore, a less oxygen

transport coating material may produce from soluble rice bran fiber (Krawczyk and others 2008), which may reduce lipid oxidation of the core ingredient during spray drying and storage. Menhaden oil is a good source of eicosapentaenoic acid (12.8-15.4%) and docosahexaenoic acid (6.9-9.1%) (Yin and Sathivel 2010), while soluble rice bran fiber (SRBF) is known for its anti oxidation activity and hypocholesterolemic effects in human. The microencapsulated menhaden oil with soluble rice bran fiber can provide potential health benefits to consumers. Menhaden (*Brevoortia tyrannus*) is an abundant marine species in the United States but is seldom consumed as a food. In 2008, more than 608.45 million kilograms of menhaden was harvested in the US, which was 16% of the total commercial fisheries (NMFS 2009). Menhaden is mainly considered as reduction fishery; thus, most of the menhaden are used for producing fish oil, fish meal and fish soluble. Purified menhaden oil was approved for use in human foods (FDA 2004). Attempts to incorporate fish oil into food formulations has had limited success because of 'fishy' flavors and lipid oxidation in the finished products (Kolanowski and others 1999). Microencapsulation of fish oil can reduce fishy smell and improve the oxidation stability of the oil thus making it suitable for addition to food.

The objectives of the study were: 1) to prepare and characterize the rheological properties of the emulsion containing menhaden fish oil with soluble rice bran fiber (SRBF); 2) to evaluate the antioxidant properties of SRBF in the emulsion containing menhaden fish oil; and 3) develop and characterize the microencapsulated menhaden fish oil with SRBF using a spray drying process, and estimate the production, evaporation rate, and energy required to spray dry the emulsion for producing microencapsulated menhaden fish oil with SRBF powder.

4.2 Materials and Methods

4.2.1 Extraction of Soluble Rice Bran Fiber

SRBF was extracted from defatted rice bran and purified using selected conditions in previous chapters. The starch-free defatted rice bran (50 g) was prepared as described in section 2.2.2 and mixed with 3% $Ca(OH)_2$ solution at ratio 29.75:1 (v/w) by Talboys laboratory 134-1 stirrer (Troemner LLC, Thorofare, NJ) in a 2 L beaker seated in 84 °C water bath for 1hr for extraction. The extracted soluble fraction was ultrafiltrated using the 10 kDa MWCO at 100 kPa pressure and freeze-dried.

4.2.2 Emulsion Preparation

The components used to prepare a stable emulsion were menhaden oil (6.7%), SRBF (1.1%), Na-caseinate (10.0%) and water (82.2%). Na-caseinate (6% moisture, 85.5% protein, 3.0% fat, 4.0% ash and 1.0% carbohydrate) and SRBF (7.1% moisture, 2.4% protein, 0.6% fat, 0.4% ash and 89.5% carbohydrate) were dissolved into water and stirred using RCT B S1 magnetic stir (IKA Labrotechnik, Janke & Kunkel GmbH & Co., Staufen, Germany) over night at room temperature separately. Purified menhaden oil (PMO) (Mid-Atlantic Bait, Inc., Lottsburg, VA) and the SRBF solution were gradually added into Na-caseinate solution and homogenized well using a hand blender (Cuisinart Inc., East Windsor, NJ, USA) for 2 min. An ultrasonic processor (500 Watt Model, Cole-Parmer Instrument Co. Vernon Hill, IL) was employed for 10 min to break down the oil droplets size in emulsion containing PMO and SRBF (EFMO) into micro scale.

4.2.3 Physical Properties of EFMO

Emulsifying stability was evaluated according to the method of Min and others (2003) with minor modifications. The emulsion sample (5 g) was placed into a 10 mL centrifugal tube

and stored at -20 ^{o}C for 2 days and then allowed to thaw at room temperature for 4 hr. The thawed sample was centrifuged at 15,000 *g* for 40 min at -2 ^{o}C and the amount of oil separated was measured. Oil recovery % was calculated as [weight of oil recovered/ 5 g of emulsion sample] x 100.

Flow properties and viscoelastic properties of EFMO were measured using an AR 2000 ex Rheometer (TA Instruments, New Castle, DE) fitted with plate geometry (steel plates with a 40-mm diameter and a gap of 400 μm). The sample was placed in the temperature-controlled parallel plate and allowed to equilibrate to 5, 15, and/or 25 ^{o}C. The shear stress was measured at shear rates from 0 to 100 s^{-1}.

The power law (Eq. 4.1) was used to analyze the flow properties of the emulsion samples.

$$\sigma = K\gamma^{n} \qquad \text{(Eq.4.1)}$$

where σ = shear stress (Pa), γ = shear rate (s^{-1}), *K* = consistency index (Pa.s^{n}), and *n* = flow behavior index. Logarithms were taken on both sides of Equation 4.1, and a plot of log σ versus log γ was constructed, and the magnitude of *K* and *n* were determined from the resulting straight line intercepts for log *K* and the slope for *n* values. The mean values of *n*, *K*, and apparent viscosity (at 100 s^{-1}) of triplicate samples were reported.

The frequency sweep (0.1 to10 Hz) test was conducted at a constant temperature of 25^{o}C to determine the viscoelastic properties of EFMO. The storage modulus and loss modulus of the emulsions were obtained using Universal Analysis (TA instrument) software and were calculated using Equation 4.2 and Equation 4.3:

$$G' = \left(\frac{\sigma_0}{\gamma_0}\right)\cos\delta \qquad \text{(Eq.4.2)}$$

$$G'' = \left(\frac{\sigma_0}{\gamma_0}\right)\sin\delta \qquad \text{(Eq.4.3)}$$

$$\tan\delta = \frac{G''}{G'} \qquad \text{(Eq.4.4)}$$

where *G'* (Pa) is the storage modulus, *G''* (Pa) is the loss modulus, tan δ is the loss tangent, σ is generated stress, and γ is oscillating strain.

The microstructure of the emulsion was determined using a light microscope (Nikon Microphot-FXA, Nikon Instrument Inc., Japan). A drop of the emulsion was observed at an objective magnification of 80. Images of the structure for the emulsion were acquired using image processing software with a CCD camera.

4.2.4 Emulsion Oxidation

Thiobarbituric acid-reactive substances (TBARS) of EFMO were analyzed to evaluate the emulsion oxidation during storage at 20 °C and 40 °C. Emulsion containing menhaden fish oil without SRBF was used as control.

TBARS of emulsion samples were measured according to Mei and others (1998) with modification. A thiobarbituric acid (TBA) solution was prepared by mixing 15 g of trichloroacetic acid, 0.375 g of TBA, 1.76 mL of 12 N HCl, and 82.9 mL of H_2O. TBA solution (100mL) was mixed with 3 mL of 2% butylated hydroxytoluene in ethanol, and 2 mL of this solution was mixed with 1 mL of emulsion sample. The mixture was vortexed for 10 sec and heated in a boiling water bath for 15 min. After being the mixture cooled to the room temperature, it was centrifuged at 3400*g* for 25 min. The absorbance of the supernatant was measured at 532 nm. Concentrations of TBARS were determined from standard curves prepared with 0-0.02 mmol/L 1, 1, 3, 3-tetraethoxypropane.

4.2.5 Microencapsulation of PMO with SRBF

The emulsion was dried to produce microencapsulated PMO with SRBF (MFMO) by a pilot plant scale FT80/81 Tall Form spray drier (Armfield, Ringwood, UK). The spray dryer includes inlet and outlet air fans, electrical air heating chamber, a tall drying chamber, and a cyclone

separator (Fig. 4.1). The inlet ambient air temperature was measured by a mercury thermometer (Ertco Inc., Dubuque, IA), the relative humidity and velocity of inlet ambient air were measured using Omega 4-in-1 multifunctional anemometer (Omega Engineering, Stamford, CT). The ambient air was blown into the air heating chamber by inlet fan where the ambient air was heated by electrical heater from 27.1 ± 0.8 ^{o}C to 180±2 ^{o}C. The heated dry air (inlet air) was blown into the top of the drying chamber. The emulsion temperature was measured using a mercury thermometer (Ertco Inc., Dubuque, IA) and fed to a spray nozzle through the hygienic progressing cavity pump at the rate of 19.90×10^{-5} kg/s.

The atomized EFMO particles at the tip of the nozzle were dried in the drying chamber by the heated air. The dried powder and air were pulled through an exhaust pipe toward to the bottom of the drying chamber by the exhaust fan and they were transported to the cyclone separator. The MFMO powder separated by the cyclone separator was collected in the cyclone collection vessel and the exhaust air and dust were expelled through filter bag to atmosphere. The internal diameter of the ambient air intake pipe and the exhaust air pipe, exhaust (outlet) air temperature, and outlet air velocity were measured. The air temperature and relative humidity of the exhaust air that left from the cyclone through exhaust fan were recorded. The emulsion, powder, and dust were analyzed for moisture content. The estimated production rate was determined and compared with actual production rate. A mass balance for air entering and the leaving the spray dryer, the evaporation rate and energy required to dry EFMO for producing MFMO powder were determined as described in Fig. 4.2.

4.2.5.1 Estimation of the Production Rate of MFMO

The dry solids flow rate was determined based on the dry solids entering and leaving the spray dryer:

$$P_i = P_o + P_d \quad \text{(Eq.4.5)}$$

where, P_i is the emulsion flow rate (kg dry solids/s), P_o is the powder flow rate (kg dry solids/s), and P_d is the dust flow rate (kg dry solids/s).

The product flow rate was estimated according to equation 4.6:

$$P_p = P_i - P_d \quad \text{(Eq.4.6)}$$

where, P_p is the estimated product flow rate (kg dry solids/s), which including powder particles stored on the chamber pipes and joints and chambers and powders collected from the vessel (P_o). The properties of the powder stored on the chamber pipes and joins and chambers were assumed as the same as those of the powder collected from the vessel.

4.2.5.2 Estimation of the Evaporation Rate of Spray-Drying MFMO

The total moisture entering the spray dryer was accounted from both the moisture in the feed and the heated dry air, while the total moisture leaving from the spray dryer was calculated from the moisture in the dried powder and dust and the exhaust air. Thus the overall moisture balance can be expressed as:

$$w_i P_i + AH_i L_i = w_o P_p + w_d P_d + AH_o L_o \quad \text{(Eq.4.7)}$$

where, w_i is the moisture content of the emulsion (dry basis, kg water/kg dry solids), w_o is the moisture content of the product (dry basis, kg water/kg dry solids), w_d is the moisture content of the dust (dry basis, kg/kg dry solids),. AH_i is the absolute humidity of the inlet ambient air (kg water /kg dry air), AH_o is the absolute humidity of the exhaust (outlet) air (kg water/kg dry air); P_i is the emulsion mass flow rate (kg dry solids/s); P_p is the product mass flow rate (kg dry solids/s). P_d is the dust mass flow rate (kg dry solids/s), L_i is the inlet ambient air mass flow rate (kg/s), L_o is the outlet air mass flow rate (kg/s).

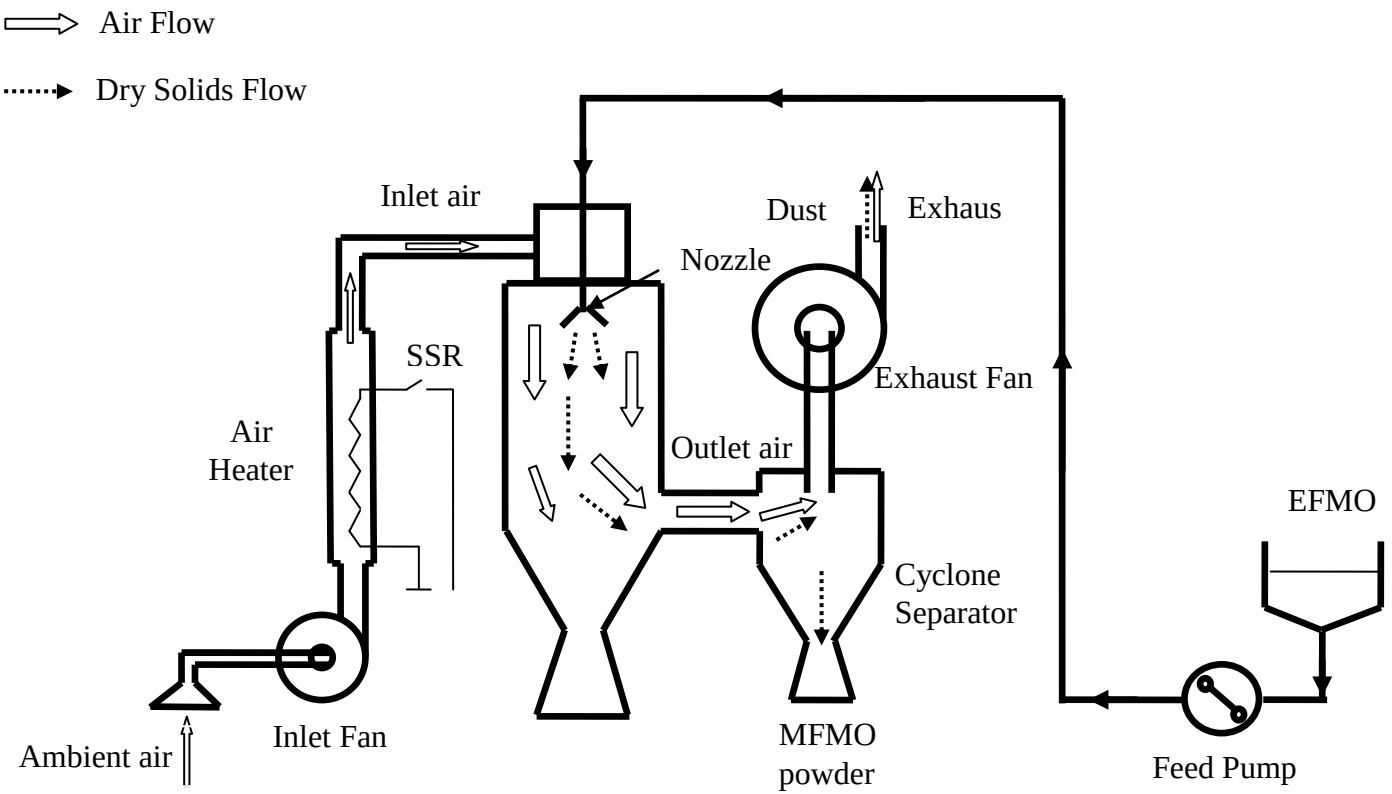

Fig. 4.1 Pilot Scale Spray Dryer System
EFMO = emulsion containing PMO and SRBF; MFMO = microencapsulated PMO with SRBF

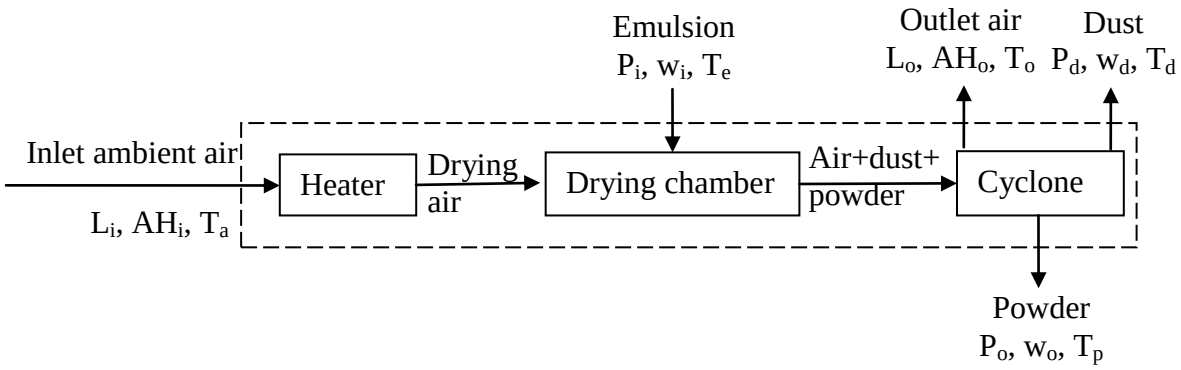

Fig. 4.2 Spray Drying Process Flow Chart

The evaporation rate (E_v, kg water/s) was estimated from moisture removed from the emulsion using equation 4.8:

$$Ev = w_i P_i - w_o P_p - w_d P_d \qquad \text{(Eq.4.8)}$$

The evaporation rate was calculated from the moisture uptake by the dry air using equation 4.9:

$$Ev = AH_o L_o - AH_i L_i \qquad \text{(Eq.4.9)}$$

The air mass flow rate was determined using equation 4.10:

$$L = \frac{V}{Vh} \qquad \text{(Eq.4.10)}$$

where L is the air mass flow rate (kg/s), V is the air volumetric flow rate (m^3/s). Vh is the specific humid air volume (m^3/kg humid air).

The air volumetric flow was calculated using equation 4. 11

$$V = v \times A = v \times \pi r^2 \qquad \text{(Eq.4.11)}$$

where, V represents the air volumetric flow (m^3/s), v represents the air average velocity (m/s), A is the pipe cross sectional area (m^2), and r is the internal radius of the pipe.

The specific dry air volume is determined from the ideal gas law:

$$Vh = (22.4 m^3/kgmol)\left(\frac{1}{29 kg/kgmol Air} + \frac{AH}{18 kg/kgmol Water Vapor}\right)\left(\frac{T^oK}{273^oK}\right)\left(\frac{1 atm}{Patm}\right) \qquad \text{(Eq.4.12)}$$

where Vh is the specific dry air volume (m^3/ kg humid air), T is the temperature (°C), and AH is the absolute humidity of air (kg water/kg dry air), which is obtained from psychometric chart (appendix A.5, Singh and Heldman 2001).

4.2.5.3 Estimation of Energy Used to Heat the Ambient Air

The heat balance was calculated based on the enthalpy given up by the hot air to dry the product and the enthalpy consumed through the drying process and heat lost from the surface of the dryer. It can be expressed as:

$$Q = L_i (Cp_{air} + Cp_{vap} AH_i)(T_i - T_a) \quad \text{(Eq.4.13)}$$

where, Q is the energy used to heat the inlet ambient air to the drying air, L_i is inlet ambient air mass flow rate (kg/s), Cp_{air} = specific heat of dry air (kJ/kg °K), Cp_{vap} = specific heat of water vapor (kJ/kg °K), AH_i is the absolute humidity of inlet ambient air (kg water/ kg dry air), T_a and T_i are inlet ambient air temperature (°K) and drying air temperature (°K), respectively.

4.2.6 Moisture, Water Activity, Bulk Density, Color and Microencapsulate Efficiency (ME) of MFMO

The stored spray dried powder stored at 4 °C was analyzed for moisture, water activity, bulk density, color, and microencapsulate efficiency. The moisture content was determined using a CEM SMART System 5 microwave moisture/solids analyzer (CEM Corporation, Matthews, NC). A calibrated Rotronic water activity meter (AwQUICK, Rotronic Instrument Corp., Huntington, NY) was used to measure the water activity of MFMO at room temperature. Bulk density was determined in triplicate using a 25 mL glass measuring cylinder and bulk density values reported as g per cm^3.

The amounts of surface and total oil were determined to calculate the ME as described by Tan and others (2005) with a slight modification. Surface oil was determined by stirring 5 g of MFMO with 50 mL hexane in a covered 100 mL beaker at 25 °C for 10 min. The suspension

was then filtered and the residue rinsed thrice by passing 20 ml of hexane each time. The surface oil was determined gravimetrically following residual powder air drying for 30 min. The total oil, which included both the encapsulated oil and surface oil, was determined using a CEM FAS-9001-3 fat analyzer (CEM, Matthews, NC). The ME was calculated as follows:

$$M_E = [\text{Total oil} - \text{Surface oil})/\text{Total oil}] \times 100\% \quad \text{(Eq.4.14)}$$

Color of the MFMO was determined using the chroma meter LABSCAN XE (Hunterlab, VA, USA). The colorimeter was standardized by white and black tiles. The data of colors were reported in CIELAB color scales (L* value is degree of lightness to darkness, a* value is degree of redness to greenness, and b* value is degree of yellowness to blueness). Chroma and hue angle value were calculated as following:

$$\text{Chroma} = [a^{*2} + b^{*2}]\ ½ \quad \text{(Eq.4.15)}$$

$$\text{Hue angle} = \tan^{-1}(b^*/a^*) \quad \text{(Eq.4.16)}$$

4.2.7 Lipid Oxidation and Fatty Acid Methyl Ester Composition of MFMO

TBARS content were tested to estimate the lipid oxidation of the microencapsulated powder. The microencapsulated powder was reconstituted as 10 % (w/w) emulsion by mixing with water and vortexing for 5 min. The measurement of TBARS were described as above.

Fatty acid methyl ester composition of samples was determined at the USDA-ARS Laboratory, University of Alaska Fairbanks, AK. Fatty acid methyl ester (FAME) composition of samples was determined after samples were extracted using a Model 200 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA) at 50°C with 100% dichloromethane. The solvent was evaporated off by passing nitrogen over the samples in a 50 °C water bath. FAMEs were prepared using a modified method of Maxwell and Marmer (1983), which used 9.5 mL of isooctane, 0.5 mL 23:0 as an internal standard and washings of 1 mL 2 mol/g KOH (1.12g/ 10 ml

MeOH) to convert the fatty acids into their methyl esters, saturated ammonium acetate was used to neutralize KOH and distilled water was used to wash ammonium acetate from isooctane layer. The liquid containing methyl ester in isooctane layer was analyzed by Gas chromatographic (GC) analysis using a GC model 7890A (Agilent) fitted with a HP-88 (100 m x 0.25 mm ID x 0.25 μm film) column. The oven program used was 90 °C for 8 min, followed by 10 °C/min to 175 °C for 10 min, 4 °C/min to 190 °C for 10 min, 5 °C/min to 210 °C for 5 min and then 20 °C/min to 250 °C for 8 min. ChemStation software was used to integrate peaks. Peaks were identified by comparing to reference standards obtained from Sigma: Supelco 37 mix, PUFA #1, PUFA #3 and cod liver oil. Data are expressed as percent of total integrated area. Data were expressed as percent of total integrated area.

4.2.8 Particle Size Distribution of MFMO

The particle size distribution was measured with a Microtrac S3500 light scattering system (MicroTrac, Largo FL) in wet operation. Isopropyl alcohol was used as carrying fluid. The system uses three fixed 780 nm solid state lasers with computer controlled single lens alignment and the measurement capability is from 0.24 to 2800 microns. During the measurement, a certain amount of powder samples was placed in the test chamber with circulating isopropyl alcohol. A period of 10 second ultra sound mixing at 20 watts was used before each test. The sample was pumped through sample cell at 40% of the maximum flow rate. Light was scattered from the tri-lasers from low to high angles (0-163 degrees). The whole light scatter pattern was collected. The volume distribution of the particle size was calculated using modified MIE-scattering technique.

4.2.9 Statistics Analysis

Mean values from the three separate experiments or replicate analysis are reported with standard deviation. The statistical significance of observed differences among treatment means was evaluated by analysis of variance (ANOVA) (SAS Version 9.2, SAS Institute Inc., Cary, NC, U.S.A), followed by post hoc Tukey's studentized range test.

4.3 Results and Discussion

4.3.1 Emulsion Stability, Rheology Properties and Light Microscope of EFMO

No oil was recovered from EFMO stored at -20 ^{o}C for 2 days, which demonstrated the emulsion sample was stable. The flow behavior index (n), consistency index (*K*) values, and apparent viscosity at 100 s^{-1} of EFMO at 5, 15 and 25 °C were listed in Table 4.1. As the temperature increased from 5 °C to 15 °C, *n* value increased and the apparent viscosity decreased; but there was no significant difference between the values at 15 and 25 °C (Table 4.1). The flow index value (*n*) for EFMO was less than 1.0, which indicated that it was a pseudoplastic fluid (Paredes and others 1989). The *n* value of EFMO was similar to reported values for some commercial emulsions and model emulsion systems (Dickie and Kokini 1983, Steffe 1992). Viscosity and *K* of EFMO was higher at 5^{o}C than those at 15 and 25^{o}C. Viscosity and fluidity of the solution are modified by the feed temperature (Zakarian and King 1982, Zbicinski and others2002, AIChE Equipment Testing Procedure 2003). It is important to know the changes of the viscosity and fluidity of the solution because they alter the spraying capacity of the spray dryer.

Dynamic rheological tests were used to characterize viscoelastic properties of EFMO. Viscoelastic properties and the frequency sweep test of EFMO are shown in Fig. 4.3. The G' (an elastic or storage modulus) and G" (a viscous or loss modulus) of the EFMO were determined as

a function of frequency (ω) at a fixed temperature of 25 oC. G' is a measure of energy recovered per cycle of sinusoidal shear deformation and G" is an estimate of energy dissipated as heat per cycle (Rao 1999). EFMO (Fig. 4.3) showed a gradual increase in both the loss modulus and the storage modulus with increasing frequency and also with a slightly higher G' than G". The tangent of the phase angle (δ) measures the energy loss compared with the energy stored in each cycle of deformation (Bryant and McClements 2000) and EFMO showed a gradual decrease in δ with increasing frequency (Fig. 4.4). The δ value could be used to explain whether the material behaves as solid (90 ^{o}) or liquid (0 ^{o}) and values between 0 ^{o} and 90 ^{o} indicate the material is viscoelastic (Bryant and McClements 2000). EFMO behaved more like a viscoelastic material because it had a higher G' and higher δ (above 45°). Moschakis and others (2005) reported that an emulsion with viscoelastic characteristic would retard the rearrangement of macroscopic phase separation, which indicated that the EFMO was stable.

Table 4.1 Flow Properties of EFMO

Temperature (°C)	*n*	*K* (Pa.s^{n})	Viscosity (Pa.s)
5	0.56±0.03^{b}	1.15±0.10^{a}	0.18±0.03^{a}
15	0.83±0.05^{a}	0.26±0.06^{b}	0.11±0.00^{b}
25	0.87±0.04^{a}	0.17±0.04^{b}	0.09±0.01^{b}

^{ab}means with different letters in each column are significantly different (P< 0.05).
n = flow behavior index; *K* = consistency index (Pa.s^{n}).

Small and well isolated spherical oil droplets were observed under light microscopy for EFMO (Fig. 4.5). Kiokias and others (2007) reported that the electrostatic repulsion between the protein molecules at the interface prevented flocculation of the droplets. The estimated droplet size range for EFMO from the light microscopy image was 1-10 μm. It is believed that small oil droplets would be enclosed and embedded more efficiently within the wall matrix of the microcapsules and result in more stable emulsion during the spray drying encapsulation process (Jafari and others 2008). According to Sala and others (2007, 2009), the variations in the oil

droplet size did not significantly influence aggregation of the oil droplets in the emulsion. Egelandsdal and others (2001) reported that the droplet size distributions were determined by other factors, such as the quantity and the quality of the emulsifier, the energy input of the homogenizer, the viscosity of the emulsion, and the temperature used during homogenization. It is important to form a stable emulsion of fish oils in the coating solution and to keep emulsions stable over a certain period of time before spraying any emulsion into a spray drying chamber to obtain stable microencapsulated oil (Sathivel and Kramer 2010).

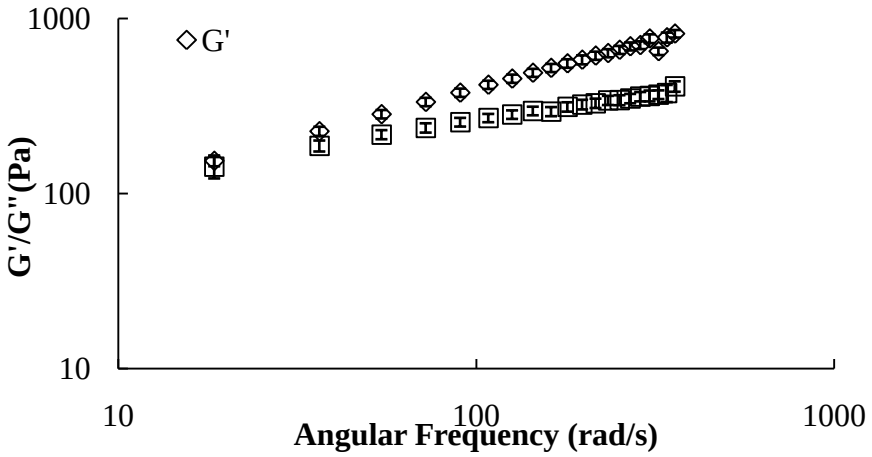

Fig. 4.3 Rheology Properties of EFMO: Storage Modulus (G') and Loss Modulus (G") vs. Angular Frequency. EFMO = Emulsion containing SRBF and PMO.

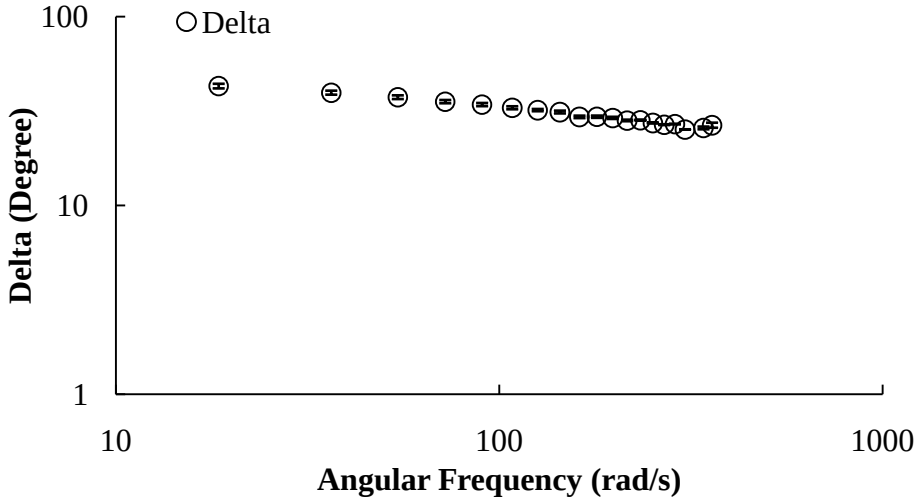

Fig. 4.4 Rheology Properties of EFMO: Phase Angle (δ) vs. Angular Frequency. EFMO = Emulsion containing SRBF and PMO. The ○= δ of EFMO.

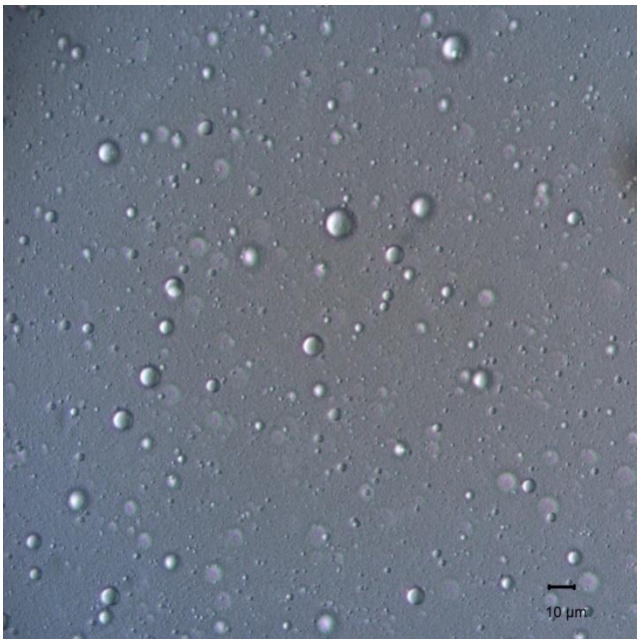

Fig. 4.5 Light Microscopy Image of the EFMO. Magnification: 80; EFMO = Emulsion containing SRBF and PMO.

4.3.2 Emulsion Oxidation

The formation of TBARS in emulsion containing PMO with or without SRBF during storage at 20 °C and 40 °C is shown in Fig. 4.6. The TBARS value of all emulsions did not significantly change during the initial 40 hr (which is not shown), but it increased significantly until the end of storage. A similar trend of TBARS value in the emulsion fortified with DHA was observed by Tippetts and Martini (2010) and they also found that the TBARS value did not increase at the first 50 hr and the TBARS value reached the maximum at 72 hr storage, when significant differences in odor were also detected.

The emulsions with/ without SRBF stored at 20 and 40 °C had similar TBARS values during first 40 hours. After 40 hours, the emulsions stored at 40 °C had higher TBARS value than those stored at 20 °C. Emulsions with SRBF had lower TBARS values than emulsion without SRBF during storage period between 40-88 hours storage regardless of storage

temperatures. This indicated that SRBF significantly reduced lipid oxidation of the emulsion containing menhaden oil during the storage.

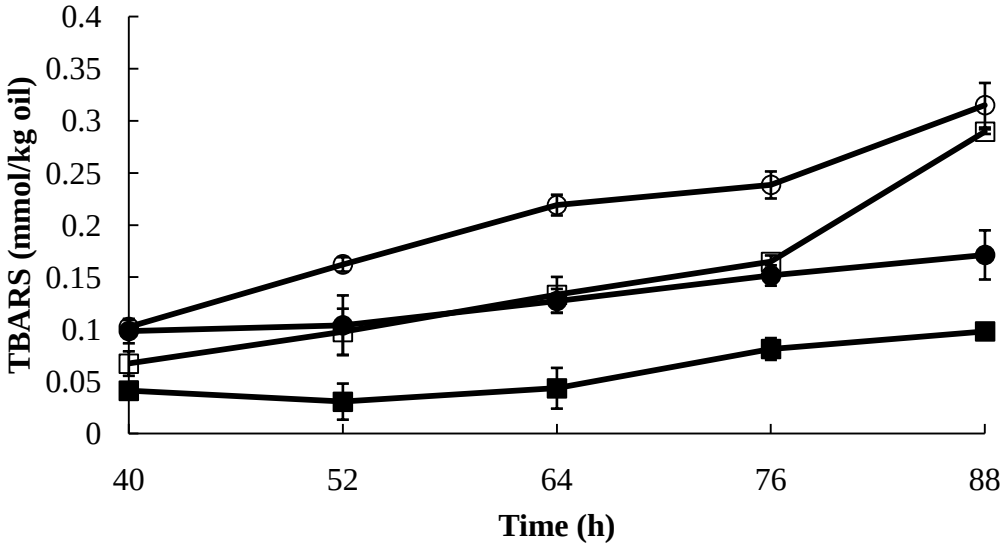

Fig. 4.6 Formation of TBARS in Emulsion Containing PMO with or without SRBF During Storage at 20 °C and 40 °C. The -□- = emulstion containing PMO without SRBF stored at 20 °C; -■- = EFMO stored at 20 °C; -○- = emulstion containing PMO without SRBF stored at 40 °C; -●- = EFMO stored at 40 °C.

4.3.3 Spray Dryer Performance

The estimation of mass flow rate of spray drying MFMO is listed in Table 4.2. The moisture contents of emulsion (EFMO), dust, and product (MFMO) on dry basis were 4.44, 0.06 and 0.06 kg water/ kg dry solids, respectively. The mass flow rates of EFMO, dust and MFMO on wet basis were 1.99×10^{-4}, 8.6×10^{-7}, and 2.45×10^{-5} kg/s, separately. The estimated powder production rate was 3.45×10^{-5} kg dry solids/s, which was higher than the actual production rate 2.31×10^{-5} kg dry solids/s. As expected some powder particles were stored on the chambers pipes and joints and chambers walls, which might be a possible reason the actual product rate was lower than estimated production rate. Huang and others (2006) have reported that a large amount powder can deposited on the outlet pipe and chamber wall during spray drying.

The calculation for the evaporation rate and energy required to spray dry MFMO is summarized in Table 4.3. The ambient air that entered the spray dryer was at 27.1 ± 0.8 °C with relative humidity of 58.9 ± 10.2 %. Based on the dry bulb temperature and relative humidity of ambient air, the absolute humidity of the ambient air was (1.32 ± 0.18) × 10^{-2} kg water/ kg dry air. The flow rate for dried air entering to the spray dryer was 1.743 × 10^{-2} kg/s which was balanced with the outlet air leaving the spray dryer (1.747 × 10^{-2} kg/s) . As expected, volumetric flow rate, absolute humidity, and specific volume increased, while relative humidity decreased during spray drying. The evaporation rate calculated from moisture uptake by the dry air and evaporation rate estimated based on the moisture content of the emulsion, product (P_p), and dust had a similar value. The evaporation rate was smaller than the value (7.8 × 10^{-3} kg water/s) reported by Huang and others (2006). Evaporation rate during the spray drying depends on a number of factors including humidity of the dry air and the temperature in the drying chamber. Huang and others (2006) have reported that a higher evaporation rate can be resulted when the feed droplets pass through a lower humidity and higher temperature drying chamber. The energy used to increase the inlet ambient air temperature from 27.1 °C to 180 °C was 2.78 kJ/s. The heat energy for heating the ambient air was generated by electric heater and the energy capacity of the electric heater was 4.5 kJ/s (FT80/81 Tall Form spray drier manual, Armfield, Ringwood, UK), which was higher than the estimated energy required to heat the ambient air. The energy consumption per unit evaporation rate for drying the emulsion was 17820.5 kJ/kg, which was significantly higher than the reported value (3114 kJ/kg) by Huang and others (2006) for drying maltodextrin suspension. A smaller evaporation rate leads to higher heat consumption (Huang and others 2006).

Table 4.2 Mass Flow Rate of Spray Drying MFMO

	Emulsion	Dust	Powder
Moisture content (%, wet basis)	81.6 ± 0.51	5.83 ± 0.17	5.92 ± 0.46
Moisture content (× 10^{-2} kg water/kg dry solids)	444.15 ± 15.28	6.19 ± 0.18	6.17 ± 0.48
Mass flow rate (× 10^{-5} kg /s, wet basis)	19.90 ± 0.09	0.09 ± 0.03	2.45 ± 0.02
Mass flow rate (× 10^{-5} kg dry solids/s)	3.53 ± 0.02	0.08 ± 0.03	2.31 ± 0.02
Estimated powder product rate (× 10^{-5} kg dry solids/s)	—	—	3.45 ± 0.03

Values are means and SD of triplicate determinations. MFMO = microencapsulated PMO with SRBF.

Table 4.3 Summary of the Calculation for the Evaporation Rate and Energy Required to Spray Dry MFMO

	Inlet ambient air	Outlet air
Temperature (^{o}C)	27.1 ± 0.8	82.6 ± 2.26
Velocity (m/s)	16.65 ± 0.35	4.12 ± 0.09
Pipe diameter (m)	0.034	0.075
Volumetric flow rate of air (× 10^{-2} m^3/s)	1.51 ± 0.04	1.82 ± 0.04
Relative humidity (%)	58.9 ± 10.2	6.7 ± 1.2
Absolute humidity (× 10^{-2} kg water /kg dry air)	1.32 ± 0.18	2.21 ± 0.18
Specific volume air (m^3/kg dry air)	0.868 ±0.001	1.042 ± 0.003
Air flow rate (× 10^{-2} kg/s)	1.743 ± 0.042	1.747 ± 0.038
Specific heat of dry air (kJ/kg K)*	1.01 ± 0.00	1.02 ± 0.00
Specific heat of water vapor for dry air (kJ/kg K)**	1.88	1.88
Evaporation rate (from emulsion, powder, and dust moisture, × 10^{-4} kg water/s)		1.5711 ±0.0499
Evaporation rate (from air moisture uptake, × 10^{-4} kg water/s)		1.5568 ± 0.0006
Energy used to heat the ambient air (kJ/s)		2.78 ± 0.08

Values are means and SD of triplicate determinations. MFMO = Microencapsulated menhaden oil containing SRBF. * Appendix A.4.4 (Singh and Heldman 2001). ** Singh and Heldman (2001).

4.3.4 Moisture, Water Activity, Bulk Density, Microencapsulate Efficiency, Microcapsules Oxidation and FAME Composition of MFMO

The moisture content of MFMO (5.81%) (Table 4.4) was much higher than the moisture content of a fish oil powder (1.4-1.7 %) reported by Jafari and others (2008), and moisture content of tuna oil powders (2.89-3.02 %) reported by Klaypradit and Huang (2008), but was lower than the moisture content of a microencapsulated fruit fiber and antioxidant hibiscus extract powder (8.1-10.7 %) reported by Chiou and Langrish (2007). The moisture content of

MFMO was also higher than the maximum moisture specification for most dried powders in the food industry (3-4g/100g) (Masters 1991), which might be due to the exposure of powder to the ambient humidity and it absorbed water before measurement. The moisture content of the microencapsulated fish oil depends on the factors determining the spray drying, including evaporation rate and viscosity of emulsion. The water activity of the MFMO (0.16) was lower than values (0.19-0.24) reported by Klinkesorn and others (2006) for spray dried microencapsulated tuna oil powder.

Table 4.4 Properties of MFMO

Parameter	MFMO
Moisture (%)	5.81±0.43
Water activity	0.16±0.01
Bulk density	0.28±0.02
Color a*	0.11±0.21
Color b*	9.11±0.89
Color c*	9.32±0.91
L*	92.41±0.43
Hue angle	97.31±1.33
Yield (%)	60.36±0.24
Surface oil (mg/g)	160.00±1.32
Total oil (mg/g)	374.97±1.26
Microencapsulate efficiency (%)	57.32±0.27
TBARS (mmol/kg oil)	0.27±0.02

Values are means and SD of triplicate determinations. MFMO = microencapsulated menhaden oil containing SRBF, a* = degree of redness to greenness, b* = degree of yellowness to blueness, c* = chroma, L* = degree of lightness to darkness, TBARS = Thiobarbituric acid-reactive substances.

Bulk density of the MFMO (0.275 g/ cm^3) was similar to reported spray dried vegetable oil powder value (0.27-0.44 g/cm^3) (Fuchs and others 2006) and spray dried fish oil powder value (0.19-0.28 g/cm^3) (Kagami and others 2003). The color intensity (chroma) and hue angle of the MFMO were 9.32 and 97.31, respectively (Table 4.4). The yield of MFMO based on the collected powder was 60.36% and Fuchs and others (2006) also reported a similar yield value (65%) for spray dried vegetable oil powder using maltodextrin and acacia gum as wall materials.

Our study also showed that estimated powder production rate was higher than actual powder production. The ME (57.3%) was lower compared with previously published value (84.5-86.9 %) for the spray dried tuna oil powder (Klinkesorn and others 2006) and (79.3 – 83.5 %) for spray dried tuna oil powders using chitosan, maltodextrin and whey protein isolate as wall materials (Klaypradit and Huang 2008).

The TBARS value (Table 4.4) of the total oil from spray dried MFMO (0.27 mmol/ kg oil) was higher than the TBARS value of EFMO (0.10 ± 0.01 mmol/ kg oil). The oxidation stability of dried powder is usually determined by total lipids (Klinkesorn and others 2006), which include encapsulated and surface oil. MFMO contained 43 % surface oil, which might be more oxidized than the encapsulated oil. Therefore, MFMO had a higher TBARS value than the TBARS value of EFMO. Lipid oxidation value of microencapsulated oil included both lipid oxidation of surface and encapsulated oils (Hogan and others 2003).

The fatty acid profiles of PMO and MFMO are shown in Table 4.5. The fatty acid composition of menhaden oil after encapsulation had a slightly higher percent of total saturated fatty acid (SAFA) and monounsaturated fatty acids (MUFA) than polyunsaturated fatty acids (PUFA). The major fatty acids in PMO and MFMO were C14:0, C16:0, C16:1n7, c18:1n9c, with EPA and DHA. The content of EPA and DHA in the MFMO were reduced from 14.49 % to 11.52% and from 6.15% to 4.51%, respectively, when PSO was microencapsulated (Table 4.5). Klaypradit and Huang (2008) used an atomizer spray drier to encapsulate tuna oil and found the encapsulation process preserved EPA and DHA, but the amount of the total of EPA and DHA still reduced from 28.3 g/100 g in crude oil to 24 g/100g.

Table 4.5 FAMEs Composition of PMO and MFMO

Fatty acid	PMO	MFMO
14:0	10.48±0.09[b]	11.78±0.14[a]
15:0	0.60±0.00[b]	0.67±0.01[a]
16:0	19.67±0.30[b]	21.84±0.46[a]
16:1n7	14.12±0.16[b]	15.65±0.19[a]
18:0	3.13±0.06[b]	3.47±0.09[a]
18:1n9c	8.36±0.11[b]	9.22±0.19[a]
18:1n7	3.36±0.05[b]	3.69±0.08[a]
18:2n6c	0.99±0.01[a]	1.01±0.02[a]
18:3n3	0.83±0.01[a]	0.78±0.04[a]
18:4n3	2.56±0.07[a]	2.20±0.19[a]
18:4n1	0.50±0.00[a]	0.44±0.02[b]
20:1n9	1.37±0.02[b]	1.46±0.03[a]
20:4n6	1.32±0.02[a]	1.14±0.08[b]
20:5n3	14.49±0.46[a]	11.52±1.14[b]
22:5n3	2.28±0.07[a]	1.80±0.18[b]
22:6n3	6.15±0.23[a]	4.51±0.48[b]
ω 3 total	26.31±0.83[a]	20.82±2.02[b]
ω 6 total	2.77±0.03[a]	2.28±0.14[b]
SAFA	33.88±0.37[b]	37.77±0.60[a]
MUFA	27.21±0.32[b]	30.02±0.47[a]
PUFA	31.42±0.83[a]	25.36±1.71[b]
ω 3 / ω 6	9.51±0.20[a]	9.17±1.32[a]
P/S	1.16±0.04[a]	0.85±0.07[b]
totoal identified	92.52±0.23[a]	93.14±0.66[a]

[ab]means with different letters in each column are significantly different ($P < 0.05$). FAMEs = fatty acid methyl esters, SAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, P/S = polyunsaturated fatty acids/ saturated fatty acids.

4.3.5 Particle Size of MFMO

The particle size distribution is shown in Fig. 4.7. The particle size of 90% MFMO ranged from 8 to 62 μm. MFMO had higher amounts of 18.5-22 and 22-21.62 microns particles while the least abundant (0.0%) particle size was 4.62 microns. The particle size of MFMO was smaller than a microencapsulated fish oil powder (300 to 700 μm) produced by spray granulation and fluid bed film coating (Anwar and others 2010) and slightly higher than reported the particle size (5 to 30 μm) for a spray-dried tuna oil powder (Klinkesorn and others 2006). The volume-length diameter ($d_{4,3}$) of MFMO was 28.48 ± 1.80 μm, which was similar to $d_{4,3}$ values of spray

dried fish oil powders using a modified starch (28.8 μm) and/or whey protein concentrate (34.0 μm) as wall materials (Jafari and others 2008). According to AIChE Equipment Testing Procedure, 2003, particle size of the powder is depended on the spray dryer operating conditions such as feed temperature, air inlet temperature, and air outlet temperature. Hogan and others (2001) reported the $d_{4,3}$ value of spray dried soya oil powder using whey protein concentrate as a wall material ranged from 15 to 22.5 μm, and found the powder particle size increased with the increasing of total solids content and/or the apparent viscosity of the emulsions. Kagami and others (2003) produced spray dried fish oil powder with particle size ranging from 18.9 to 22.4 and found the particle sizes of powders were not affected by different wall systems and oil loading rate.

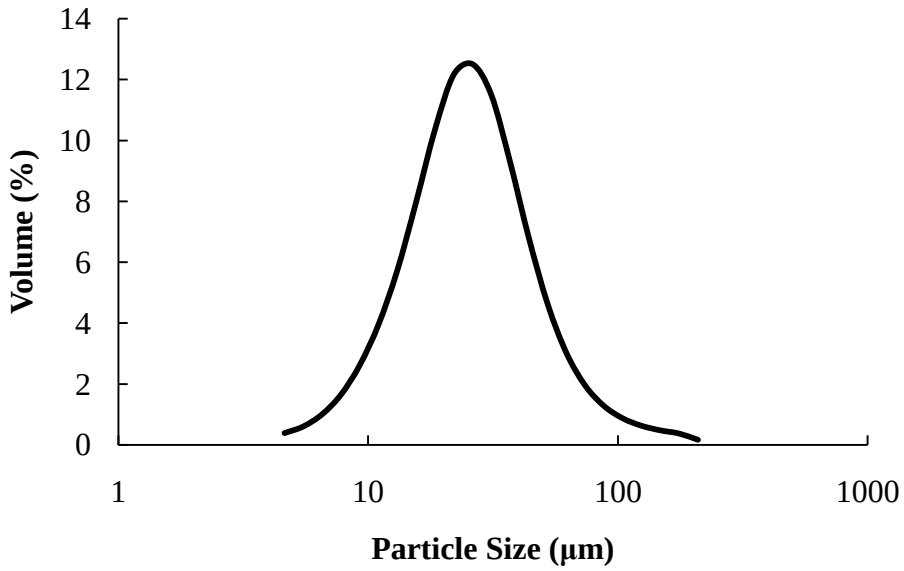

Fig. 4.7 Particle Size Distribution of the MFMO

4.4 Conclusions

Emulsion containing purified menhaden oil and soluble rice bran fiber exhibited high stability. Viscosity and flow behavior index of EFMO increased as the temperature increased. EFMO was a pseudoplastic fluid and it behaved more like a viscoelastic material. EFMO had

small and well isolated spherical oil droplets with an oil droplet size of 1-10 μm. EFMO showed better oxidative stability than emulsion containing PMO without SRBF at both low and high temperature. Estimated MFMO production rate was higher than actual production rate. Evaporation rates calculated based on moisture uptake by dry air and moisture content of EFMO, MFMO, and dust were similar. The energy used to heat the inlet ambient air was lower than energy capacity of the electrical heater of the spray dryer. MFMO contained polyunsaturated fatty acids and the EPA and DHA in MFMO were 11.52% and 4.51%, respectively. The particle size of 90% MFMO ranged from 8 to 62 μm, and the volume-length diameter of MFMO was 28.5 μm. This study demonstrated that the microencapsulated PMO with SRBF can be effectively produced. Dry powder containing PMO with SRBF could provide potential health benefits for humans.

CHAPTER 5 SUMMARY AND CONCLUSIONS

Defatted rice bran (DRB) is a byproduct of rice bran oil extraction from whole rice bran and an excellent source of dietary fiber and other components. Soluble rice bran fiber (SRBF) extracted from defatted rice bran is known for its antioxidant, anti-tumor, immune-modulation activities and hypocholesterolemic effects in human. The extraction of non-starch polysaccharides is significantly affected by the extraction conditions such as temperature, time, ratio of water to raw material, solvent concentration. Therefore, it is difficult to estimate the optimize SRBF yield based on just the one-factor-at-a-time (single factor) approach not only because it is time consuming, but also interactions between extractions conditions may not be included in the determination of the yield and/or quality. A study is needed to determine optimum extraction conditions to obtain a desirable yield or quality of SRBF from DRB and understand the degree of interactions between the independent variables. The goal of this study is designed to extract the SRBF from DRB, purify the extracted SRBF and also develop microencapsulated menhaden oil (PMO) containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with SRBF.

The specific objectives of the study were to: (1) determine the effects of single factor such as temperature, time, $Ca(OH)_2$ concentration, and the ratio of $Ca(OH)_2$ solution to DRB on SRBF yield; (2) optimize the effects of the ratio of extraction solution to defatted rice bran, concentration of extraction solution, and extraction temperature on the SRBF yield using response surface methodology with a three level and three independent variables Box-Behnken factorial design; (3) evaluate the effects of molecular weight cut off and transmembrane pressure of ultrafiltration membranes on the permeability of the solution containing SRBF; (4) determine the performance of ultafiltration for separating the small molecular weight sugars and minerals

from solution containing SRBF; (5) prepare and characterize the rheological properties of the emulsion containing PMO with SRBF; (6) evaluate the antioxidant properties of SRBF in the emulsion containing PMO; and (7) develop and characterize the microencapsulated PMO with SRBF using a spray drying process, and estimate the production rate and energy required to spray dry the emulsion for producing microencapsulated menhaden fish oil with SRBF powder.

The second chapter was devoted to extract the SRBF from DRB using a single factor experiment and based on the single factor experiment, the independent variables including the ratio of $Ca(OH)_2$ solution to DRB , concentration of $Ca(OH)_2$ solution, extraction temperature, and their levels were selected and used for determining optimum extracting conditions for the SRBF using response surface methodology. The study showed that an estimated optimum yield of SRBF (7.89%) could be extracted from DF with 3% $Ca(OH)_2$ solution to DRB ratio 29.75:1 and stirred for 1 hr at 84^{o}C and also $Ca(OH)_2$ solution concentration was the most effective factor among the conditions used to extract SRBF.

In chapter three, the extracted SRBF solution from DRB was purified using ultrafiltration process with 1, 5, and 10 kDa MWCO membranes at 100 kPa to remove minerals, monosaccharides and other small molecules. The study showed that the conventional processing steps, such as dialysis and alcohol precipitation, for removing mineral, monosaccharides and other small molecules from SRBF, could be replaced with the ultrafiltration technology. The 10 kDa MWCO membrane at a pressure of 100 kPa was effective to purify SRBF solution compared with 1 and 5 kDa MWCO membranes at the same pressured.

The final chapter was designed to develop a microencapsulated PMO with SRBF. The emulsion prepared with the small and well isolated spherical droplets of PMO containing SRBF showed a pseudoplastic fluid and viscoelastic characteristics, which indicated that the emulsion

was stable and it could be used spray dry to produce a stable powder. The estimated microencapsulated PMO with SRBF powder (MFMO) production rate using spray dryer was 3.45×10^{-5} kg dry solids/s and was higher than the actual production rate 2.31×10^{-5} kg dry solids/s. The energy required to increase the inlet ambient air temperature from 27.1 to 180 °C and evaporation rate for during spray drying the emulsion was 2.78 kJ/s and 7.8×10^{-3} kg water/s, respectively. MFMO contained polyunsaturated fatty acids and the EPA and DHA in MFMO were 11.52% and 4.51%, respectively. The particle size of 90% MFMO ranged from 8 to 62 μm, and the volume-length diameter of MFMO was 28.5 μm.

In summary, the SRBF can be extracted from DRB and purified using ultafiltration methods. The optimum extraction conditions for SRBF can be estimated using the response surface methodology. The conventional purification steps of SRBF including dialysis and alcohol precipitation could be replaced with ultrafiltration technology. This study demonstrated that the microencapsulated PMO with SRBF can be effectively produced. Dry powder containing PMO with SRBF could provide potential health benefits for humans.

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APPENDIX: SPRAY DRYER PERFORMANCE CALCULATION

Estimation of the Production Rate of MFMO

$$P_i = P_o + P_d$$

$$P_o = P_i - P_d$$

$$P_i = 3.56 \times 10^{-5}\,\text{kg/s}$$

$$P_p = P_i - P_d = 3.56\times10^{-5} - 0.08\times10^{-5} = 3.48\times10^{-5}\,\text{kg dry solids/s}$$

Air flow - Inlet

When the inlet ambient air temperature was 26.5 °C with relative humidity 69.7%, the absolute humidity in the air (AH_i) was obtained as 0.0152kg water/kg Dry air from psychometric chart.

$$Vh_i = (0.00283 + 0.00456 \times AH_i) \times T_i = (0.00283 + 0.00456 \times 0.0152) \times (26.5 + 273) = 0.868\text{m}^3/\text{kg}$$

$$V_i = v_i \times A = v_i \times \pi r^2 = 16.58 \times 3.14 \times 0.017^2 = 0.0150\ \text{m}^3/\text{s}$$

$$L_i = \frac{V_i}{Vh_i} = 0.0150/0.8\ 68 = 0.0173\text{kg/s}$$

Air flow – Outlet

When the inlet ambient air temperature was 80.0 °C with relative humidity 8.0%, the absolute humidity in the air (AH_o) was obtained as 0.0242kg water/kg Dry air from psychometric chart.

$$Vh_o = (0.00283 + 0.00456 \times AH_o) \times T_o = (0.00283 + 0.00456 \times 0.0242) \times (80 + 273) = 1.038\text{m}^3/\text{kg}$$

$$V_o = v_o \times A = v_o \times \pi r^2 = 4.07 \times 3.14 \times 0.0375^2 = 0.0180\text{m}^3/\text{s}$$

$$L_o = \frac{V_o}{Vh_o} = 0.0180/1.0\ 38 = 0.0173\text{kg/s}$$

Estimation of the Evaporation Rate of Spray-Drying MFMO

$$w_i P_i + AH_i L_i = w_o P_p + w_d P_d + AH_o L_o$$

Evaporation rates based on moisture content of emulsion, dust, and powder:

$$Ev = w_i P_i - w_o P_p - w_d P_d = 4.353 \times 3.56 \times 10^{-5} - 0.062 \times 3.48 \times 10^{-5} - 0.064 \times 0.08 \times 10^{-5} = 1.548 \times 10^{-4} \text{ kg/s}$$

Evaporation rates based on moisture uptake by dry air:

$$Ev = (AH_o - AH_i)L = (0.0242 - 0.0152) \times 0.0173 = 1.557 \times 10^{-4} \text{ kg/s}$$

Estimation of Energy Used to Heat the Ambient Air

$$Q_i = L_i (Cp_{air} + Cp_{vap} H_i)(T_i - T_{amb}) = 0.0173 \times (1.01 + 1.88 \times 0.0152) \times (180 - 26.5) = 2.76 \text{kJ/s}$$

VITA

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