Value-added processing of rice bran focusing on dietary fiber modification

by

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ABSTRACT

Rice bran (RB) is an underutilized byproduct of rice milling industry. RB is rich in insoluble dietary fiber (IDF) but poor in soluble dietary fiber (SDF). Recently, SDF derived from RB has been proven for its superior antioxidant and prebiotic activities which confer human health benefits (digestive, cardiovascular, nerve health, etc.). Moreover, SDF can improve sensory and physicochemical properties (texture, color, uniformity, water binding capacity, hydration, etc.) of SDF-enriched foods. Therefore, conversion of RB-IDF to RB-SDF would be to utilize this affordable byproduct to add value to the rice bran processing industry as a common functional food ingredient. The aim of this study was to maximize soluble pentosan content (a major SDF component) in RB, by investigating the effect of physical (extrusion) and enzymatic (xylanase) technologies in individual and combined ways on water-washed rice bran and its soluble compositions. A water washing procedure was necessary to remove water solubles as a strategy to increase the proportion of total dietary fiber (IDF + SDF) that could be converted to SDF after treatment. Even though water washing wasted native SDF along with starch and other solubles, preparing the sample this way was practical due to the large subsequent increase in IDF. The sequentially combined process of extrusion and enzyme treatments, compared to the individual and simultaneously combined treatments, significantly increased total solubility and soluble pentosan content of the final RB product. The warm-water-soluble pentosan content of treated RB was 6.5% by the sequentially combined process, 4% by either parallel combined process or extrusion alone or xylanase treatment alone. The hot-water-soluble pentosan of treated RB achieved a higher level of 10.5% by the sequentially combined process, 4.8% by extrusion alone, and 6.5% by xylanase treatment alone. A maximum total hot water solubility of 25% was achieved, of which 10.5% was pentosan, when water-washed rice bran was treated with extrusion and enzyme in sequence, representing an approximately four fold increase compared to

untreated RB. Overall, washing rice bran with water was shown to be an efficient method to remove non-dietary fiber components. This study will likely represent the first published example for rice bran demonstrating an alternative to enzymatic methods used conventionally (e.g. amylase, protease, lipase) to hydrolyze non-dietary fiber compounds for further fiber processing.

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LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemists
ACS	American Chemical Society
AOAC	Association of Official Analytical Chemists
Ara/Xyl	Arabinose/xylose ratio
AX	Arabinoxylan
CAF	Cycloartenol ferulic acid ester
C-DRB	Commerical-defatted rice bran
CEC	Cation exchange capacity
CHD	Coronary heart disease
DF	Dietary fiber
DP	Degree of polymerization
DSC	Differential Scanning Calorimetry
EU	European Union
FAO	Food and Agricultural Organization of United Nations
FAOSTAT	Food and Agricultural Organization of United Nations' Statistics
FBC	Fat binding capacity
FDA	Food and Drug Administration
FFA	Free fatty acid
GRAS	Generally Recognized as Safe
GRC	Glucose retardation capacity
HDL-C	High density lipoprotein cholesterol
HPLC	High performance liquid chromatography
IDF	Insoluble dietary fiber
IRRI	International Rice Research Institute
LDL-C	Low density lipoprotein cholesterol
L-DRB	Lab-defatted rice bran
LSD	Least Significant Difference

MMT	million metric tons
NRB	Native rice bran
OZ	γ-oryzanol
RBH	Rice bran hemicellulose
RBIDF	Rice bran insoluble dietary fiber
RBO	Rice bran oil
RBSDF	Rice bran soluble dietary fiber
SAS	Statistical Analysis System
SC	Swelling capacity
SDF	Soluble dietary fiber
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning Electron Microscopy
SRB	Stabilized rice bran
TBARS	Thiobarbituric acid reactive substances
T_c	Conclusion temperature
T_o	Onset temperature
T_p	Peak temperature
USDA	The United States Department of Agriculture
v/v	Volume by volume
VAD	Vitamin A Deficiency
w/v	Weight by volume
w/w	Weight by weight
wb	Wet basis
WBS	Water binding capacity
WOF	Warmed-over flavor score
WS	Water solubility
WU	Water uptake

CHAPTER 1. INTRODUCTION AND OBJECTIVES

1.1 Introduction

Rice is the world's second largest cereal crop after maize in terms of annual production with over 738 million metric tons recorded in 2012 (FAOSTAT 2012). Rice is a staple food for more than half the world's population and about 90% of the world's rice is produced and consumed in Asia. In recent years, rice has become an important staple throughout Africa too.

Domesticated rice is designated as either *Oryza sativa* or *Oryza glaberrima*. *O. sativa* is the leading species, grown in Asia, America, Australia and part of Africa, whereas *O. glaberrima* is grown only in West Africa on a small scale.

Rice (*Oryza sativa*) is divided into three subspecies, namely *Indica, Japonica,* and *Javonica*. Of the three, *Indica* is usually grown in tropical climates like India, Vietnam, Thailand, and Southern China, and is the predominant species of rice on more than 80% of rice-producing land (Champagne, 2004). *Indica* rice grains are long and fluffy when cooked. *Japonica* is usually grown in temperate climates like Australia, China, Japan, California, and Egypt. The cooked grains are round, short, and sticky, chewy and moist. *Javonica* is medium-grained and grown in tropical climates. *Indica* and *Japonica* are the most predominant species in the rice industry.

The major forms of rice are rough rice (paddy), brown rice, and milled rice. Rough rice is the unprocessed grain obtained from paddy rice fields, whereas brown rice is rough rice after hull removal. Additionally, removing the underlying bran layers during milling produces milled rice. Milled rice is the most preferred form for consumers due to its superior organoleptic features (appearance, taste, flavor, aroma and texture).

Rice bran is a by-product of rice milling which removes the hull and bran from rough rice. According to FAO (Food and Agriculture Organization of the United Nations), the world's rice milling industry annually produces 63 to 76 million tons of rice bran which is mainly used as animal feed. Rice bran is usually not consumed as human food due to its high insoluble fiber content, possible hull contamination (Luh, 1991) and its tendency to go rancid quickly (Juliano,

1985a). Less than 10% rice bran is heat stabilized and used in value-added processing for health food. Rice bran is rich in protein, lipid, dietary fiber, vitamins, minerals, and antioxidants. It contains 13.2-17.3% protein, 17-22.9% crude fat, 9.5-13.2% crude fiber, 9.2-11.5% ash, 16.1% starch, and 27.6-33.3% dietary fiber (Pomeranz & Ory, 1982). Although rice bran has been mainly used as animal feed, it can be made edible for human consumption with the application of suitable technologies (Saunders, 1990).

The food industry considers cereal bran products from rice and wheat good sources of insoluble dietary fiber (IDF). However, having more soluble dietary fiber (SDF) in the bran is beneficial in both in terms of food production (SDF hydrates and blends well with the food matrix), and bettering terms of conferring human health benefits (gut health, diabetic management, heart health, etc). In food, SDF can affect texture, gelling, thickening, and emulsifying properties. In addition, rice bran also contains bioactive components such as ferulic acid and gamma-oryzanol with antioxidant activities, primarily bound to dietary fiber. Physical and enzymatic treatments may free-up these bound phytochemicals resulting in a better food product.

Therefore, the primary focus of this research is to enhance the SDF content of rice bran and to enhance its free phytochemical content. It is expected that the above treatments would convert a portion of IDF into their soluble form, and thus improve the SDF content of rice bran.

1.2 Thesis objectives and hypotheses

Extrusion and enzyme treatments to rice bran may enhance its soluble dietary fiber content as well as the content of free phytochemicals. Furthermore, the resulting changes in the above composition may enhance the physicochemical, functional, nutritional characteristics.

1. To better understand the compositions in rice bran (native, stabilized, and defatted)

Hypothesis: Rice bran obtained from different sources (BUNGE Milling Inc., RiceBran Technologies, and Riceland Foods Inc.) and rice bran treated with different pre-processing methods will have differences in composition.

2. To optimize a simple water-washing protocol for rice bran in order to enhance the total dietary fiber in this product.

Hypothesis: Water washing will remove some starch, protein, lipid and some water-soluble components, thus enhancing the dietary fiber content in rice bran fiber concentrates.

3. To study the effect of enzymatic treatments on the fiber composition of water washed rice bran products.

Hypothesis: Treatment of rice bran fiber concentrates with Xylanase will degrade/depolymerize the chains of arabinoxylan, thus increasing the soluble content in these products. Xylanase enzymes obtained from different providers will have different effectiveness on rice bran fiber hydrolysis.

4. To study the effect of physical treatment (extrusion) on the fiber composition and functional/physicochemical properties (water holding capacity, lipid binding capacity, etc.) of defatted rice bran products.

Hypothesis: Extrusion cooking may modify the dietary fiber profile - increasing the soluble portion and influencing the water holding capacity of bran products.

5. To study the combined effect of enzyme and physical treatments on the fiber composition of rice bran products.

Hypothesis: A combination of two approaches namely enzyme treatment and extrusion treatment may achieve the dietary fiber modification better than either approach alone.

CHAPTER 2. LITERATURE REVIEW

2.1 Rice production and consumption

Rice is commonly cultivated in more than one hundred countries around the world, with a total harvested area in 2009 of approximately 158 million hectares, producing over 700 million tons of paddy rice and 470 million tons of milled rice annually. Roughly 90% of the rice in the world is grown in Asia (nearly 640 million tons) while sub-Saharan Africa yields about 19 million tons and Latin America accounts for about 25 million tons. Almost all rice in Asia and sub-Sahara Africa is grown on 0.5-3 hectare small farms (IRRI).

Asia is the major producer of rice worldwide. Among the top five rice producing countries, China is the largest producer with nearly 205 million metric tons (MMT), followed by India with 158 MMT, Indonesia with 69 MMT, Bangladesh with 51 MMT, and Vietnam with 44 MMT (**Figure 2.1**). A large amount of rice from these countries is used to feed their large populations. India, Vietnam, and Thailand have been the top rice exporters in recent years with 10.3 MMT, 7.7 MMT, and 6.9 MMT respectively (**Figure 2.2**).

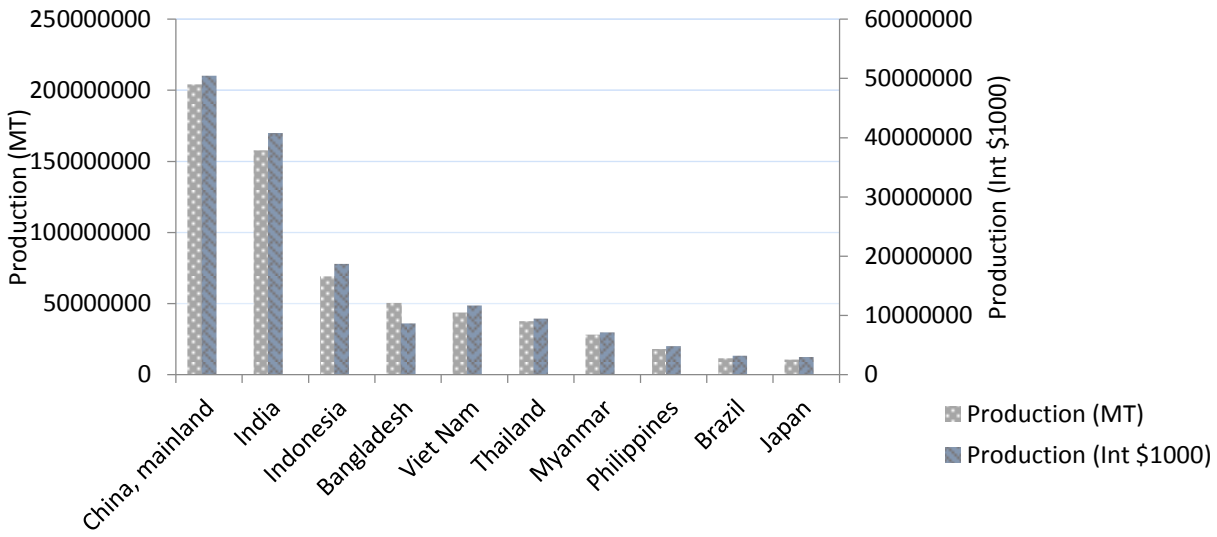


Figure 2.1 Global top 10 rice producers in 2012
Source: FAOSTAT 2012, http://faostat.fao.org/site/339/default.aspx

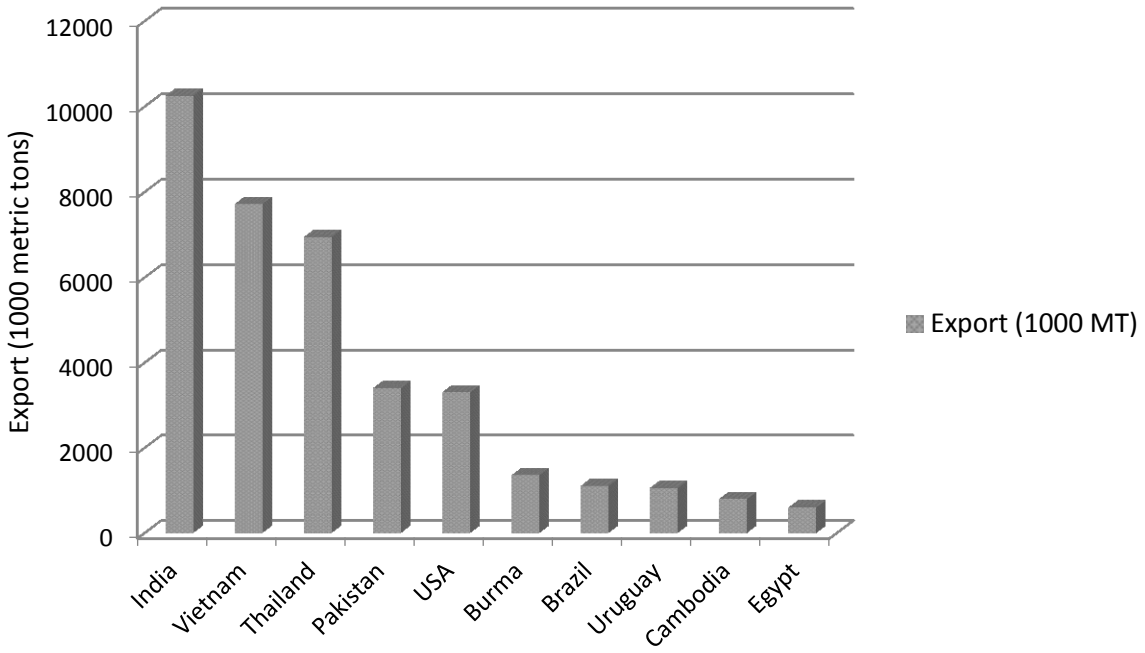


Figure 2.2 Global top 10 rice exporters in 2012
Source: USDA Rice Yearbook 2014. http://www.ers.usda.gov/data-products/rice-yearbook-2014.aspx#.U_0qzvldWSo

World rice production and utilization figures from 2005 to 2014 as recorded by the USDA (the United States Department of Agriculture) are presented in **Figure 2.3**. This period witnessed a steady increase in both production and consumption. Generally, the global rice production met consumption demands during this period of time. In developing and densely populated Asian countries, rice is consumed as the staple food. The data from FAO stated that more than 50% of the global population relies on rice as their main caloric source. Although there is scientific evidence claiming that brown rice offers more health benefits than white milled rice, the latter is predominantly consumed. Not only consumed as a staple food, rice is also utilized in the form of noodles, fermented rice, soups, breakfast cereals, snacks, and puffed rice. Additionally, rice is used in the beer and wine industry as a source of starch.

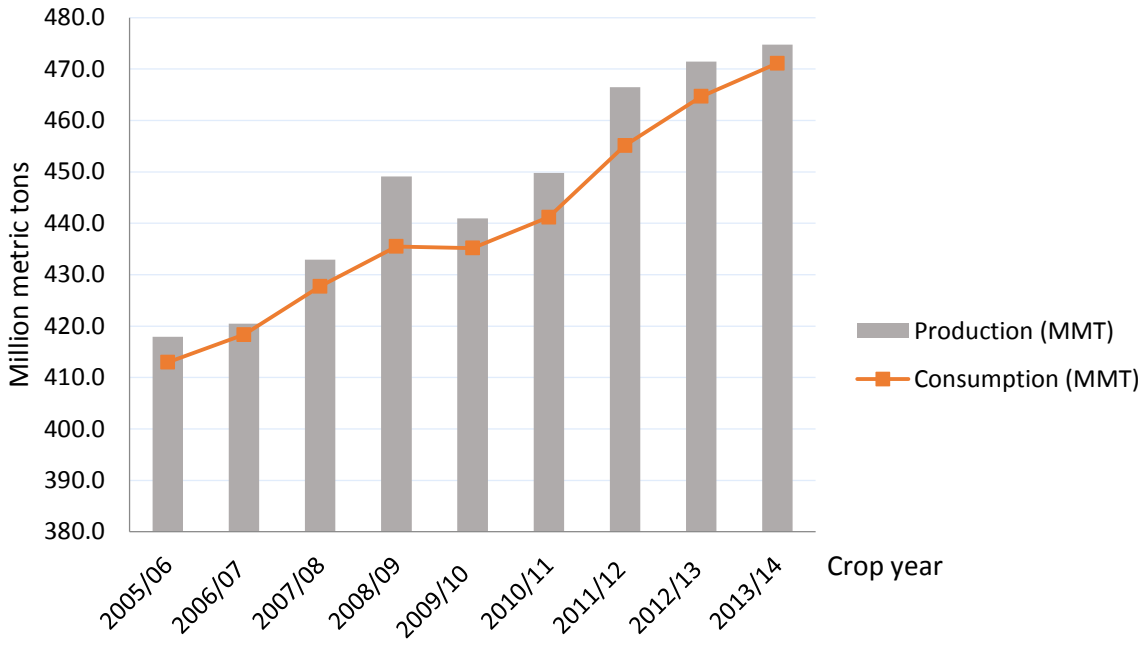


Figure 2.3 World milled rice production and utilization 2005-2014
Source: USDA Rice Yearbook 2014.

Annually, the rice milling industry produces tons of by-products such as rice hulls, rice bran, rice polishings, and broken rice. Rice harvested from paddy fields, is fully wrapped by a tough, fibrous hull. Rough rice grains are dried in order to reduce the moisture for storage and further processing. The first stage of the milling process is removal of the hull, which yields brown rice. The next step is removal of the bran layer, which yields white rice (milled rice). Over 600 million metric tons of paddy rice is milled each year worldwide, generating approximately 382 million metric tons of brown rice, and 337 million metric tons of white rice for human consumption (Kahlon, 2009). Accordingly, global production of 60 to 68 million metric tons of rice bran is available for animal and human use or is discarded as waste.

2.2 Rice anatomy and composition

2.2.1 Rice anatomy

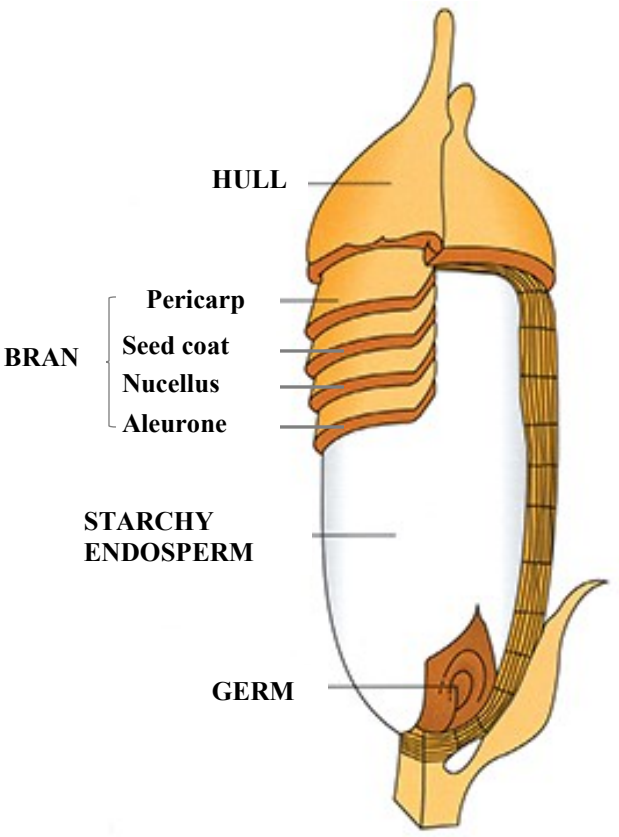


Figure 2.4 Structure of a rice grain
Source: USA Rice Federation http://www.menurice.com/all-about-u-s-rice/how-rice-is-grown/rice-anatomy/

The gross structure of the mature rough rice grain is shown in **Figure 2.4**. Like other cereal grains, the primary parts of the rice grain are the hull, bran, endosperm, and germ of embryo (Juliano, 1985b). The hull (or husk) is an outer coat of the kernel (caryopsis), which functions as a protective layer against insect infestation and unexpected changes in the moisture content of the grain due to humidity fluctuation of the environment (Marshall & Wadsworth, 1994). The hull accounts for 18-20% (weight basis) of the rough rice. It consists of two modified leaves, namely the lemma which covers the dorsal part of the grain and the palea which covers

the ventral portion. The two leaves join together longitudinally. The hull is high in fiber and crude ash but low in protein, starch, and lipid.

Under the hull layer is the bran which is composed of the pericarp, seed coat, nucellus, and aleurone (**Figure 2.4**). The bran accounts for 5-8% (weight basis) of rough rice (Juliano, 1985b). It is the most nutritious part of the caryopsis. The bran contains a great balance of dietary fiber, fat, protein, starch, phytochemicals, vitamins and minerals.

Removal of the bran from brown rice exposes the thin subaleurone layer and starchy endosperm. The milling process which removes the subaleurone layer and a small part of the endosperm is called polishing. This polish fraction consists of 3-4% by weight of brown rice (Juliano, 1985b). The subaleurone layer is rich in protein bodies and contains a small amount of starch granules (Marshall & Wadsworth, 1994). The endosperm comprises a large amount of starch, some protein bodies, and almost no lipid bodies (Marshall & Wadsworth, 1994).

The embryo is situated on one side of the endosperm towards the base of caryopsis. The embryo is not firmly attached to the endosperm, contains a short axis with the plume at its apex bound on the inner side and the root at its base (Tateoka, 1964).

2.2.2 Rice composition

The composition of rough rice and its fractions are subject to varietal, environmental, and processing variability (Champagne, Wood, Juliano, & Bechtel, 2004). **Table 2.1** presents the chemical composition of rough rice, brown rice, and milled rice.

Table 2.1 Composition (%) of rough rice, brown rice, and milled rice

Constituent	Rough rice	Brown rice	Milled rice
Moisture	14	14	14
Starch	53.4	66.4	77.6
Protein (N x 5.95)	5.8-7.7	4.3-18.2	4.5-10.5
Crude fat	1.5-2.3	1.6-2.8	0.3-0.5
Crude fiber	7.2-10.4	0.6-1.0	0.2-0.5
Crude ash	2.9-5.2	1.0-1.5	0.3-0.8
Carbohydrates	64-73	73-87	77-98

Source: Champagne et al. (2004)

2.2.2.1 Starch

Starch is highly concentrated in the endosperm of the rice kernel, and accounts for approximately 78% (wet basis) or 90% (dry basis) of the total milled rice (**Table 2.1**). Starch consists of highly branched amylopectin and essentially linear amylose. In rice, the amylose content is categorized into five classes: waxy (0-2%), very low amylose (3-9%), low amylose (10-19%), intermediate amylose (20-24%), and high amylose (above 24%) (Juliano, 1971). Rice with low amylose content tends to cook tender, sticky, and glossy; whereas rice with high amylose content exhibits high volume expansion and correlates with dry, firm, and fluffy characteristics (Juliano, 1971). The former is usually referred to as Indica (sub-species of rice), whereas the latter is usually referred to as Japonica (Juliano, 1971).

Japonica rice contains more very short chain amylopectin with degree of polymerization (DP) from 6 to 10, and less chains with DP between 13 and 22 than Indica rice (Umemoto, Nakamura, Satoh, & Terashima, 1999). However, these authors did not observe a significant difference in the distribution of longer chains (DP>24) between the two varieties. When measured by differential scanning calorimetry (DSC), the very short amylopectin chain is negatively correlated with gelatinization onset (T_o), peak (T_p) and conclusion (T_c) temperature of rice starch while the longer chains had a positive correlation (Vandeputte, Vermeylen, Geeroms, & Delcour, 2003).

2.2.2.2 Dietary fiber

The dietary fiber content varies between rice varieties. Generally, pigmented varieties contain higher amounts of dietary fiber compared to non-pigmented (Savitha & Singh, 2011). In brown rice, the bran contains 71% of the total dietary fiber while the endosperm consists of 10%, and the polish comprises 19% (Resurreccion, Juliano, & Tanaka, 1979). The three major parts of rice dietary fiber are cellulose, hemicellulose, and lignin. It is reported that nonwaxy brown rice cell-wall preparation contains less hemicellulose than cellulose (23% as appose to 32%), whereas waxy brown rice cell-wall preparation contains more hemicellulose than cellulose (42% as appose to 27%) (Lai, Lu, He, & Chen, 2007).

2.2.2.3 Protein

Generally, brown rice is believed to have the lowest protein content among the common cereals; however, its protein utilization and digestible energy are the highest amongst these (Juliano, 1985b). Rice protein content varies significantly by variety (Juliano & Villareal, 1993). Protein content from *Oryza sativa* varieties ranged from 4.5 to 15.9%, and protein content from *Oryza glaberrima* varieties was between 10.2-15.9%. Asian rice varieties exhibited the greatest protein content compared to varieties from other continents (Australia, America, Europe, Africa) (Juliano & Villareal, 1993). Based on Osborne's protein classification, rice proteins are divided into four groups, namely albumin (water-soluble), globulin (salt-soluble), prolamin (alcohol-soluble), and glutelin (alkali-soluble). In milled rice, albumin (1-3%) and globulin (1-7%) proteins are mainly concentrated at the kernel's surface whereas glutelin (88-93%) and prolamin (1-4%) proteins are distributed in the kernel's center (Houston, Iwasaki, Mohammad, & Chen, 1968). Brown rice protein is comprised of 18.8-20% albumin and globulin, 12.5-14.5% prolamin, and 66.0-67.7% glutelin (Asano, Hirano, Isobe, & Sakurai, 2000).

2.2.2.4 Lipids

Lipids account for 2.3% of brown rice, 18.3% of rice bran, and 6.3% of polished rice (Fujino, 1978). Neutral lipid (storage lipid) is distributed mainly in the bran whereas polar lipid (functional lipid) is predominantly distributed in the endosperm (Fujino, 1978). The common lipid in rice bran is triglycerides, and in endosperm is free fatty acids (Fujino, 1978).

In terms of cellular distribution and association, rice lipids can be classified into nonstarch lipids, predominantly in the bran, and starch lipids in the starch granules of the rice kernel. Non-starch lipids contain 82-91% neutral lipids (of which 73-82% are triglycerides), 7-10% phospholipids and 2-8% glycolipids (Choudhury & Juliano, 1980). The predominant fatty acids in rice starch are linoleic, oleic and palmitic acids (Choudhury & Juliano, 1980). Nonwaxy milled rice has more starch lipid content and less nonstarch lipid content than waxy milled rice (Choudhury & Juliano, 1980). Recent research on rice oil has given much attention to γ-oryzanol, a mixture of steryl and other triterpenyl esters of ferulic acid (4-hydroxy-3-methoxy cinnamic acid) due to its health benefits (Chandrashekar, Kumar, Ramesh, Lokesh, & Krishna, 2014; S. B. Ghatak & S. J. Panchal, 2012; S. B. Ghatak & S. S. Panchal, 2012; Scavariello & Arellano, 1998; Wilson, Nicolosi, Woolfrey, & Kritchevsky, 2007).

2.2.2.5 Vitamins

Vitamins are micronutrients produced by plants. The rice kernel contains little or no vitamin A, C, or D. Therefore, Vitamin A Deficiency (VAD) is a prevalent disease in some Asian countries where their diets are fully dependent on rice (Juliano, 1993). A new rice variety called Golden Rice which is genetically produced to have β-carotene (a precursor of vitamin A) in its endosperm is now available. This modification was considered a strategy to combat VAD and should be viewed as a complement to food fortification and supplementation (Dawe, Robertson, & Unnevehr, 2002).

2.2.2.6 Minerals

Minerals account for 2.9-5.2% of rough rice, 1-1.5% of brown rice, and 0.3-0.8% of milled rice (Champagne et al., 2004b). Rice minerals are more abundant in the outer layers than in the inner portion (Itani, Tamaki, Arai, & Horino, 2002). It is reported that brown rice minerals distributions are 42% in the bran, 26% in the polish, and 32% in the endosperm (Resurreccion et al., 1979). Wang et al. (2011) reported that the six minerals in the brown rice followed the order Mg > Ca > Mn > Zn > Fe > Se. Accordingly, P, Mg, Ca, Mn, and Fe are highly concentrated in the outer layer of the rice kernel, whereas Zn and Se appear to be evenly distributed throughout the caryopsis (Wang et al., 2011).

2.2.2.7 Phytochemicals

Rice (mainly rice bran) phytochemicals include a wide array of bioactive substances such as oryzanol, phytosterols, tocotrienols, squalence, polycosanols, phytic acid, ferulic acid, inositol hexaphosphate etc. (Devi & Arumughan, 2007). Devi and Arumughan (2007) extracted phytochemicals from defatted rice bran with four solvents (ethanol, methanol, ethyl acetate, and hexane), and found that oryzanols, tocols and ferulic acid were present in the various solvent extracts. The extracts contained from 3263 ppm to 7841ppm of oryzanols, 421 ppm to 5782 ppm of ferulic acids, and from 10012 ppm to 55027 ppm of total phenols. These phytochemicals have protective effects on cells against oxidative damage, thus preventing cancers, and cardiovascular and nerve diseases (Kehrer, 1993). However, some of these bioactive compounds are heat labile and are often lost during heat processing treatments (Pascual et al., 2013).

2.3 Rice grain processing

2.3.1 Parboiling rice

Parboiling is a hydro-thermal treatment done on paddy rice, which includes soaking, heating, and drying, in order to promote the milling, nutritional, and organoleptic characteristics in rice. In other words, parboiling means precooking full-hull rice without disturbing its size and shape (Bhattacharya, 2004). As the name implies, parboiled rice is rice that has been “par”-tially “boiled” or partially cooked. Parboiling is thought to have originated from India, but the exact time and how the ancient parboiling was started, still remain unknown. For years, South Asia has been the world’s biggest producer of parboiled rice (Bhattacharya, 2004). The use of parboiled rice appears to have been increasing in recent years due to its nutritional and easy-to-cook properties.

Soaking

The first step in the parboiling process is to hydrate the paddy rice in excess water until it is saturated. The purpose of hydrating the rice is to enable the starch gelatinization of the rice kernel on the subsequent heating step (Bhattacharya, 2004). The rate of moisture migration is dependent on the soaking temperature. Elbert et al. (2001) reported that an increase in soaking temperature increased the time to attain the equilibrium moisture of rice grain. At relatively low

soaking temperatures, the activation energy of diffusion was low. The rigid structure of rice and the intact starch granules prevented the grain from absorbing water. As the soaking temperature increases above 77^{o}C (gelatinization temperature of rice), the activation energy of diffusion increases four-fold, and therefore more water can be absorbed into the rice grain, and thus the diffusion of water is a controlling factor for parboiling. The presence of the husk on paddy rice may be an important barrier in the soaking process as it imparts effective resistance to water absorption (Thakur & Gupta, 2006). However, when the grain moisture exceeds 30-32% (wb) and a temperature above 70^{o}C, the husk splits open, resulting in an upsurge in hydration (Bhattacharya, 2004), this is called over-imbibition and deformation of the grain.

With increasing moisture content, the density of milled rice and brown rice decreases, but that of paddy rice increases paradoxically (Bhattacharya, 2004). This is due to the void space between the endosperm and the husk which enables the endosperm to expand without disturbing the overall grain volume (Bhattacharya, 2004). As the moisture content increases from 13 to 30% moisture, brown rice volume rises around 30%, while rough rice rises only 9%. However, when it reaches 32% moisture, the paddy volume steeply increases (Bhattacharya, 2004).

Bhattacharya (2004) believes that the soaking process is affected by a wide range of variables such as surface area and volume of grain, the texture of husk, tightness of lemma closing, and the gelatinization temperature. He suggests that determination of soaking conditions is more practical than calculation which was applied by many researchers. The method for determining the optimal soaking conditions is to soak paddy rice at varied temperatures for various times, drain it, then steam it for 5-10 minutes, dry, and mill it. If soaking temperatures are below 65^{o}C, then it is not necessary to control the soaking time as the equilibrium moisture content under these conditions does not exceed 30-32% (wb) and therefore no splitting of the husk occurs. However, a long soaking time may cause the rice to ferment and germinate. Ideally soaking should be performed at 70^{o}C or higher and with good control of time.

Heating or steaming

The second step of the parboiling process is heating the soaked paddy rice in order to gelatinize the starch. Bhattacharya and Rao (1966a) suggested that a two-minute steaming time at atmospheric pressure is sufficient for starch gelatinization. It is essential to take the heat input into account as it has a significant impact on the subsequent milling quality (Bhattacharya, 2004). The color of parboiled rice is strongly affected by the severity of heat treatment, time and pressure during steaming (Bhattacharya & Rao, 1966b). Increasing the time and pressure of steaming brought out a significant color-inducing effect making it deeper and darker. This discoloration is mainly due to nonenzymatic Maillard browning of the outer bran layers and the endosperm during steaming (Lamberts, Brijs, Mohamed, Verhelst, & Delcour, 2006). Maillard precursors (reducing sugars and free amino acid from the bran layer) leached out during soaking react together under high temperature to form Maillard yellow or red pigments (Lamberts et al., 2006). In addition, the bran pigments diffuse to the endosperm to contribute to the parboiled rice color (Lamberts et al., 2006). Some heating techniques were applied such as mild heating at 80^{o}C in a closed box soaked in a bath, heating in a closed rotating drum by flue gases in the jacket, or by thermic fluid, by electrical resistance, by ohmic heating, by microwave, and even by hot sand or air (Bhattacharya, 2004).

Drying

The purpose of drying pre-cooked rice is to adjust and lower the moisture content to a suitable level for subsequent processing. After heating and steaming, the moisture content of parboiled rice is usually 35% (wb), which is approximately 16% after drying. At this moisture content, the moisture distribution becomes more uniform (Velupillai & Verma, 1986). When the moisture content of parboiled rice is 15% or lower, rice grain breakage occurs during the subsequent milling process and therefore decreases the quality and the yield of whole grains (Bhattacharya & Swamy, 1967). Rapid drying with hot air may also cause unsatisfactory milling quality, whereas slow drying in the shade enable good head yield (whole kernels of milled rice) (Bhattacharya & Swamy, 1967). The milling breakage did not exceed 2% if the parboiled rice was dried in two passes with a tempering in between (2 hours if hot, 8 hours if at room

temperature) in the moisture range of 15 to 19%, followed by hot conditioning (storage in a heated bottle at 80^{o}C) (Bhattacharya & Swamy, 1967).

2.3.2 Rice milling

The objective of rice milling is to remove the husk, bran, germ and broken kernels. Rice milling consists of several steps in the following sequence 1) cleaning, 2) dehusking, 3) debranning, 4) polishing, and 5) grading or removal of broken grains.

2.3.2.1 Cleaning

Cleaning is the first step in rice milling and is done to remove immature rough rice, unfilled grains, and foreign materials (stones, mud balls, straw, metal, glass, grass, etc.). The differences in size, density, magnetic conductance, frictional force, and optical characteristics of these impurities allow the cleaning process to be performed in different stages (Bond, 2004). First, the wire-meshed drum sieve is used to remove impurities which are dramatically longer than paddy rice. The paddy grains go through the openings of the drum sieve while the longer impurities remain on the sieve and are discharged at the end. Following this, a scalperator (**Figure 2.5**) is used to remove impurities with lower density than paddy rice. A moving column of air blows up lighter materials such as dust, unfilled grains and empty husks separately from the stream of rice. These light materials then settle in an aspiration chamber and exit the machine.

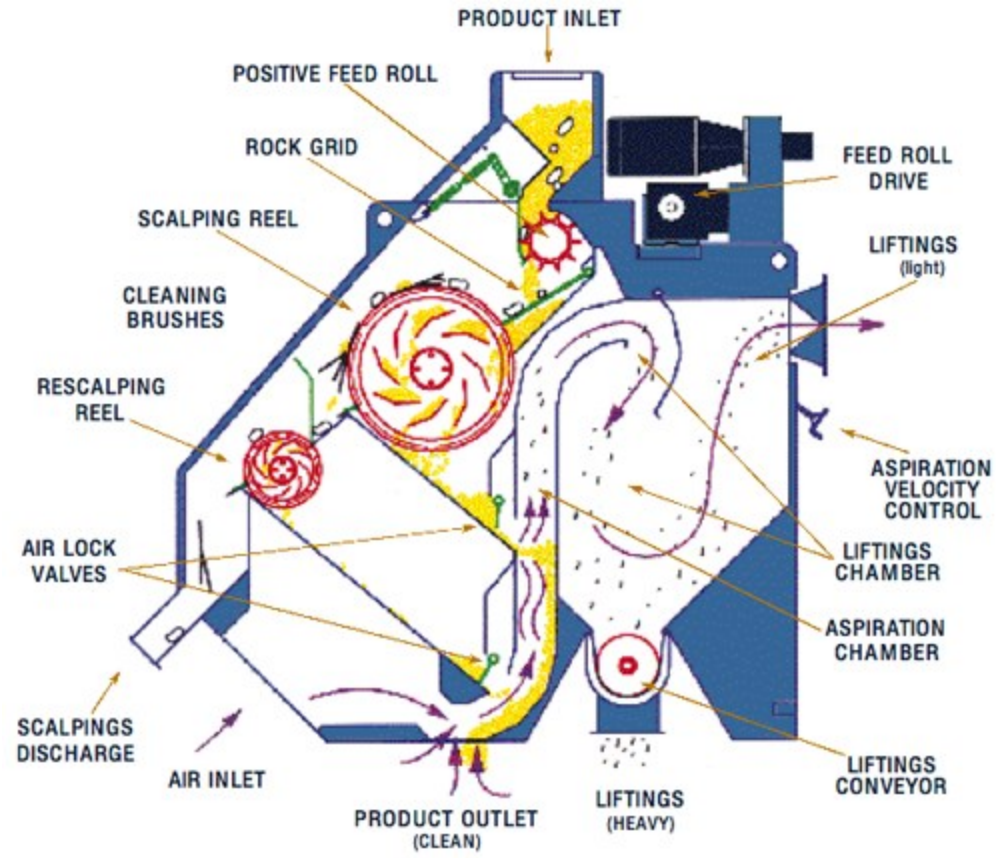


Figure 2.5 The scalperator
(Source: Carter Day International, Minneapolis, MN). www.carterday.com.

The next step in cleaning is to remove slightly larger impurities such as straws, soybeans, large seeds, and in the meantime remove smaller impurities such as sand and small weed seeds. This step is implemented by a paddy cleaner with a series of upper and lower decks.

Lastly other materials such as stones, mud pieces, and glass are eliminated by a de-stoner. The de-stoner also separates these materials from paddy rice based on their density. Rice is flown away from a textured deck surface by air column while dense objects stay on the surface. Metal is usually removed at the final stage of cleaning with the use of magnets.

2.3.2.2 Dehusking

Rice husk is not edible and possibly contaminated with pesticides, therefore it must be eliminated from paddy rice (Arendt & Zannini, 2013). The presence of a void space between the husk and caryopsis allows the grain to be dehusked without abrasion to the pericarp (Razavi &

Farahmandfar, 2008). The husk is usually removed by a machine called rubber-roll hullers. This machine consists of two identical rubber rolls, one rotating clockwise and the other counter clockwise (Arendt & Zannini, 2013), which move at different speeds. This allows the paddy grains to fall between the rolls and encounter a shear action, which cause the husks to be stripped off. Rice breakage is a significant concern during dehusking. The rolling speed, rice variety, and grain humidity are major factors affecting the rice breakage (Arendt & Zannini, 2013). After husking, the detached husks are removed by aspiration due to their lower density as compared to the grains. The husks are thus lifted by the air and settled in a chamber where they are discharged (Bond, 2004).

Although the husking efficiency in commercial practice is high (approximately 90%), the remaining % of paddy grains still remains in the brown rice kernels and must be separated accordingly (Bond, 2004). The paddy grains can be separated from brown rice using a paddy separator. This separator consists of several textured trays which allow heavier brown rice to settle on their surface and then move to the end of the trays. The lighter paddy grains settle on top of the brown rice stream, and move to the other end of the trays due to oscillatory motion (Bond, 2004).

2.3.2.3 Debranning

Debranning is a process in which the bran layers and germ layers are removed from the rice kernel. The bran layers and germ of brown rice contain a significant amount of oil, fiber, and lipase. These components make brown rice spoil easily and non-preferable to rice consumers. Therefore, debranning is in demand to prolong the shelf-life of brown rice and improve its sensory characteristics among consumers. The two commonly used debranning machines are the abrasive cutting type and the friction type (Bond, 2004). The abrasive cutting type uses a coarse surface to break and peel the bran off the brown rice kernel while the friction type uses the pressure and movement between the grains to generate the friction to peel off the bran (Bond, 2004).

2.3.2.4 Polishing

Even after rice has gone through debranning, some bran may still adhere loosely on the surface of rice kernels, and further processing is needed. The purpose of polishing is to remove

the remaining bran from the milled rice. Generally, the polishing machine has a cone shape covered with rolling leather strips which gently brush the kernel surface and remove the adhered bran (Arendt & Zannini, 2013). Polished rice becomes shinier, glossier, and highly preferable in the food market. Some industrial processing plants economically combine debranning and polishing into one machine.

2.3.2.5 Grading/ removal of broken grains

Abrasion and friction during from debranning process inevitably break some grains into small pieces. In addition, some bran and dust particles still remain in the milled rice after debranning and even polishing. During the grading process, broken rice is separated from head milled rice (whole kernel rice) by vibrating sieves (**Figure 2.6**) or rotary cylinders, whereas bran and dust are removed by air aspiration (Arendt & Zannini, 2013). The vibrating grading operator uses a rotary or vibratory motion to expose head rice to a sieving surface. Broken rice or foreign materials fall through the sieve opening and are discharged.

Figure 2.6 Rotary vibrating operator
(Source: Weiicu Integrating Global Trade Leads, China). www.weiku.com.

2.4 Rice bran – composition, health benefits, food applications

2.4.1 Rice bran composition

Rice bran is composed of 4 different layers which are the pericarp, seed coat, nucellus and aleurone. The bran portion accounts for 5-8% of the brown rice weight (Juliano, 1985a). Rice bran contains 17-22.9% crude fat, 13.2-17.3% protein, 9.5-13.2% crude fiber, 9.2-11.5% ash, 16.1% starch, and 27.6-33.3% dietary fiber (Pomeranz & Ory, 1982). Due to the diversity in composition, rice bran is believed to be the most nutritious fraction in rice.

2.4.1.1 Rice bran oil

Rice bran oil (RBO) is the oil which has been extracted from the germ and bran fractions. RBO has been widely produced and used in many Asian countries like Japan, Thailand, India, Korea, Indonesia, and China as industrial oil. Particularly, Thailand exported 35,448 tons of RBO in 2010 (FAOSTAT, 2010). However, in recent years, Western markets have shown growing interest in rice bran oil due to its recognized health benefits (Kim & Godber, 2001). In Japan, the healthy rice bran oil is in constant demand of roughly 80 thousand tons annually (Sugano & Tsuji, 1997). Besides traditional utilization as a vegetable oil, rice oil is also widely used in the pharmaceutical and food industries due to its nutraceutical and therapeutical properties (Prasad, Sanjay, Khatokar, Vismaya, & Swamy, 2011). High levels of antioxidants and phytosterols lead RBO to open its range of uses (Esa, Ling, & Peng, 2013). It is superior to other vegetable oils due to its inclusion of omega-3 and omega-6 fatty acids, especially oryzanol and high amount of unsaponifiables (Krishna, Khatoon, & Babylatha, 2005).

Rice bran consists of 15-22% oil by weight (Orthoefer, 1996). RBO contains around 96% of saponifiables and 4% unsaponifiable matters which include antioxidants and micronutrients. The saponifiable lipids are composed of 68-71% triglycerides (TG), 2-3% diglycerides (DG), 5 - 6% monoglycerides (MG), 2-3% free fatty acids (FFA), 2-3% waxes, 5-7% glycolipids and 3-4% phospholipids (McCaskill & Zhang, 1999). The unsaponifiables are rich in tocopherol, tocotrienol, oryzanol, phytosterols, polyphenols and squalene. RBO has a higher content of unsaponifiable matters than other vegetable oils which have a content of less than 1-2%. RBO is a balanced oil which contains mono-unsaturated and polyunsaturated fatty acids which greatly contribute to its nutritional properties. Moreover, there is a high content of antioxidants in RBO,

which improves the shelf-life compared to other cooking oils. Additionally, RBO has a low viscosity and thus when used to cook, the oil absorbed into food is reduced, reducing the overall caloric value in foods.

The composition of unsaponifiables present in rice bran oil is presented in **Table 2.2**. Of the components found in the unsaponifiable fractions, oryzanol represents about 20% of unsaponifiable matter in RBO (Rong, Ausman, & Nicolosi, 1997). The name oryzanol was chosen when Kaneko and Tsuchiya in 1954 first isolated it in rice bran oil (Oryza sativa L.). According to Seitz in 1989, the most accessible natural source of γ-oryzanol is rice (Seitz, 1989). Oryzanol is a mixture of ferulic acid esters of sterols (campesterol, stigmasterol and β-stigmasterol) and triterpenoid alcohols (cycloartenol, 24-methylenecycloartanol, cyclobranol). It is believed to be the main component responsible for reducing the harmful form of cholesterol – low density lipoprotein (LDL) without reducing the good form of cholesterol – high density lipoprotein (HDL) (Minhajuddin, Beg, & Iqbal, 2005). When oryzanol is extracted and purified, it is a white or slightly yellowish, tasteless crystalline powder with little or no odor and which melts at 137.5-138.5^{o}C (Xu & Godber, 2000). It is insoluble in water, slightly soluble in diethyl ether and n-heptane, and practically soluble in chloroform (Bucci, Magri, Magri, & Marini, 2003).

Approximately 1.7% (v/v) tocotrienol is found in the unsaponifiable matters of RBO (deDeckere & Korver, 1996). The content of tocotrienols in RBO ranged from 72 to 1157 ppm depending on rice bran sources and refining methods. Crude rice bran oils were found to contain 19-46 mg of α-tocopherol per 100 g of oil, 1-3 mg of β-tocoperol, 1-10 mg of γ-tocopherol, and 0.4-0.9 mg of δ-tocopherol, for a total of about 50 mg/100 g (Kanematsu et al., 1983), plus 14-33 mg of α-tocotrienol and 9-69 mg of γ-tocotrienol per 100 g of oil (Tanabe, Yamaoka, & Kato, 1981; Tanabe, Yamaoka, Tanaka, Kato, & Amemiya, 1982). The content and biological activities of tocotrienol are higher than those of tocopherols (Qureshi, Sami, Salser, & Khan, 2001). It is reported that approximately 1% (v/v) of the unsaponifiable fraction of RBO is α-tocopherol. HPLC analysis of RBO showed that 1 g of RBO contains 3.02 mg of α-tocopherol (Qureshi, Mo, Packer, & Peterson, 2000). The major forms of tocopherols in RBO are α-tocopherol (5,7,8-trimethyltocol), γ-tocopherol (7,8-dimethyltocol) and δ-tocopherol (8-methyltocol) (Xu & Godber, 1999).

Tocopherols and tocotrienols known as tocols collectively make up vitamin E. Those components cannot be synthesized in the human and animal bodies and primarily come from plants. Tocopherols and tocotrienols differ by the number and positions of methyl groups and the fused chromonol ring, and the absence and presence of the three double bonds in the isoprenoid side chain. Tocotrienol has 3 double bonds at the 3', 7' and 11' positions of the hydrocarbon tail. These bonds offer tocotrienols greater fluidity which makes it easier for the body to incorporate them into cell membranes (Yap, Yuen, & Wong, 2001). Two novel tocotrienols d-P21-T3 (desmethyl tocotrienol) and d-P25-T3 (didesmethyl tocotrienol) have been isolated from stabilized rice bran (Qureshi et al., 2000).

Table 2.2 Composition of unsaponifiables present in rice bran oil

Sterol	**%**
Plant sterol	43
Campesterol	-
Stigmasterol	-
β-sitosterol	-
Triterpene Alcohols	28
24-Methylene Cycloartenol	-
Cycloartenol	-
Aliphatic alcohols, hydrocarbons	19
4-Methyl Sterols	10

Source: Itoh, Tamura, and Matsumot (1973)

Crude RBO has higher levels of non-TGs compared to other vegetable oils; however, these non-TGs are mostly removed during the refining processes. The losses in chemical refining are 2.5 - 3 times the FFA content of the crude oil (Van Hoed et al., 2006).

The free fatty acids (FFAs) in RBO include unsaturated fatty acids such as oleic acid (38.4%), linoleic acid (34.4%), and linolenic acid (2.2%), and saturated fatty acids such as palmitic acid (21.5%) and stearic acid (2.9%) (Rukmini & Raghuram, 1991a). Krishna et al. (2001) and Chang and Huang (1998) reported that the saturated, monounstaturated and polyunsaturated fatty acid ratio in RBO is roughly 1:2.2:1.5. Due mainly to high amount of

linoleic acid, RBO is known as the hypocholesterolemic vegetable oil in both animals and humans.

Waxes are also found in the RBO as part of the saponifiables. Waxes are esters of long-chain fatty acids and alcohols. Rice wax comprises about 15% alkanes, 35% esters, 10% aldehydes, and 40% long chain primary alcohols (Bianchi, Lupotto, & Russo, 1979). Waxes tend to form stable emulsions during oil refining and thus increase oil losses during processing (Mishra, Gopalakrishna, & Prabhakar, 1988). Generally, the higher the extraction temperature, the greater the quantity of wax removed from the bran when hexane is used as the solvent. Waxes have low iodine values, high melting points (82-84^{o}C), and have been classified into hard and soft fractions. The hard fraction contains fatty alcohols such as C-24, C-26, and C-30, saturated fatty acids such as C-22, C-24 and C-26, and normal alkanes of C-29 and C-31. Soft fraction comprises C-24 and C-30 alcohols, C-16 and C-24 saturated fatty acids, and C-21 and C-29 normal alkanes (Nicolosi & Rogers, 1993).

2.4.1.2 Rice bran protein

The protein content in rice bran is about 10-15% and it consists of water-soluble (albumin), salt-soluble (globulin), alcohol-soluble (prolamin), and alkali-soluble (glutelin) fractions. The content of these protein fractions in rice bran varies among rice varieties. For example, it was reported that one Japanese rice variety displayed a protein composition of 37% albumin, 31% globulin, 2% prolamin, and 27% glutelin (Fabian & Ju, 2011). Hamada (1997) reported that proteins from the defatted brans of USA representative rice cultivars contained 34% albumin, 15% globulin, 6% prolamin, and 11% acid-soluble glutelin.

Albumins

Rice bran albumins account for 2-6% of the total seed protein and about 37% of the rice bran protein (Fabian & Ju, 2011). Like other albumins, they are readily soluble in water due to sufficient net charge and the deficiency of extensive disulfide cross-linking or aggregation (Hamada, 1997). It is reported that relative molecular weight (MW) of endosperm rice albumins range from 10 to 200 kDa (Iwasaki, Shibuya, Suzuki, & Chikubu, 1982) whereas MW of rice bran albumins are 100 kDa (Hamada, 1997).

Amongst storage proteins in rice bran, albumins are believed to give the greatest nutritional value, since they are readily absorbed and utilized by the body (Mawal, Mawal, & Ranjekar, 1987). One study showed that a 16-kDa rice albumin may have antioxidant activity by preventing Cu^{2+} from inducing LDL oxidation (Wei, Nguyen, Kim, & Sok, 2007). The antioxidant activity of rice albumins is similar to that of serum albumin, and the reason might be that rice albumins have N-terminal amino acid sequences being homologous to that of serum albumin (Nakase et al., 1996).

Isolation of pure albumin from rice bran is still challenging work because there is always a certain amount of salt co-extracted with albumin and globulin. As a result, to obtain pure albumin for special applications and to determine its properties, complicated purification steps such as repetitive precipitation, ultracentrifugation, and dialysis are required (Hamada, 1997; Iwasaki et al., 1982; Mawal et al., 1987).

Globulins

Globulins which are rich in sulfur account for about 31% of the storage protein in rice bran. They are readily soluble in salt solution. Rice globulins have varying sizes of polypeptide chains, linked together by inter-chain disulfide bonds (Hamada, 1997). By using SDS-PAGE Krishnan, White, and Pueppke (1992) specified the 25 kDa and the 16 kDa polypeptides. The 25 kDa fraction is the major polypeptide of rice globulin (Krishnan et al., 1992; Pan & Reeck, 1988). Rice bran globulin fraction has the highest antioxidant activity compared to other fractions (Chanput, Theerakulkait, & Nakai, 2009). Of all antioxidative peptides isolated from rice bran globulin protein, 19 peptides composed 6-30 amino acid residues with molecular weight from 670-3,611 Da, in which Tyr-leu-Ala-Gly-Met-Asn sequence showed the strongest antioxidative properties (Adebiyi, Adebiyi, Yamashita, Ogawa, & Muramoto, 2009).

Prolamins

Rice bran prolamins are the least sizeable fraction among storage proteins (Adebiyi, Adebiyi, Hasegawa, Ogawa, & Muramoto, 2009). They are readily soluble in 60-70% aqueous ethanol and almost insoluble in water, but easily soluble in acids or alkali (Fabian & Ju, 2011). Molecular weight of rice prolamin polypeptides is about 10-53 kDa (Adebiyi, Adebiyi,

Hasegawa, et al., 2009). Those polypeptides are classified into two major groups, namely 15.5 and 14.2 kDa. Both groups have similar amino acid composition with high levels of glutamic acid, alanine, glycine, and arginine, and low levels of lysine and histidine (Shyur, Wen, & Chen, 1994). Generally, rice prolamins are not of interest for extraction due to their low prevalence.

Glutelin

Glutelins, which are soluble in alkali, constitute about 11-27% of the total protein in rice bran. The glutelin bodies have fine ultrastructure resulting from the accumulation of different classes of proteins, and have a complex internal organization. Rice glutelins are comprised of high MW proteins ranging from 45 to 150 kDa when extracted with 0.1 M NaOH, to prevent hydrolysis. If using a stronger alkali solution, there will be two groups of glutelins obtained, namely α-polypeptides with MW of 34-39 kDa and β-polypeptides with MW of 21-23 kDa (Krishnan et al., 1992).

2.4.1.3 Rice bran dietary fiber

Dietary fiber is the edible parts of plants or analogous carbohydrates which are resistant to digestion and absorption in the human small intestine and are partially fermentable in the large intestine. It is not hydrolyzed by enzymes secreted by the human digestive system but can be digested by micro flora in the gut. Dietary fiber consists of cellulose, hemicellulose, pectins, gums, lignins, and resistant starch. These components are classified into two groups based on their solubility in water. The natural gel-forming fibers like pectins, gums, and part of hemicelluloses are soluble in water whereas the structural or matrix fibers like lignins, cellulose, and some hemicelluloses are insoluble. FDA has accepted three health claims related to dietary fiber intake and reduced risk of heart disease and cancer: 1) the reduced risk of cancer claim for fiber containing grain products, fruits, and vegetables (FDA 1993a), 2) the reduced risk of coronary heart disease (CHD) claim for fruits, vegetables, and grain products that contain fiber, in particular soluble fiber (FDA, 1993b), and 3) soluble fiber from certain foods and risk of coronary heart disease (FDA, 2008).

Rice bran is an excellent source of dietary fiber ranging from 20 to 51% depending on the product (Saunders, 1990). Rice bran fiber consists of mostly insoluble fractions, and 7-13% soluble fractions (Anderson, Deakins, Floore, Smith, & Whitis, 1990). Defatted rice bran

contains 27% cellulose, 37% hemicellulose, and 5% lignin (Hernandez, 2005). The bran hemicellulose consisted mainly of highly branched arabinoxylan and xyloglucan. Arabinoxylans consists predominantly of the pentoses arabinose and xylose, and are therefore classified as pentosans. The acidic arabinoxylan component in rice bran appears to have more doubly-branched xylose residues in the main chain and also more complicated side chains than the endosperm arabinoxylan. Xyloglucan was also isolated from the crude hemicellulose but the amount of β-(1,3),(1,4) glucan was very small compared to the endosperm hemicellulose (Shibuya, Nakane, Yasui, Tanaka, & Iwasaki, 1985). Glucose, arabinose, xylose and galactose were the main monosaccharides found in rice bran hemicelluloses (Gremli & Juliano, 1970; Mod, Conkerton, Ory, & Normand, 1979; Shibuya & Iwasaki, 1985).

2.4.2 Rice bran health benefits

2.4.2.1 Rice bran oil and phytochemicals

Effects of fatty acids.

Rice bran oil contains approximately 76% unsaturated fatty acids which are broken down as 38.4% oleic acids, 34.4% linoleic acids and 2.2% linolenic acids. Saturated fatty acids make up 24% of the total fatty acids, which include 21.5% palmitic acid and 2.9% stearic acids. Numerous studies have demonstrated that diets enriched in saturated fatty acids lead to increased serum total cholesterol and low density lipoprotein cholesterol (LDL-C) levels, whereas diets enriched in unsaturated fatty acids lead to lowered serum LDL-C. The mechanism associated with the hypocholesterolemic action of the unsaturated fatty acids are not well understood, even though studies (Kuo, Rudd, Nicolosi, & Loscalzo, 1989; Nicolosi et al., 1990; Spady & Dietschy, 1985, 1988) would suggest that unsaturated fatty acids prevent the down-regulation of the LDL receptor normally observed during intakes of saturated fat and cholesterol.

Effects of unsaponifiable components

The minor components of rice bran oil such as gamma oryzanol, phytosterol, and other phytosterol conjugates are believed to have antioxidant properties against free radicals which deactivate the natural by-products of oxidative metabolism (Nakayama, Manabe, Suzuki,

Sakamoto, & Inagaki, 1987; Rukmini & Raghuram, 1991b; Sakamoto, Tabata, Shirasaki, Inagaki, & Nakayama, 1987)

Gamma oryzanol has been proven to be a multifunctional nutraceutical due to its antioxidant properties (Xu, Hua, & Godber, 2001), and its ability to lower cholesterol (Akihisa et al., 2000; Xu et al., 2001), reduce cholesterol absorption (Lloyd, Siebenmorgen, & Beers, 2000), increase HDL cholesterol (Cicero & Gaddi, 2001), retard platelet aggregation (Seetharamaiah, Krishnakantha, & Chandrasekhara, 1990), and inhibit tumor promotion (Kim, Kang, Nam, & Friedman, 2012). A supplementation of oryzanol to the RBO-containing diet led to a substantial decrease in the serum cholesterol of rats (Seetharamaiah & Chandrasekhara, 1989). In this study, oryzanol extracted from rice bran was added to the rat diets in crystalline form varying amounts to find out the optimal dosage. Rats were randomly fed diets which were enriched with 1% cholesterol, 0.15% bile salts, and either with 0.2, 0.5, 1.0 and 2.0% oryzanol or no oryzanol. A control group was provided with a cholesterol-free diet. The result stated that 0.5%-oryzanol-fed animals showed the lowest cholesterol levels compared to the other groups. In a similar study, 0.5% oryzanol was enriched with 1% cholesterol in diets, compared to 10% refined rice bran oil containing traces of oryzanol. The oryzanol-supplemented diet was associated with lower total cholesterol levels (Seetharamaiah & Chandrasekhara, 1989).

Another report also indicated a beneficial effect when replacing regular cooking oil with RBO for 15-30 days, which led to considerable reductions in the total cholesterol and triglycerides in 12 hypercholesterolemic and hypertriglyceridemic subjects. The cholesterol-lowering effect of γ-oryzanol was described in hyperlipidemic patients who were given 300 mg per day γ-oryzanol for three months (Cicero & Gaddi, 2001). Futhermore, the addition of a 60 g mixture of rice bran oil and safflower oil at the ratio of 70:30 to the diets of 10 female subjects for seven days resulted in lowering total cholesterol as compared to either of the oils alone (Suzuki & Oshima, 1970a, 1970b).

Sakamoto et al. (1987) investigated the hypolipidemic effects of γ-oryzanol (OZ) and cycloartenol ferulic acid ester (CAF) on the hyperlipidemia of male Sprague-Dawley rats. The rats were fed a high cholesterol diet and were then were given a daily dose of OZ and CAF at 10 mg/kg for 6-12 days administered either orally or intravenously. The results showed that

intravenous administration of OZ and CAF significantly inhibited the increase in total serum cholesterol, phospholipid, and free cholesterol in the rats. It was concluded that intravenous administration of OZ and CAF may increase the excretion of lipids in the blood.

Another study regarding the antioxidant effectiveness of microencapsulated γ-oryzanol was conducted on high cholesterol-fed Sprague-Dawley rats (Suh, Yoo, Chang, & Lee, 2005). The levels of total serum and liver cholesterol and LDL cholesterol in the blood samples of these rats were significantly decreased, and HDL cholesterol increased. Not only that, the results also indicated that the microencapsulated γ-oryzanol could effectively protect the lipids and cholesterol from heat-induced oxidation. Therefore, microencapsulation is a promising technique to protect the antioxidant properties of γ-oryzanol from heat-induced lipid oxidation.

In a comparison between the hypolipidemic effect of γ-oryzanol and that of ferulic acid, (two major unsaponifiables in RBO), results showed that γ-oryzanol has a greater cholesterol-lowering activity (Wilson et al., 2007). In this study, hamsters were divided into 4 groups and fed a hypercholesterolemic diet (HCD) containing coconut oil, RBO, oryzanol, and ferulic acid. After 10 weeks, the plasma total cholesterol and low density lipoproteins significantly decreased in the RBO-, oryzanol-, ferulic acid-fed rats; the greatest decrease was seen in oryzanol feed. It was also found that the oryzanol-fed hamsters excreted significantly more coprostenol and cholesterol in their feces than the ferulic acid-fed hamsters. However, ferulic acid may have higher antioxidant capacity because it maintains vitamin E levels better than RBO and oryzanol.

Other unsaponifiable fractions in RBO such as cycloartenol (CA) and 24-methylene cycloartenol also play a role in lowering cholesterol levels. A study was conducted by Rukmini and Raghuram (1991a) to test the hypolipidemic effect on hypercholesterolemic rats. The rats were fed CA and 24-methylene cycloartanol in amounts present in RBO for 8 weeks. The results indicated that CA significantly reduced cholesterol and triglyceride levels. It was explained that the accumulation of CA in the rat liver may have inhibited cholesterol esterase activity, which in turn resulted in lowering circulating cholesterol levels. It is known that CA has a similar structure to cholesterol and therefore the ability to compete with the binding sites of cholesterol and its metabolized derivatives.

Vitamin E which includes α-tocopherol, α-tocotrienol, γ-tocopherol and γ-tocotrienol also contributes to the hypocholesterolemic effect of rice bran. All components in vitamin E and oryzanol exhibited remarkable antioxidant activity in the inhibition of cholesterol oxidation (Xu et al., 2001). Even though γ-oryzanol has higher activity than each component in vitamin E, due to its higher content in rice bran, the role of vitamin E as an inhibitor of cholesterol oxidation is essential and worth consideration. Tocotrienols in rice bran has greater antioxidant activity than tocopherols (Qureshi et al., 2000).

- Mechanism of hypocholesterolemic action

Numerous research studies have shown that gamma oryzanol can lower the cholesterol levels in blood and reduce the risk of coronary heart disease. There are several mechanisms by which unsaponifiable matters improve the serum biochemical profile. Such mechanisms include interrupting the absorption of intestinal cholesterol rather than increasing the excretion of fat and neutral sterols (Kahlon, Chow, Chiu, Hudson, & Sayre, 1996; Nagao et al., 2001) and increasing fecal steroid excretion by interfering with choleterol absorption (Ikeda, Nakashimayoshida, & Sugano, 1985; Sharma & Rukmini, 1986). The findings suggest that the hypocholesterolemic activity of gamma-oryzanol is due in part to impaired apical uptake of cholesterol into enterocytes and perhaps a decrease in HMG-CoA reductase activity (Makynen, Chitchumroonchokchai, Adisakwattana, Failla, & Ariyapitipun, 2012); or in some cases, inhibition of lipid metabolism (Sakamoto et al., 1987).

2.4.2.2 Rice bran protein

Due to its high quality, rice bran protein represents a great potential for food and nutraceutical applications. The unique property of rice bran protein is its hypoallergenic and anticancer activities (Fabian & Ju, 2011; Kawamura & Ishikawa, 1993; Shoji et al., 2001). In Kawamura and Ishikawa (1993) study, rice bran protein extracted by alkali solution with a molecular weight greater than 0.5 kDa showed lethal activity against 3T3 transformed cells but no significant effect on normal cells. Moreover, Shoji et al. (2001) indicated that a 57-kDa rice bran protein has the potential of an anti-cancer agent since its interruption of cancer cell adhesion is related to the prevention of growth, invasion and metastasis. Thus, rice bran protein is

considered safe in infant food formulas or potential immunotherapy of individuals suffering from rice-induced oral or inhalant immediate hypersensitivity.

Rice protein is believed to have the highest nutritional value, as compared to other cereal grains, owing to its high content of essential amino acids such as lysine and threonine which are generally deficient in cereals (Mawal et al., 1987; Shih, 2004). Even according to (Wang, Hettiarachchy, Qi, Burks, & Siebenmorgen, 1999), the amino acids in rice bran protein were better than casein and soy protein isolates in accomplishing the amino acid requirement for 2-5 year old children.

2.4.2.3 Dietary fiber

Rice bran contains significant amounts of dietary fiber. Rice bran can be used as a dietary fiber source when stabilized (Randall et al., 1985). Several studies suggested that the hypocholesterolemic effect of rice bran is contributed by dietary fiber (Saunders, 1990). One of the active constituents of dietary fiber is a water soluble polysaccharide fraction. Mod et al. (1978; 1979) isolated and chemically characterized the water- and alkali-soluble hemicelluloses from rice bran. It has been reported that hemicellulose B preparation isolated from defatted rice bran had the potential to scavenge cholesterol and bile acid (Hu & Yu, 2013). Hemicellulose is the complex mixture of polysaccharides that can be extracted from most plant cell walls with dilute alkali (Aspinall, 1959). Hemicellulose was fractionated into hemicellulose A and hemicellulose B. Hemicellulose A is precipitated from the extract on neutralization while hemicellulose B is precipitated on the addition of ethanol to the neutralized solution (Southgate, 1977).

Like most beta-linked fibers, rice bran fiber is not digested by human intestinal enzymes and not expected to be absorbed completely. Rice bran increases the viscosity of the gastrointestinal contents (Dikeman, Murphy, & Fahey, 2006), which attenuates blood glucose and lipid concentrations. Rice bran can be fermented by the colonic microflora to produce acetate, propionate, and butyrate which promote colon health. Rice bran fiber also has fecal bulking effects to promote intestinal regularity (Miyoshi, Okuda, Oi, & Koishi, 1986; Tomlin & Read, 1988). Symptoms of fiber deficiency such as constipation can be improved by consuming rice bran fiber.

Accumulating evidence indicates that the intake of dietary fiber brings out numerous beneficial effects against diseases such as gastrointestinal disease, cardiovascular diseases, lowering blood cholesterol, diabetes and colon cancer (Brown, Rosner, Willett, & Sacks, 1999; Park & Floch, 2007; Tabatabai & Li, 2000; Theuwissen & Mensink, 2008; Zeng, Lazarova, & Bordonaro, 2014). Soluble fibers are supposed to act like a sponge, and absorb water in the intestine. They blend the food with water forming a gel and thereby slow down the rate of digestion and absorption (Abdul-Hamid & Luan, 2000). In general, 1 g of soluble fiber can reduce total cholesterol by around 0.045 mmol/L, and reduce the risk of coronary heart disease by 29% for each daily 10 g intake (Brown et al., 1999; Rimm et al., 1996). Insoluble fiber is effective in promoting the feeling of fullness, stool mass, bulk, and reducing constipation. Grains are excellent sources of insoluble fiber, while fruits, vegetables, legumes are good sources of soluble fiber (Dreher, 2001).

The hypolipidemic effect of dietary fiber extracted from rice bran has been investigated by numerous research studies. Topping et al. (1990) researched the effect of rice bran, wheat bran dietary fiber and fish oil on the lipid mechanism of male adult rats. They suggested that the combination of rice bran plus fish oil appears to have more beneficial effects on lipid metabolism than wheat bran plus fish oil. Plasma and hepatic triacyglycerols and hepatic lipogenesis and cholesterol were reduced significantly by fishoil- and ricebran-based diets. Hepatic low density lipoproteiṇ (LDL) receptor activity was considerably lower by feeding rice bran.

The effects of rice bran fiber on laxation

The dietary fiber content of rice bran varies depending on the degree of milling or on the amount of starch in the bran. Stabilized rice bran contains approximately 20-25% dietary fiber and 2% soluble fiber. It is generally accepted that even though soluble dietary fibers are efficient cholesterol lowering components, they have little effect on laxation which is expressed as the increase in stool weight. (Tomlin & Read, 1988) reported that rice bran was more likely to increase the stool weight and stool frequency than wheat bran, but both had comparable hastening effects on the transit time. The mechanism of the effective stool bulking from rice bran might be explained due to a high content of retrograded starch.

The hypolipidemic effects of rice bran

A research study conducted by Kestin et al. (1990) compared the effects of dietary fiber from wheat bran, rice bran and oat bran on lowering serum lipids, blood pressure, and improving glucose metabolism. Twenty four mildly hypercholesterolemic men had diets enriched with the three cereal bran dietary fiber at 11.8 g/day for 4 weeks. The results indicated that wheat and rice bran showed little effect on plasma cholesterol while oat bran lowered significantly total cholesterol, and mainly reduced LDL cholesterol. Rice bran- and oat bran- based diets significantly increased HDL cholesterol and lowered the concentration of plasma triglycerides more than wheat bran and oat bran. However, there were no apparent differences in blood pressure between the groups of baseline-fed diets and the three cereal rice bran dietary fiber diets. The results also suggested that the hypocholesterolemic effect of rice bran is not only caused by functional components in the rice bran oil, but also by the rice bran dietary fiber.

Anticarcinogenic activies of rice bran fiber

Many clinical studies have been conducted to demonstrate the anticarcinogenic effect of rice bran. Aoe et al. (1993) reported that soluble rice bran hemicellulose (RBH) may prevent 1,2-dimethylhydrazine (DMH)-induced large bowel carcinogenesis in Fischer 344 rats. Rats were fed a baseline diet or a diet containing 2% or 4% RBH. A week later, the rats were given an injection of DMH at weekly intervals for 20 weeks and were stopped giving the injection for 7 weeks before being autopsied. The results showed that the incidence and number of colon tumors per rat were both significantly lower in rats fed RBH.

Similarly, Verschoyle et al. (2007) reported that rice bran interfered with the development of tumors in tumor-linked glycoprotein. Genetic mice that were fed rice bran showed significantly reduced numbers of intestinal adenomas and hemorrhages compared to low-fiber diet and no-fiber diet fed mice. This suggests that the fibrous constituents of the rice bran inhibit carcinogenesis. Other studies have also reported anticarcinogenic properties of rice bran (Cai et al., 2004; Fan, Morioka, & Ito, 2000; Ghoneum & Gollapudi, 2005; Katayama et al., 2003; Kong et al., 2009; Luo, Li, Yu, Badger, & Fang, 2004; Miyoshi et al., 2001; Nam et al., 2005; Norazalina, Norhaizan, Hairuszah, & Norashareena, 2010).

2.4.3 Food applications of rice bran

Rice bran has been increasingly applicable in the food industry due to its health-promoting properties. A few studies endeavored to evaluate rice bran protein as a food ingredient in order to enhance quality and nutrition. Khan et al. (2011) enriched weaning food with rice bran protein isolates through drum drying and the pregelatinized starchy ingredients. The formulation had good organoleptic quality and met standards for supplementary infant foods. This enriched weaning food could substantially contribute to the daily essential amino acid requirements. In another study, rice bran protein concentrate was enriched into bread by replacing 10% of wheat flour. The protein content of enriched bread (16.5%) was found to have no negative effects on the sensory quality (Sadawarte, Sawate, Pawar, & Machewad, 2007). Moreover, rice bran protein hydrolysates may be used as nutritional supplements, functional ingredients, flavor enhancers, coffee whiteners, cosmetics, personal care products, confection products, and beverages (Fabian & Ju, 2011).

γ-oryzanol is a major bioactive compound of rice bran oil (RBO), which accounts for approximately 1.5% of crude RBO (Manjula & Subramanian, 2008). Due to its strong antioxidant properties, γ-oryzanol has been used widely in foods, nutraceuticals, pharmaceuticals, and cosmetics. Beef patties with added γ-oryzanol exhibited higher oxidative stability during 4^{o}C storage than control beef patties. γ-oryzanol-containing beef had the lowest TBARS values (Thiobarbituric acid reactive substances, a by-product of lipid peroxidation), WOF scores (warmed-over flavor scores, a rancid flavor), C7-oxidized cholesterol derivatives, hydroperoxide and hexanal concentrations (Kim, Suh, Yang, & Lee, 2003).

Rice bran oil has higher thermal and oxidative stability than sunflower oil, hence it can be a preferred replacement for deep-fat frying, baking, and storage. An blend of 60% rice bran oil and 40% sunflower oil showed good thermal stability during repeated deep frying of potato chips, had lower cost, and higher storage stability (Sharma, Kaur, Sarkar, & Singh, 2006). Rice bran oil was also mixed with soybean oil to reduce lipid peroxidation in a fried dough with rice flour during storage (Chotimarkorn & Silalai, 2008). Winterized rice bran oil may be used to make salad dressing and mayonnaise, while hydrogenated rice bran oil is suitable for specialty shortening and margarine formulations (Orthoefer & Eastman, 2004).

Rice bran can be used as a fiber ingredient in food and beverage formulations even though there are some limitations such as mouth-feel. Over the past few years, numerous new fiber ingredients have been developed to improve mouth feel and functionality such as water holding. Rice bran fiber has been expected to become a replacement for conventional fiber ingredients in populations who have deficient fiber sources. Hemicellulose B, which is more highly branched and more soluble than hemicellulose A, has been reported to have many biological activities including lowering blood cholesterol and preventing colon cancer (Hu & Yu, 2013). Thus its potential in functional food applications has been researched and developed. Hu et al. (2009) added defatted rice bran hemicellulose B to bread and observed the chemical and functional properties of bread. The outcome indicated that defatted hemicellulose B of rice bran had high water- and fat-binding and swelling capacity, but low viscosity. The addition of hemicellulose B of defatted rice bran at 1%, 2%, and 3% decreased the loaf volume significantly and increased the firmness of bread, while remaining at an acceptable level.

Similarly, enrichment with dietary fiber from stabilized bran flour (SRBF) to pizzas was observed (de Delahaye, Jimenez, & Perez, 2005). The sensory properties of pizzas were not significantly affected when adding 5% of SRBF. During sixty-day storage at -18^{o}C, the content of dietary fiber was increased if the enrichment level increased, while the starch content was decreased. However, the water absorption and stability of pizzas decreased.

One of the rising concerns regarding the consumption of rice bran fiber is its safety. However, recently it has been accepted as a GRAS ingredient (Generally Recognized as Safe) by the FDA. It was determined to have natural biological origin, and nutrient properties without known detrimental effects and health hazards. Moreover, increased intake of dietary fiber has been recommended by the USDA Dietary Guidelines Committee, and rice bran fiber is an excellent source of dietary fiber.

2.5 Rice bran processing

2.5.1 Stabilization and oil extraction

2.5.1.1 Stabilization

The instability of rice bran during storage is its greatest limitation to its use as a food ingredient. The milling process activates lipase, an enzyme endogenously present in the bran or produced by microbes. The lipases break the oil into free fatty acids which are easily oxidized to form rancidity, causing bad smell, bitter taste and subsequently making it unsuitable for consumption. Therefore, it is necessary to process the food material through stabilization techniques in order to inhibit or destroy the lipase activity, and reduce oil losses which directly degrade into free fatty acids. There are a variety of techniques available such as cold storage, drying, steaming, chemical treatments, and expelling which are used to reduce the instability of rice bran. Rice bran stabilized by extrusion cooking can be stored up to one year at less than 22^{o}C in gas-permeable packaging, and the recommended storage life is expected to be 3 to 4 months (Carroll, 1990; Randall et al., 1985). Well-stabilized rice bran can be a good source of essential protein, unsaturated fatty acid, tocopherol, tocotrienol, oryzanol, and phenolic compounds.

Heat treatments are commercially the most commonly used stabilization method. However, heat methods may lower valuable components in bran, remove substantial moisture, and may be unable to completely inactivate enzymes. Parboiling is another method which leads to bran stabilization by destroying lipase activity (Nasirullah, Krishnamurthy, & Nagaraja, 1989). Therefore, extrusion cooking has been developed to reduce the loss during heating. This method can denature lipases permanently (Ramezanzadeh et al., 1999), and the bran can be safely stored up to 4 months in contrast to dry heat methods (Carroll, 1990; Randall et al., 1985).

Microwave heating has brought many advantages and improvement for rice bran stabilization. Microwave energy is an inexpensive, but efficient source of heat in comparison with other thermal treatments. Microwaving may reduce the detrimental effects on the nutritional value and bran color. Water must be added to the bran to obtain a 21% or more moisture content before microwave heating, and the process may take 3 minutes to denature lipase enzymes (Tao, Rao, & Liuzzo, 1993).

In a recent study, ohmic heating was used to stabilize rice bran and to improve rice bran oil extraction yield as compared to microwave heating (Lakkakula, Lima, & Walker, 2004). Ohmic or electric heating occurs when there is an alternating current passing through a food sample, and heat is generated by the sample's electrical resistance. Both microwave heating and ohmic heating are effective methods to stabilize rice bran, but they normally require the addition of moisture to the sample before the treatment. Ohmic heating increased the extracted total lipid to a maximum of 92% compared to 53% of total lipids extracted from the control samples.

Prabhakar and Venkatesh (1986) also developed an acid-stabilized method for rice bran based on the principle that lipases act slowly at low pH. These authors used hydrochloric acid to treat the rice bran by sprinkling or spraying the acid. The bran feed quality does not seem to be affected by the acid treatment, and the stabilized bran storage life may be about 3 months without mold growth. Acid and heat may be combined together to extend the storage time of rice bran. A 0.1-2.0% acetic acid was added to parboiled rice bran to maintain the bran stability for at least 6 months at ambient conditions (Tao, 2001).

2.5.1.2 Oil extraction and purification

Generally, it is difficult to produce rice bran oil (RBO) due to its high FFA, waxes, bran fines and pigment content. These factors lead to high refining losses. Therefore, lowering refining loss in RBO processing has received attention from oil researchers.

A solvent extraction process using hexane is the most commonly used method in RBO extraction (Gastrock, Vix, Aquin, Graci, & Spadaro, 1955). In this solvent extraction method, the miscella which is the mixture of extracted oil and solvent contains 70-75% (w/v) solvent content. The obtained crude oil consists of more than 80% triglycerides along with various impurities such as waxes, gums, FFA, and pigments. The impurities cause poor color and haziness in the oil appearance. They may be catalysts of poisoning, and may cause a slow rate of hydrogenation if the oil is used for making hydrogenated shortenings. Therefore, they must be removed by refining process before the oil become edible. Another technique for oil extraction is using supercritical carbon dioxide extraction. This method produces RBO with lighter color, lower phosphorous content, waxes and free fatty acid, and more essential fatty acids and oryzanol (Kuk & Dowd, 1998).

The primary steps in the RBO processing are filtration of bran fines, degumming, dewaxing, deacidification, bleaching, and deodorization.

a) Fines removal

Crude rice bran oil contains about 0.5% bran fines (Orthoefer & Eastman, 2004) Removal of the fines before degumming is necessary to obtain better oil quality and yield. The removal may be implemented by self-opening separator or filtration of crude oil at ambient temperature (Ghosh, 2007).

b) Degumming

RBO extracted from a solvent process contains significant amounts of gums and other mucilaginous matters which deposit in the storage tanks. They usually exist in combination with oryzanol, thus increasing refining losses by emulsifying considerable amounts of neutral oil which are lost during the soap stock. There are different degumming methods available, including water degumming, acid degumming, super and TOP degumming (Dijkstra & Opstal, 1989), surface-active compound degumming such as lauryl sulfate or sodium oleate (Bhattacharyya, Chakrabarty, Vaidyanathan, & Bhattacharyya, 1983). Enzymatic degumming has been so far probably the best process for reducing the phosphorous content of crude oil (Roy, Rao, & Prasad, 2002). Phospholipase A2 was used to catalyze the non-hydratable phosphatides (gums) into hydratable lysophospholipids which were then removed by centrifugation. This process produced no color deterioration of degummed oil in comparison to the conventional degumming process as it was implemented at low temperature. In addition, the oil content of the gums from enzymatic degumming is only 25-30% compared to 50-60% in the conventional one. More importantly, the oryzanol content in crude RBO remains almost intact during the enzymatic degumming process.

c) Dewaxing

Removal of waxes in RBO can be done with or without additives. The conventional method is to heat the RBO to around 90^{o}C to destroy existing crystals and then cool with stirring to around 20^{o}C before allow it to mature for 4 hours. Waxes are removed by plate and frame filtration. Dewaxed oil is likely to become cloudy in cold storage (Ghosh, 2007).

The addition of additives like calcium chloride to RBO is also a method to separate waxes from oil. This method may remove about 60% of wax from the oil (Ghosh, 2007). Haraldsson (1983) reported that dewaxing of RBO may be achieved by keeping the refined oil at low temperature in the presence of soap stock before centrifugation. Another way is to cool the oil to 8^{o}C then add 5% water and a small amount of sodium lauryl sulfate, and agitate for four hours to disperse the wax crystals in the water phase. The mixture is then separated by centrifugation.

Dewaxing of the RBO miscella phase is also described. Rice bran miscella is chilled in a compartment fitted with a 1-10 rpm stirrer to form wax crystals, which are then separated by centrifugation. This process can remove over 90% of RBO waxes (Cavanagh, 1976). It is more suitable for large solvent refining plants than smaller ones.

Dewaxing of RBO is also possible by using acetone as a solvent in which the oil is soluble but the wax is insoluble (De & Bhattacharyya, 1998). This method is done by one of two ways. The RBO waxes are extracted from the settled waste of the RBO tank, or a solvent is added to the oil phase to deposit the wax content and then filtered.

Dewaxing can be done simultaneously with degumming. The process uses water and an aqueous solution of $CaCl_2$, followed by low temperature crystallization of gums and waxes together, followed by centrifugation (Rajam, Soban Kumar, Sundaresan, & Arumughan, 2005). This process is more economical due to the elimination of one step from the whole process.

d) De-acidification (refining)

De-acidification is one of the most difficult steps of rice bran oil production due to its FFA, wax, and unsaponifiable matters. A conventional refining process uses alkali after degumming and/or dewaxing, relying on the end use of the oil. However, a problem associated with this process is the high losses during refining (Aryusuk, Puengtham, Lilitchan, Jeyashoke, & Krisnangkura, 2008).

Alkali refining

Alkali refining normally leads not only to oil loss, but also to loss in the nutritional components present in RBO. These high losses may be explained in different ways. One of the

explanations is that the foots or soap formed from crude RBO tend to emulsify the oil under the refining conditions (Cousins, Prachankadee, & Bhodhiprasart, 1955). Another explanation is due to that the presence of saponins (Hartman & Dosreis, 1976) but there is no concrete evidence to support this statement. In a recent study (Singh & Singh, 2009), the FFA from degummed RBO was significantly reduced by re-esterifying it with glycerol. It is recommended to use a weak aqueous solution of alkali along with an indicator to monitor the pH value during the neutralization process to reduce losses (Ichimatsu and Ichimatsu, 1995; Hidaka and Tsuchiya, 2000).

Miscella refining

Miscella refining is a deacidification method normally used for RBO containing high FFA. A miscella refining process involved hexane and alkali solution showed its efficiency in extent of deacidification, refining loss and color (Bhattacharyya, Majumdar, & Bhattacharyya, 1986). Miscella refining produces refined oil with lower refining loss, lighter color, and eliminates the need for water washing of the refined oil or miscella (Bhattacharyya et al., 1986; Cavanagh, 1976). In this process, the extracted miscella can be directly degummed, dewaxed and refined without desolventization. The miscella refining is suggested to be done at the solvent extracting plant as soon as possible after the oil is extracted from the source material. A miscella with 40-58% oil content (w/v) is most likely processed in miscella refining plant. The cost of the equipment is higher than a conventional refining plant and control of the process is more difficult (Bhattacharyya et al., 1986).

Mixed solvent refining

The most appropriate refining method for high FFA RBO is the mixed solvent process using hexane as the primary solvent and ethanol or isopropanol as the secondary solvent. Bhattacharyya et al. (1987) was patented the refining of high FFA RBO by a mixed solvent alone or by mixed solvent extraction followed by alkali neutralization.

Steam refining

Steam refining of high FFA oils has been applied in Europe for years. Steam refining not only reduces refining loss but also does not affect micronutrients in RBO, especially oryzanol,

and eliminate the soap production (Kim, Kim, Cheigh, & Yoon, 1985). It also eliminates the environmental problems which alkali and solvent refining bring about. However, acid value and color of steamed refined oil were not as good as those of caustic refined oil (Kim et al., 1985).

Re-esterification of FFA

A new approach for high FFA RBO refining includes re-esterification of FFA with glycerol after degumming and dewaxing. Bhattacharyya and Bhattacharyya (1989) used a fungal lipase enzyme from *Mucor miehei* to esterify the FFA in degummed, dewaxed RBO with glycerol in order to obtain the refined oil. The result was very encouraging. The enzyme was used to synthesize triglycerides from fatty acids and glycerol, thus de-acidifying high FFA RBO. This is called biorefining process.

e) Bleaching

RBO always contains chlorophyll, carotenoids, and the oxidized products of tocopherols and metallic salts of fatty acids. Therefore, bleaching is done to remove these components from the RBO. Bleaching is generally done after degumming, dewaxing, and deacidification, but if the RBO is treated with stream refining, then it should be applied immediately after degumming. The bleaching should be done before any alkali treatment because chlorophyll in RBO tends to be stabilized by alkali and heat, thus it becomes very hard to remove (Cowan, 1976). Earth bleaching under high vacuum and high temperature (around 110^{o}C) is commonly used. In addition to removing pigments by the ion exchange properties of bleaching, earth bleaching helps reduce the amount of oxidation in the products.

f) De-odorization

Deodorization of degummed, dewaxed and deacidified RBO is performed in the typical way used for other vegetable oils (Cowan, 1976). In general, conditions of deodorization involve a temperature between 200 and 220^{o}C and a pressure of 6-10 mmHg. If RBO is processed with steam refining due to high FFA content, then the deodorization is done simultaneously with deacidification. In this case, the temperature and vacuum used in steam refining is higher (around 250^{o}C and 1-3 mmHg).

2.5.2 Dietary fiber extraction

Soluble dietary fiber extraction

Rice bran soluble dietary fiber (RBSDF) is more applicable than its insoluble counterpart in food use. Aoe et al. (1993) extracted RBSDF from defatted rice bran using alkali treatment and acid hydrolysis methods . They removed starch from defatted rice bran (DRB) by digesting it with glucoamylase, then recovered the residue by filtration, followed by water washing and air drying the residue. The starch-free residue was then blended in a colloid mill with alkali or acid solutions, and extracted by shaking for 4 hours at 60^{o}C. After extraction, the extract was centrifuged and neutralized with acetic acid or sodium hydroxide. The neutralized supernatant was centrifuged again before being dialyzed under running tap water for 3 days to remove contaminants. After dialysis, the extract was precipitated with 95% ethanol and collected by centrifugation and then freeze-dried.

They found that the soluble dietary fiber extracted from alkali solutions consisted mainly of arabinose and xylose with a Ara/Xyl ratio of 1.0:1.1. However, for soluble fibers extracted from hydrochloric acid, the ratio of arabinose to xylose was lower, 1.0:1.0. RBSDF extracted with calcium hydroxide had the lightest color, while that extracted with sodium hydroxide had the darkest color. $Ca(OH)_2$ gave a desirable composition and yield of RBSDF, plus hypocholesterolemic properties. Consequently, calcium hydroxide appeared to be appropriate for rice bran soluble dietary fiber extraction.

A recent study by Wan et al. (2014) followed up the work of Aoe et al. (1993) with the objective of finding the optimum RBSDF extraction conditions using response surface methodology . They investigated the influential factors of the extraction such as ratio of $Ca(OH)_2$ solution to defatted rice bran, concentration of $Ca(OH)_2$, and extraction temperature on the yield of RBSDF. They observed that the highest yield of RBSDF was obtained at a ratio of 3% $Ca(OH)_2$ to defatted rice bran 29.75:1 (mL/g). The optimum extraction time was 1 hour stirring and the temperature was 84^{o}C.

Hemicellulose is the major component of soluble dietary fiber in rice. Dating back to 1970, the extraction process of water-soluble hemicelluloses from milled rice has been

conducted by Cartano and Juliano (Cartano & Juliano, 1970). Defatted rice flour was blended with water and mechanically stirred for 1 hour at 4^{o}C. The suspension was centrifuged, and the supernatant was collected. The residue was re-blended with water and rigorously stirred, and then centrifuged. The combined supernatants were heated at 90oC for 3 minutes and cooled down before filtered by Celite-aid filtration. The filtrate was dialyzed with distilled water at 4^{o}C for 6 days and lyophilized to a white powder.

Insoluble dietary fiber extraction and fractionation

Insoluble dietary fiber (IDF) from rice bran has not received as much attention as soluble dietary fiber (SDF) in the food industry. However, it is essential to isolate and fractionate IDF compounds into individual parts as some of the physiological effects of fiber rely on these individual components (Claye, Idouraine, & Weber, 1996). Therefore, Claye et al. (1996) reported a procedure of extraction and fractionation of several cereal by-products including rice bran (**Figure 2.7**). Briefly, defatted rice bran samples were first depleted of starch and protein by using amyloglucosidase and trypsin enzymes respectively. The next step was removing pectic substances by using 0.5% (w/v) ammonium oxalate solution at 85% for 2 hours. This depectinated residue was dried for further extraction of hemicellulose, cellulose, and lignin. The residue was mixed with 5% potassium hydroxide, flushed with nitrogen, shaken for 24h, and then centrifuged. The supernatant which contained hemicellulose was collected for the next separation, and the residue which contains lignocellulose was collected for further fractionation. The hemicellulose-containing supernatant was treated with 50% acetic acid to adjust the pH to 5.0-5.5 and centrifuged. The residue from centrifugation was hemicellulose A, while the supernatant was precipitated with 95% ethanol to obtain hemicellulose B. Cellulose was extracted from the lignocellulose-containing residue part by applying buffered potassium permanganate ($KMnO_4$) as a delignifying agent. Lignin was obtained by hydrolyzing the lignocellulose-containing residue portion with 72% H_2SO_4 for 30 hours.

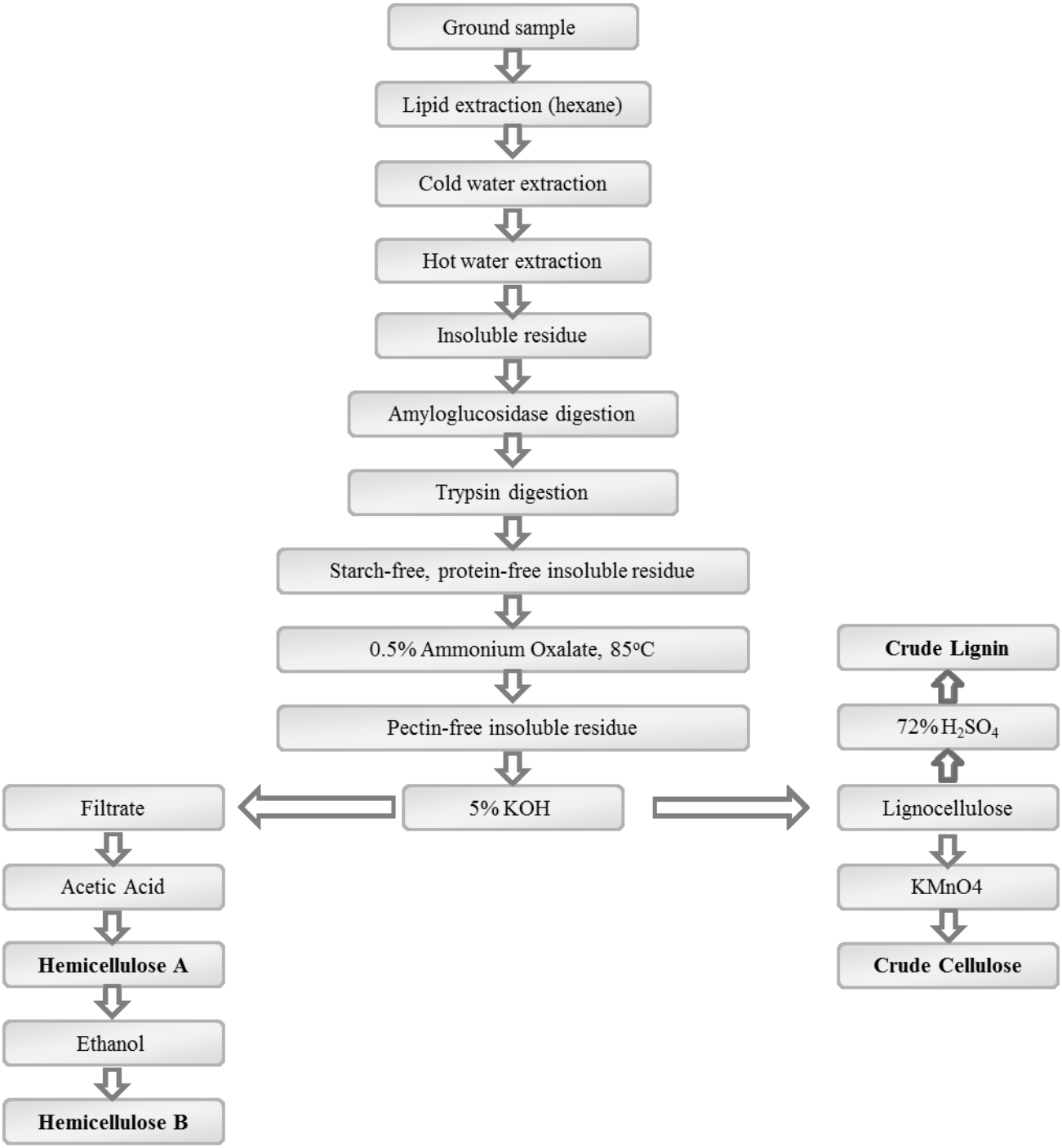


Figure 2.7 Extraction and fractionation of dietary fiber from cereals (Claye et al., 1996)

2.5.3 Xylanase treatments on rice bran

Dietary fiber (DF) is one of the most important components of cereals due to its health benefits. Rice bran is rich in DF, with arabinoxylans (AX) being the main non-cellulose polysaccharide present. AX is a polymer of xylose backbone with arabinose substitutions. The hydrolysis of AX with AX degrading enzymes produces arabinoxylan oligosaccharides (AXOS) and free sugars. AXOS has recently been receiving increased interest from researchers due to its health-promoting properties.

Generally, there are two groups of xylanases, namely endo-xylanase (endo-β-(1,4)-D-xylanase and β-(1,4)-xylosidase) and exo-xylanase. Endo-β-(1,4)-D-Xylanases (EC 3.2.1.8) are the most important arabinoxylan degrading enzymes. They cleave the internal β-(1,4) bonds between xylose in the main chain of the cereal cell wall arabinoxylans (**Figure 2.8**) and have a significant effect on the end-products. Most xylanases are produced by fungi and bacteria although they can also be found in plants, insects, snails, crustaceans, marine algae, and protozoa (Dornez, Gebruers, Delcour, & Courtin, 2009).

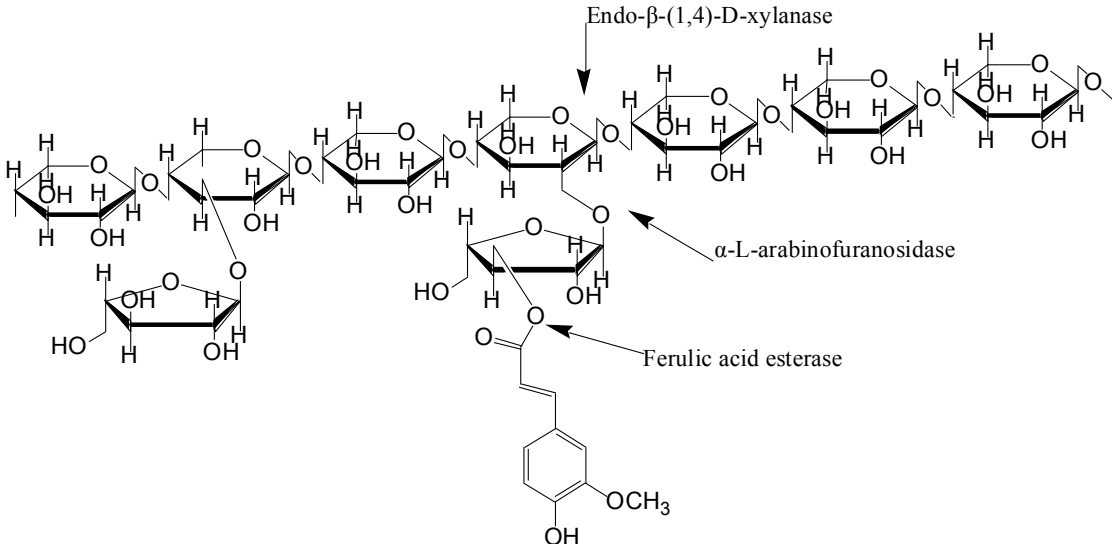


Figure 2.8 Structure of Arabinoxylan (AX) and the action of xylanolytic enzymes

Recently, Lebesi and Tzia (2012) conducted an endoxylanase treatment to rice bran in order to improve its properties and a cake's nutritional and quality characteristics. The ground rice bran (35%) was slurried with distilled water (65%) and treated with endoxylanase (70 ppm – 700 ppm) for 30 minutes at 40^{o}C and pH 5.5. The slurry was then added to the cake batter. They

found that the water binding and holding capacities of treated rice bran were decreased when its soluble dietary fiber content increased. Batter enriched with xylanase-treated rice bran showed increased viscosity, gelatinization temperature, volume and porosity. The cake's crumb firmness and water activity were reduced.

Rice bran xylooligosaccharides (XO) obtained by enzymatic treatment were compared to other cereals and millet brans (wheat, maize, and ragi) in terms of their yield, composition, and antioxidant activity (Veenashri & Muralikrishna, 2011). The procedure included extraction of water-insoluble polysaccharides, starch removal, and enzymatic treatment. The bran (100 g) was dispersed with water (700 mL) at room temperature before centrifugation (3000 xg for 20 minutes). The residue was dried by a solvent exchange method with ethanol (70%, 80%, and 90%), methanol and diethyl ether. The dried water-insoluble polysaccharides were then treated with termamyl and glucoamylase to eliminate the associated starch. Starch-free water-insoluble polysaccharides (1 g) were treated with xylanase (100 mg, 250 U) in phosphate buffer (50 mL, pH6.0, 0.1 M) at 50^{o}C for 4 hours. The digesta was centrifuged to collect the supernatant followed by precipitating with 3 volumes of ethanol. High molecular weight polysaccharides were precipitated in ethanol while xylooligosaccharides (DP 2-10) were still present in the ethanol. The xylooligosaccharide-containing supernatant was concentrated and stored at 4^{o}C. Among four brans, rice bran had the lowest yield of xylooligosaccharides (3.31%) while wheat bran had the highest yield (40%). The ratio of arabinose to xylose was 3.6:1, 1:2.43, 1:5.13, and 1:1.25 for rice, ragi, wheat and maize respectively. This result indicated that rice bran AX chain was highly substituted with arabinose as opposed to the other three counterparts. The antioxidant coefficient of rice bran xilooligosaccharides was found to be better than that of wheat, ragi, and maize as determined by the β-carotene emulsion assay (Veenashri & Muralikrishna, 2011).

To optimize the breakdown of arabinoxylans, a combination of xylanases is necessary. Kormelink and Voragen (1993) investigated the degradation of rice bran AX by combining xylan-degrading enzymes (endo-β-(1,4)-D-xylanase, β-(1,4)-xylosidase, β-(1,4)-D-arabinoxylan arabinofuranohydrolase (AXH), and acetyl xylan esterase (AE)). They also compared AX degradation of rice bran with that of wheat flour, oat spelt and wood. Enzyme concentration was 0.1 μg/mL each, and incubation conditions were at 30^{o}C for 1 or 24 hours. They found that rice bran AX was the highest branched in comparison with wheat, oat, and wood AX due to the

highest amount of arabinose. Rice bran AX contained both O-2 or O-3 single-branched xylopyranosyl residues and O-2 and O-3 double-branched xylopyranoyl residues. The high degree of branching hindered some actions of endo-xylanase and β-xylosidase on rice bran AX, while promoting the action of AXH. The combination of the four enzymes resulted in the highest extent of hydrolysis after 24 hours while releasing arabinoxylan-oligosaccharides.

2.5.4 Extrusion

Extrusion cooking is a popular and important technique used in food processing, especially for the cereal-based products (Vasanthan, Jiang, Yeung, & Li, 2002). Extrusion cooking is a thermal process that involves the application of high heat, high pressure, and shear forces to a raw material (Riha, Hwang, Karwe, Hartman, & Ho, 1996). The extrusion of cereal-based products has advantages over other physical processing methods because of low cost, short time, high productivity, versatility, energy saving, and unique product shape (Faraj, Vasanthan, & Hoover, 2004). The high pressure and high temperature used in the process result in the alteration of physical, chemical, structural, and nutritional properties of the extruded products. Nutritional effects of extrusion cooking depend on various factors, including the type of extruder, process parameters and screw combination (Björck & Asp, 1983).

There are two types of extruders: single-screw and twin-screw (co-rotating and counter-rotating) (Yacu, 2011). Twin-screw extruders have more advantages than single-screw extruders in terms of feeding, mixing, heat transfer, residence time distribution, displacement transport, and pumping performance. A scheme of a twin-screw extruder is presented in **Figure 2.9**. The basic configuration of an extruder includes a feed delivery system, tempering or preconditioning phase, extruder barrel components, and different die configurations.

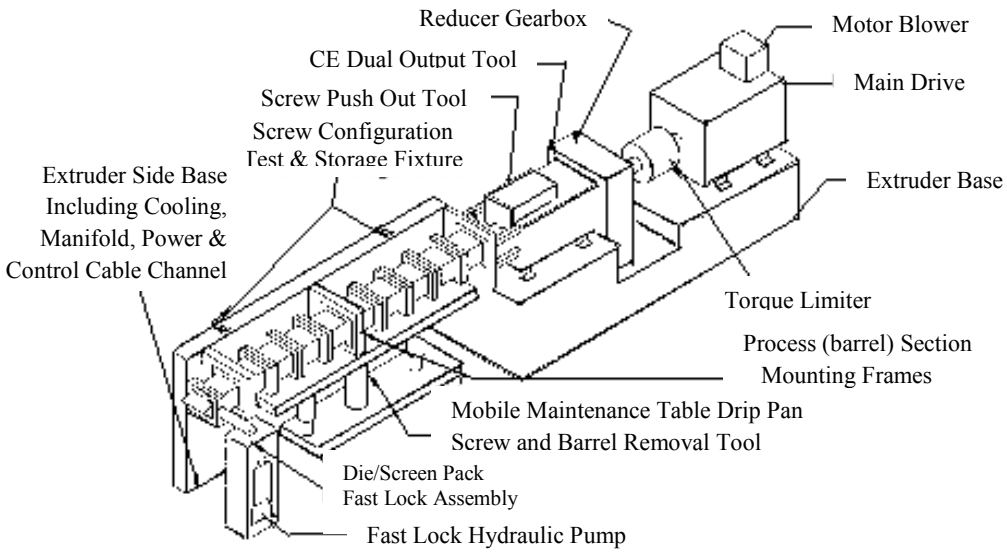


Figure 2.9 A scheme of twin screw extruder
(Source: Extruder Technologies, Inc., NJ, USA). Http://extrudertechnologies.cdmeyer.net/

Numerous research studies have been done on effects of extrusion cooking on the composition of cereals or cereal by-products. Bjorck et al. (1984) reported that extrusion cooking could increase the soluble dietary fiber content (SDF) in wheat flour by 10-35% (Bjorck, Nyman, & Asp, 1984). Extruded barley flour exhibited the same trend in SDF content (including resistant starch) as reported by Vasanthan et al. (2002). The increase from 5.6 to 7.2% in SDF was explained by the transformation of some insoluble dietary fiber (IDF) into SDF during extrusion and the formation of additional SDF by transglycosidation (Vasanthan et al., 2002). A previous study by Ostergard et al. (1989) also investigated the changes in barley flour fiber after extrusion. They found an increase from 13 to 18% of SDF upon extrusion cooking, and the decrease of starch content due to the formation of resistant starch RS3 (Ostergard, Bjorck, & Vainionpaa, 1989).

In terms of physico-chemical properties of extruded products, extrusion cooking has a significant influence on the improvement of water solubility (WS), water uptake (WU), water/fat binding capacity (WBC/FBC), swelling capacity (SC), cation exchange capacity (CEC), and glucose retardation capacity or the delay of glucose adsorption (GRC). Ralet et al. (1990) indicated that extruded wheat bran had an increase from 20 to 40% in WS along with an 8 to 16% increase in SDF. WU increased from 270 g to 375 g/100 g at a low intensity of extrusion, slightly increased at an intermediate intensity, and gradually decreased at severe intensity. It was

explained that a moderate extrusion treatment could disrupt the structures of wheat bran, generating pores for water to enter. Therefore, a drastic extrusion cooking could result in collapsed structures and therefore lower water uptake rate. Extruded wheat bran had a weak ion-exchange probably due to the solubility of some charged molecules and phytic acid-containing materials (Ralet et al., 1990).

Several research studies have been done on the compositional, physico-chemical, structural, and nutritional properties of rice and rice bran. Most recently, Daou and Zhang (2012) conducted a research work on the physico-chemical properties of physically treated rice bran dietary fiber. They carried out the extrusion treatment using a twin-screw laboratory extruder with barrel temperature 50-75-95-100^{o}C (feed end to die), a screw speed of 90 rpm and a moisture content of 18%. The solubility and viscosity of the extruded rice bran fiber significantly increased upon extrusion. They explained that the increase in solubility was related to the reduced particle sizes of the treated samples. The increase in viscosity was mainly due to the increase in soluble dietary fiber and the smaller particle sizes. The WBC of the extruded fiber in neutral and alkali pH marginally increased due to the mild conditions used. These allowed the fiber structure to open up for water to penetrate and bind with the free hydroxyl group of cellulose. They did not observe a significant difference in SC between extruded and untreated fibers. The changes in CEC were found to be insignificant upon extrusion treatment, and therefore did not influence the mineral bioavailability in the human gastro-intestinal tract. In addition, the GRC of extruded rice bran fiber was very high after 30 minutes of staying in gastrointestinal conditions. This indicated that the modified fiber remarkably delayed the glucose diffusion across the dialysis membrane, and therefore delayed the glucose absorption. However, between 60 minutes and 6 hours, the glucose retention capacity of fiber was reduced due to the saturation process.

Gualberto et al. (1997) also investigated the effects of extrusion cooking on the dietary fiber profiles of cereals brans from rice, wheat, and oat (Gualberto, Bergman, Kazemzadeh, & Weber, 1997). These authors used a twin-screw extruder with varied screw speed (225, 305, and 450 rpm), barrel temperature at 162^{o}C, and feed rate at 150 lb/h. Their results showed that protein and ash contents were not influenced by extrusion, while fat content was reduced. Fat reduction was probably due to the volatilization of some fatty acids under high temperature, and

partially due to the formation of starch-lipid or protein-lipid complexes which are not determined by the lipid assay using hexane. Insoluble dietary fiber (IDF) content in all brans decreased whereas soluble dietary fiber (SDF) increased dramatically. The screw speed was found to have an effect on IDF, with the more extreme screw speed causing a lower IDF reduction. There are several ways to explain the decrease in IDF and increase in SDF during a physical treatment such as extrusion cooking. Extruder shear force is able to break the chemical bonds in IDF macro-molecules transforming them into smaller molecules which are then soluble. The pressure inside the extruder is higher at lower screw speed, and the higher pressure may have a higher effect on the solubility of IDF than the shear rate during high-performance extrusion. In addition, resistant starch which is categorized as a soluble dietary fiber could be formed during extrusion due to the different temperatures used in the extrusion barrel. Additionally, some complexes probably formed between polysaccharides and lipid, such as starch-lipid or protein-lipid matrices, which are neither hydrolyzed by starch-degrading and protein-degrading enzymes nor extracted with lipid-extraction solvent. Therefore these complexes are finally determined as IDF. However, at a very severe screw speed condition (450 rpm), there was a decrease in SDF content compared to less severe ones. The authors explained that the shear stress generated by high screw speed might have degraded SDF composition to smaller particles which could adhere to larger IDF molecules. This study also indicated that extrusion treatment did not affect the phytate content which is usually associated with fiber and minerals. Nevertheless, a reduction in phytate content was observed after extrusion in previous studies (Alonso, Rubio, Muzquiz, & Marzo, 2001; Andersson, Hedlund, Jonsson, & Svensson, 1981; Fairweathertait, Portwood, Symss, Eagles, & Minski, 1989; Lombardiboccia, Dilullo, & Carnovale, 1991; Ummadi, Chenoweth, & Uebersax, 1995). It is reported that extrusion cooking could hydrolyze phytate to liberate phosphate molecules which constituted part of the total inositol phosphates in extruded legumes and therefore improve the availability of minerals after extrusion (Alonso et al., 2001).

2.6 Future potential for rice bran and commercial prospective

As described in the preceding discussion, rice bran offers high nutritional value from its components such as oil, protein, dietary fiber, and phytochemicals. Nevertheless, rice bran rarely reaches our plates as a food or food ingredient. Brown rice which contains rice bran has not been widely accepted due to its hard texture and gritty taste. Therefore, the introduction of rice bran

into existing meals may be a promising approach which allows consumers to continue eating their preferred foods, while consuming bioactive nutrients and health-promoting components (Borresen & Ryan, 2014). Rice bran fortification into weaning foods was performed by a few researchers (Khan et al., 2011) due to its unique fat and protein content, high digestibility and hypoallergenic properties. More research will need to be conducted in the future to develop this trend as it is a promising opportunity (Borresen & Ryan, 2014).

Recently food scientists have been working on rice bran value-added processing in order to effectively utilize as many healthy components as possible. Soluble dietary fiber has been increasingly applicable in food formulation due to its physico-chemical properties and health benefits compared to insoluble dietary fiber. Some research studies have investigated the prebiotic properties of rice bran-induced oligosaccharides, which open up an additional source of prebiotics obtained from a cheap by-product material (Herfel et al., 2013; Kataoka et al., 2008; Komiyama et al., 2011).

MGN-3 is an arabinoxylan extracted from rice bran that is treated enzymatically with an extract from Shiitake mushrooms (*Basidiomycetes mycelia)*. The chemical structure of MGN-3 contains a xylose main chain and an arabinose side chain (**Figure 2.10**). It is commercially known as Biobran, manufactured and provided by Daiwa Pharmaceutical Co. Ltd., Setagaya, Tokyo, Japan (Ghoneum, 1998). The method for manufacturing MGN-3/BioBran include three steps (**Figure 2.11**): (1) extraction of polysaccharides from defatted rice bran hemicellulose; (2) manufacture of multiple shiitake-derived enzymes used to treat the extracted polysaccharides; and (3) partial hydrolysis of rice bran hemicelluloses by the carbohydrate-hydrolyzing enzymes obtained from shiitake mushrooms. Finally, the compound is treated with high heat and pressure. MGN-3 has become known as a dietary supplement which may strengthen the immune system.

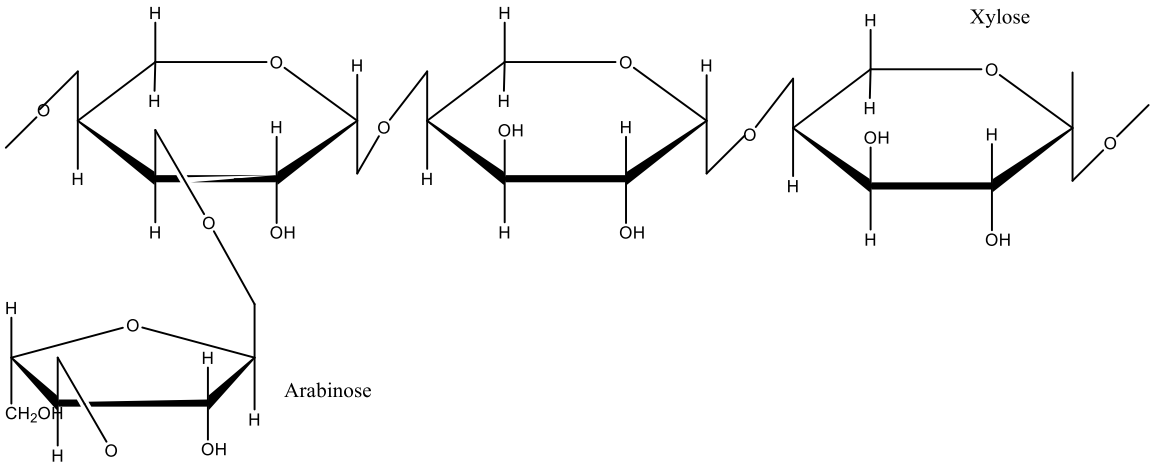


Figure 2.10 Chemical structure of MGN-3/BioBran

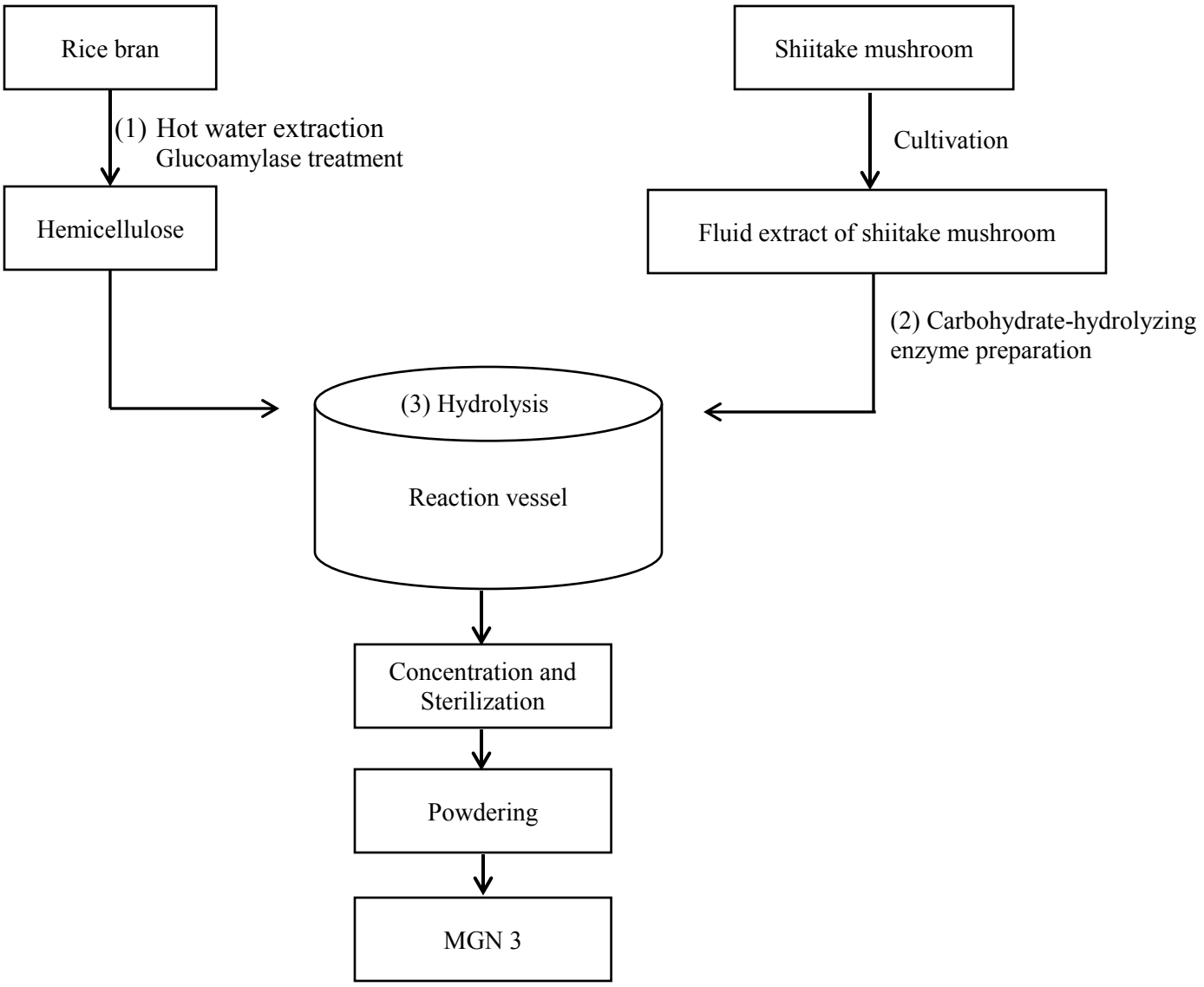


Figure 2.11 Three steps of manufacturing MGN-3/Biobran

CHAPTER 3. MATERIALS AND METHODS

3.1 Material

- Rice bran samples

Full-fat rice bran, defatted rice bran and stabilized rice bran samples used in this research, were collected from BUNGE Milling Inc. (Woodland, CA, USA), Riceland Foods Inc. (Stuttgart, AR, USA), and RiceBran Technologies (Scottsdale, AZ, USA), respectively. The samples were placed in bulk bags and stored at 4^{o}C to minimize degradation from biochemical and bacterial factors. The full-fat and stabilized rice bran were defatted by ethanol in the lab for further experiments (the protocol of rice bran defatting will be described in section 3.2.2). The bran collected after fat removal was called Lab-defatted rice bran (L-DRB), as distinguished from Commercial-defatted rice bran (C-DRB).

- Xylanase Enzymes

Commercial Xylanase enzymes used in the research included Enzeco Xylanase Concentrate with Oil (Origin from *Trichoderma ressei*, Enzyme Development Corporation, New York, NY, USA), Multifect 720 Xylanase (14,000-18,000 IU/mL, Origin from *Bacillus licheniformis*, Genencor International Inc., Rochester, NY, USA), ALI Xylanase (15,000XU/G, Origin from *Trichoderma ressei*, American Laboratory Inc., Omaha, NE, USA), and Bio-cat Xylanase (15,000XU/G, Origin from *Trichoderma ressei,* Bio-Cat Inc., Troy, VA, USA).

- Proximate analysis kits

Assay kits for the determination of total starch, dietary fiber, and phytate and phosphorus were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland).

- Chemicals

All chemicals and solvents used in this research were of American Chemical Society (ACS) certified grade.

3.2 Sample preparation

3.2.1 Grinding rice bran

Rice bran samples (native, stabilized, and defatted) obtained commercially were not finely ground and uniform due to the presence of broken rice and small pieces of husk. Therefore, they were ground in a Retsch Mill (ZM200 Ultra Centrifugal, Retsch Solutions in Milling & Sieving, Haan, Germany) with a 0.5 mm sieve to ensure their uniformity.

3.2.2 Defatting rice bran

Native rice bran and stabilized rice bran were defatted in a lab-scale with anhydrous ethanol. A preliminary defatting process was conducted with different ratios of ethanol volume (mL) to rice bran weight (g) and different extraction times in order to determine the best conditions. Rice bran (20 g) were contained in tall-wall beakers and mixed well with 60 mL, 80 mL, or 100 mL anhydrous ethanol. The beakers were then covered with aluminum foil, and placed in a 50^{o}C water bath for 1, 2, 3, and 4 hours. The mixture was stirred every 30 minutes. Fat, some phytochemicals and pigments were solubilized in the solvent, so the liquid phase was green due to the presence of chlorophylls. The beakers containing the rice bran and ethanol were then removed from the water bath and filtered under vacuum through a Whatman filter paper No. 1.

3.2.3 Washing rice bran

Native rice bran, stabilized rice bran, commercial defatted rice bran and laboratory defatted rice bran samples were washed with water to remove starch, some fat, soluble fiber, water-soluble protein, and some soluble ash. The protocol for rice bran washing is presented in **Figure 3.1**. Briefly, rice bran (50 g) was mixed with 250 mL water, and blended at 22000 rpm for 2 minutes by a Waring® blender (Waring Commercial, Torrington, CT, USA). The slurry was sieved with a test sieve (W.S. Tyler Canada, St. Catharines, ON, Canada) through 63 micron and 75 micron openings on a coarse sieve shaker (Model RX-812 CAN, W.S. Tyler, OH, US). The residue remaining on the sieve after screening was mainly insoluble dietary fiber, and was called fiber retentate 1. The retentate 1 was then rewashed three more times to collect fiber

retentates 2, 3, and 4. All fiber concentrates were then oven dried at 50°C overnight, and analyzed for composition.

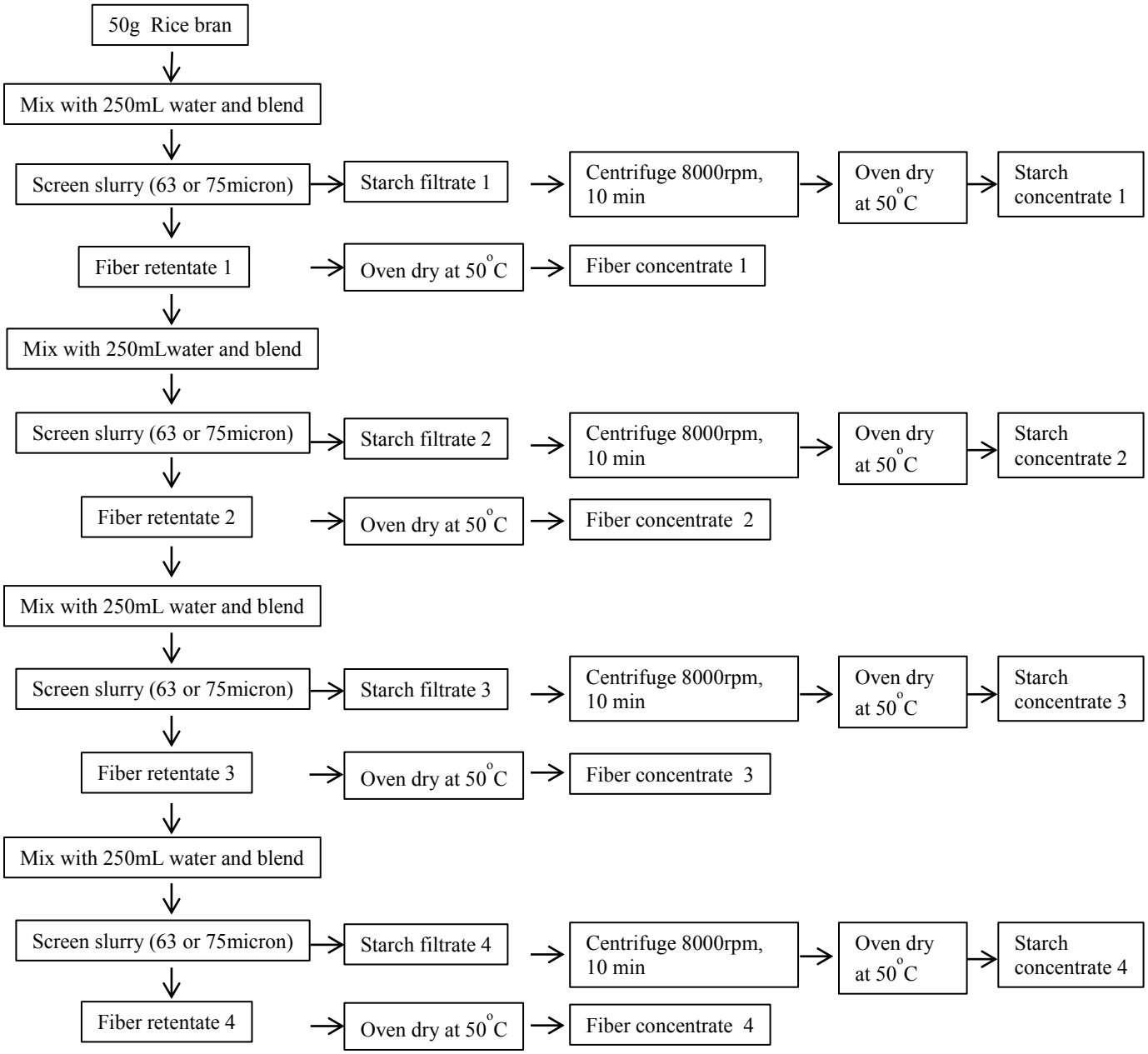


Figure 3.1 Water washing protocol for rice bran

3.3 Scanning electron microscope (SEM)

The morphology of untreated and treated fiber concentrates were studied by Scanning Electron Microscopy (SEM). Fiber samples were mounted on circular aluminum stubs with double-sided adhesive carbon tape. The excess fiber was trimmed away by air-blowing. The sample-containing stubs were then coated with 20 nm gold in a vacuum evaporator JEOL (JEOL Ltd., Akishima, Tokyo, Japan). The samples was then examined and photographed in a JEOL (JSM 6301 F*V) Scanning Electron Microscope (JEOL Ltd., Tokyo) at an accelerating voltage of 5 kv.

3.4 Proximate analysis

3.4.1 Total starch and water-soluble saccharide determination

Total starch

Analysis for total starch was accomplished by using the Megazyme Total Starch Assay Kit based on AOAC Method 996.11 and AACC Method 76.13 (Megazyme International Ltd., Wicklow, Ireland). Briefly, 30 mg of sample was dispersed in 0.2 mL of 80% ethanol in a 50 mL Corning centrifuge tube. Three mL of thermostable α-amylase in MOPS buffer (50 mM, pH 7.0) was added in each tube and heated in a boiling water bath for 6 minutes. The tube was then transferred to a 50^{o}C water bath and allowed to equilibrate. A 4-mL volume of sodium acetate buffer (200 mM, pH 4.5) was added to the tube, followed by 0.1 mL amyloglucosidase (20 U). The tube contents were mixed by a vortex mixer and incubated in a water bath at 50^{o}C for 30 minutes. The hydrolyzed sample was centrifuged at 3000 rpm for 10 minutes and 1 mL of the supernatant was withdrawn into two glass tubes. Two reagent blanks which contained 0.1 mL of distilled water each were prepared. Three mL of glucose oxidase/peroxidase reagent (GOPOD) was added to each glass tube, and they were incubated in a 50^{o}C water bath for 20 minutes. The solution was then read using a spectrophotometer at the absorbance of 510 nm.

The starch content was calculated as follows:

$$\text{Starch \%} = \Delta A \; x \; F \; x \; \frac{FV}{0.1} \; x \; \frac{1}{1000} \; x \; \frac{100}{W} \; x \; \frac{162}{180}$$

where

ΔA = Absorbance (reaction) read against reagent blank

F = $\frac{100\ (\mu g\ of\ D-glucose)}{absorbance\ for\ 100 \mu g\ of\ glucose}$ (conversion from absorbance to μg)

FV = Final volume

0.1 = volume of sample analyzed

$\frac{1}{1000}$ = conversion from μg to mg

$\frac{100}{W}$ = Factor to express "starch" as percentage

W = weight in mg "as is basis"

$\frac{162}{180}$ = Adjustment from free-glucose to anhydro D-glucose (as occur in starch)

Water-soluble starch

Water-soluble starch is soluble in hot water at 100^{o}C or warm water at 37^{o}C. The protocol of water-soluble starch determination was based on that of total starch determination. For hot-water-soluble starch, 100 mg of sample was mixed well with 10 mL of distilled water, sealed and then boiled in hot water bath at 100^{o}C for 10 minutes. For warm-water-soluble starch, 100 mg of sample was mixed well with 10 mL of distilled water and incubated in continuously shaking water bath at 37^{o}C for 1 hour. Then the solutions were centrifuged at 6500 rpm for 10 minutes. The supernatant (1 mL) was then withdrawn from the centrifuged solution, mixed with 1 mL of thermo-stable α-amylase in MOPS buffer (50 mM, pH 7.0), and boiled for 6 minutes. The mixture was then added with 2 mL of sodium acetate buffer (200 mM, PH 4.5), followed by 0.1 mL amyloglucosidase (20 U). The next steps were performed the same as total starch determination.

3.4.2 Total protein and water-soluble protein determination

Total protein

Rice bran samples were analyzed for total nitrogen using a Truspec carbon/nitrogen determinator automated dry combustion analyzer (Leco Corporation, St. Joseph, MI, USA). The

Truspec CN complies with AOAC method 992.23 and AACC method 46-30. Total protein was calculated by multiplying total N by 6.25 (conversion factor).

Water-soluble protein

Soluble protein either in hot water (100^{o}C) or in warm water (37^{o}C) was determined by Coomassie (Bradford) Protein Assay Kit (Thermo Scientific Inc., IL, USA). For hot-water-soluble proteins, 100 mg of sample was mixed well with 10 mL of distilled water and boiled in a hot water bath at 100^{o}C for 10 minutes. For warm-water-soluble proteins, 100 mg of sample was mixed well with 10 mL of distilled water and incubated in a continuously shaking water bath at 37^{o}C for 1 hour. Then the solutions were centrifuged at 6500 rpm for 10 minutes. The supernatant (0.1 mL) was then withdrawn from the centrifuged solution and mixed with 1 mL of Coomassie reagent. The mixture was left standing for 10 minutes before reading the absorbance in a spectrophotometer set at 595 nm.

3.4.3 Fat determination

Crude fat was measured using the Goldfisch Extraction Apparatus (Labconco Corporation, Kansas, MO, USA) (AACC method 30.25, 2004). Rice bran (2 g) was weighed into an extraction thimble and covered with a small amount of glass wool. An extraction beaker was also weighed, and then filled with 40 mL petroleum ether. The thimble was attached to a clamp in the condenser unit, followed by the extraction beaker. The machine was then run for 5 hours. Once extraction was completed, the beaker was removed from the apparatus, and dried in a 110^{o}C oven for 20 minutes. The residue in the beaker after drying was crude fat from the rice bran. A beaker with petroleum ether and an empty extraction thimble was used as blank.

The fat content was calculated as following:

$$\%\ Lipid = \frac{W_{beaker+extract} - W_{blank\ residue} - W_{beaker}}{W_{sample}}\ x\ 100$$

where

$W_{beaker+extract}$ = Weight of beaker and extract after extraction

$W_{blank\ residue}$ = Weight of blank residue after extraction

W_{beaker} = Weight of empty beaker

W_{sample} = Weight of sample (2 g)

3.4.4 Soluble and insoluble dietary fiber determination

The contents of IDF, SDF and TDF were determined by the Megazyme Total Dietary Fiber Assay Kit which is based on the enzymatic-gravimetric methods AOAC Method 985.29, AOAC Method 991.42, AOAC Method 991.43, AOAC Method 993.19, AACC Method 32-05.01, AACC Method 32-06.01, AACC Method 32-07.01 and AACC Method 32-21.01. In brief, 0.3 g of dried sample were treated with 50 µL of thermostable α-amylase in 10 mL MES-TRIS buffer (pH 8.2) in a boiling water bath for 35 minutes in order to gelatinize, hydrolyze and depolymerize starch. The mixture was then cooled down in a 60°C water bath, and the tube was rinsed with 15 mL of distilled water. Then, the mixture was digested with 100 µL of protease enzyme in a 60°C water bath for 30 minutes. After protein depolymerization, the mixture's pH was adjusted to 4.1-4.8 by using 0.56 N hydrochloric acid. Then, 100 µL of amyloglucosidase was added to the mixture and incubated in a 70°C water bath for 30 minutes. The hydrolyzed mixture was then filtered and washed with 60°C distilled water through a Celite-in-bed crucible. The residue in the crucible (IDF) was washed with 95% ethanol, dried in 103°C oven, and the protein and ash contents were determined. The filtrate and water washes were combined and added with four volumes of preheated 95% ethanol to precipitate the SDF for 1 hour, then filtered and washed with 78% ethanol and 95% ethanol before drying in 103°C oven. Protein and ash contents of the SDF residue were determined. The SDF content was the weight of dried SDF residue minus the weight of protein and ash. The total dietary fiber content was calculated as the sum of IDF and SDF.

3.4.5 Ash and moisture determination

The ash content was estimated by standard AOAC method 923.03 (AOAC, 2000). Moisture content was determined using a Satorious Moisture Analyzer (Model: MA45, Satorious Corporation, Goettingen, Germany). This moisture analyzer has a digital weighing scale with a pan. Sample (0.5g) was delivered over the pan before beginning analysis. As the cover was closed, a ceramic infra-red heater on the top cover transmitted heat to the sample while a sensitive thermometer measured the temperature of the heated chamber. The heating temperature

was set to 105°C. A microcontroller circuit detected the change in sample weight during heating and detected the end point of analysis when the rate of change of weight fell under a specific value.

$$\% \text{ Ash} = \frac{residue\ weight}{sample\ weight} x\ 100$$

$$\% \text{ Moisture} = \frac{sample\ weight - residue\ weight}{sample\ weight}\ x\ 100$$

3.4.6 Phytic acid and phosphorus determination

Phytic acid and phosphorous content were determined using a Megazyme kit which complies with AOAC Method 986.11 (AOAC, 2000). Briefly, 0.3 g of rice bran was mixed with 30 mL of 0.66 M hydrochloric acid shaken for 3 hours at room temperature. The mixture was centrifuged at 13000 rpm for 10 minutes. The supernatant (0.5 mL) was collected and mixed with 0.5 mL of 0.75 M sodium hydroxide. This neutralized extract was divided into two portions to analyze total phosphorous and free phosphorus. To measure total phosphorus, 0.05 mL of the extract was diluted with 0.6 mL of distilled water, mixed with 0.2 mL of sodium acetate buffer (pH 5.5) and 0.02 mL of phytase enzyme, and incubated in a 40°C water bath for 10 minutes. A 0.2 mL volume of glycerine buffer (pH 10.4) was then added to the mixture, followed by 0.02 mL of alkaline phosphatase suspension, and placed in a 40°C water bath for 15 minutes. The reaction was stopped by adding 0.3 mL of 50% (w/v) trichloroacetic acid, centrifuged at 13000 rpm for 10 minutes. To measure the free phosphorus content, 0.05 mL of neutralized extract was mixed with 0.62 mL of distilled water and 0.2 mL of sodium acetate buffer (pH 5.5), then incubated in a water bath at 40°C for 10 minutes. Volumes of 0.02 mL of distilled water and 0.2 mL of glycerine buffer (pH 10.4) were added to the mixture, and it was incubated for 15 minutes. The reaction was also stopped by adding 0.3 mL of trichloroacetic acid, and then centrifuged at 13000 rpm for 10 minutes. Colorimetric determination was done by mixing 1 mL of either total phosphorous or free phosphorous supernatant with 0.5 mL of color reagent, and incubating in a water bath at 40°C for 1 hour. The absorbance was read using a spectrophotometer at the absorbance of 655 nm against a water blank.

3.4.7 Total pentosan and soluble pentosan determination

The total pentosan and soluble pentosan content in rice bran were measured based on the orcinol-HCl method of Hashimoto, Shogren, and Pomeranz (1987) with some modifications.

Total pentosan

About 30 mg of rice bran flour was mixed with 2 mL of 2 N hydrochloric acid and hydrolyzed at 100^{o}C for 2.5 hours. The solution was then neutralized by adding 2 mL of 2 N sodium carbonate. A 2-mL volume of yeast solution (25 mg/mL in 0.2 M sodium phosphate buffer, pH 7.0) was added, and the mixture was fermented in a 37^{o}C water bath for 2 hours. The mixture was then diluted to 30 mL using distilled water, and centrifuged at 3000 rpm for 10 minutes. Then, the supernatant (0.1 mL) was withdrawn to mix with 0.9 mL of water, 0.1 mL of 1% orcinol in ethanol and 1 mL $FeCl_3$ in concentrated HCl. The reaction took place in a boiling water bath for 30 minutes. Its absorbance was read using a spectrophotometer set at 670 nm against reagent blank. A standard curve of xylose concentrations was performed on every analysis.

$$\% \text{ Total pentosan (as is)} = \frac{(As-Ab)\ x\ m\ x\ 6\ x\ 0.88\ x\ 5}{W\ x\ 1000} x100$$

where

As: Absorbance of sample

Ab: Absorbance of blank

6: Total volume of solution (2 mL HCl + 2 mL Na_2CO_3 + 2 mL yeast)

m: Slope of xylose standard curve

W: The sample weight

0.88: Adjustment from free pentose to andrydro pentose (132/150)

1/1000: Conversion from micrograms to milligrams

100: Factor to express pentosan content as a percentage of sample

5: Dilution factor (6 mL to 30 mL).

Water-soluble pentosan

The protocol for the water-soluble pentosan determination was similar to that of total pentosan described above. Instead of 30 mg used for total pentosan, the sample weight required was 100 mg. Washed rice bran (100 mg) was mixed with 10 mL distilled water and heated in boiling water for 10 minutes with occasional shaking (or in water bath set at 37^{o}C with continuous shaking for 1 hour). The solution was cooled down to room temperature before centrifuging at 5700 rpm for 10 minutes. The supernatant was the portion which contained the soluble pentosans. The supernatant (1 mL) was gently withdrawn and mixed with 2 mL 2 N HCl in sealed tubes, then incubated in the 100^{o}C oven for 2.5 hours to hydrolyze the pentosan. After acid hydrolysis, the solution was cooled down and added with 2 mL Na_2CO_3 to neutralize, followed by 2 mL yeast solution (25 mg/mL in sodium phosphate buffer), then incubated in water bath at 37^{o}C for 2 hours. The digesta was then diluted to 10 mL with distilled water and centrifuged at 5700 rpm for 10 minutes. A 1-mL aliquot was taken out to mix with 0.9 mL distilled water, 0.1 mL orcinol (1% w/v), and 1 mL $FeCl_3$ in concentrated HCl. The reaction occurred in boiling water within 30 minutes and the absorbance was read at 670 nm against a reagent blank. A series of xylose standards (10 µg, 20 µg, 30 µg, 40 µg, and 50 µg) was prepared along with samples as well.

3.4.8 Free pentose determination

Free pentose content was determined according to a combination combination of the orcinol-HCl method of Hashimoto et al. (1987) and the method of Tauber and Kleiner (1932). Briefly, 150 mg of rice bran flour was mixed with 5 mL of distilled water and heated in a boiling water bath for 10 minutes. After cooling down to room temperature, the solution was centrifuged at 65000 rpm for 10 minutes. The supernatant (1 mL) was withdrawn to mix with 0.5 mL yeast solution (25 mg/mL in distilled water). The solution was then fermented for 2 hours in a 37^{o}C water bath with occasional vortex mixing. Following complete fermentation, the sample was centrifuged at 6500 rpm for 10 minutes. The supernatant (0.1 mL) was collected and mixed with 0.1 mL of acid copper monose reagent, heated in a boiling water bath for precisely 8 minutes and cooled down for 3 minutes. Then, 0.1 mL of color reagent was added. The monosaccharides

reduced the cupric ions in the acid copper reagent to cuprous oxides which had brick-red color. The cuprous oxides then reduced the phosphomolybdic acid (color reagent) to phosphomolybdous acid which had a blue color. The blue mixture was then diluted with 1 mL of distilled water before reading absorbance at 520 nm against a reagent blank. A standard curve using xylose was performed at every analysis.

3.4.9 Water solubles

Water-soluble content of treated rice bran fiber was estimated using a Sartorious Moisture Analyzer (MA45). The sample (100 mg) was dissolved in 10 mL of distilled water and incubated in a water bath set 100^{o}C for 10 minutes with occasional shaking (or in a water bath set at 37^{o}C with continuous shaking for 1 hour) to release soluble components. The solubles may contain monosaccharides, disaccharides, low molecular-weight soluble fibers, high-molecular-weight soluble fibers, starch, fat, albumin protein, water-soluble ash, and phytic acids. The solution was then centrifuged at 6500 rpm for 10 minutes and 5 mL of the supernatant was collected to analyze for the moisture content using the MA45. The soluble content was the residue on the pan after removing all the moisture from the sample.

3.4.10 Ethanol and water solubles

Ethanol and hot/warm-water solubles were determined based on precipitation properties of high molecular-weight polymers in ethanol. The soluble polymers in hot-water solution were precipitated with 50% ethanol for 1 hour, then centrifuged for 10 minutes. A 5-mL aliquot was withdrawn from the supernatant to analyze the moisture content and residue content in the Sartorious moisture analyzer MA45.

3.5 Xylanase treatments

Commercial defatted rice bran and lab-defatted rice bran were used in this enzymatic treatment. Commercial defatted rice bran was obtained from Riceland Inc. (Stuttgart, AR, USA) with 5% fat remains in the bran. Lab-defatted rice bran was collected from the ethanol defatting of stabilized rice bran which was obtained from RiceBran Technologies (Scottsdale, AZ, USA). Both were washed with water before being treated with enzymes.

Four commercial xylanase enzymes used in this study came from different companies, namely Enzeco@ Xylanase concentrate with oil (Enzyme Development Company, New York, NY, USA), Multifect 720 Xylanase (Genercor International Inc., Rochester, NY USA), ALI Xylanase (American Laboratory Inc., Omaha, NE, USA), and BIO-CAT Xylanase (BIO-CAT Inc., Troy, VA, USA).

In duplicates, 20 g of water-washed rice bran fiber was mixed with 80 mL of distilled water and 0.2 g (Enzeco Xylanase, ALI Xylanase, and Bio-Cat Xylanase) or 2 mL (Mutifect 720 Xylanase) of enzymes. The mixture was then incubated at 55^{o}C for 5 hours. At the end of the enzyme treatment, the digesta was steamed for 30 minutes to inactivate the enzymes, and then dried overnight in a 80^{o}C oven. The enzyme-treated rice bran fibers were analyzed for chemical compositions of hot-water (100^{o}C) and warm-water (37^{o}C) solubles. Such compositions included total solubles, pentosan, pentose, ethanol and hot-water solubles, soluble starch, protein, phytic acid and phosphorous, and ash.

3.6 Extrusion treatment

A laboratory co-rotating intermeshing twin screw extruder Model C.W. Brabender PL2200 Plasti-Corder DIGI-SYSTEM (Brabender Instuments Inc., South Hackensack, NJ, USA) was used to perform the extrusion study. The screw assembly, configuration and the zone temperature profile used for this study are presented in **Figure 3.2**. The barrel temperature of the first three zones was kept at 60^{o}C in order to provide a suitable temperature for xylanase action. The die temperature was set at 100^{o}C to inactivate xylanase enzyme, sterilize the extruded product, and accommodate the flash evaporation of moisture from the extruded product. The washed rice bran (100 g) was mixed with water at various ratios (25%, 30%, 35%, 40%, 45% and 60% w/w) and left to equilibrate overnight at 4^{o}C. The equilibrated sample was then thoroughly mixed with 1%, 2% (w/w) or no xylanase (control), allowed to stand for 30 minutes, before extrusion cooking. Extruded products were dried overnight in an oven at 55^{o}C, ground in a Retsch Centrifugal Mill with a ring sieve of 0.5 mm opening.

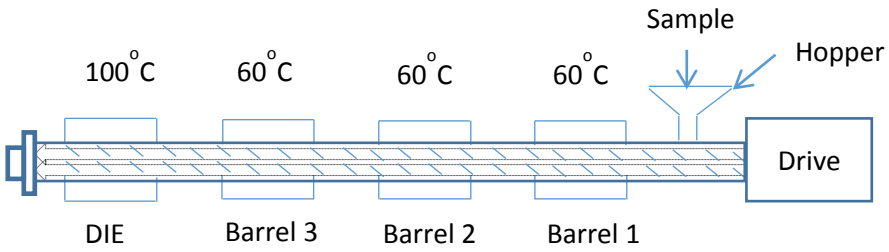


Figure 3.2 A schematic diagram of a twin screw extruder

3.7 Statistical analysis

All experiments were carried out in a complete randomized design (CRD) with at least two replicates. The results of proximate analyses were performed at least in duplicates. Data was statistically analyzed for one-way and multi-way ANOVA using SAS statistical software, version 9.3 (SAS ® Institute Inc., Cary, NY, 2013). The multiple comparison of means was accomplished by the Least Significant Difference (LSD) test at $\alpha = 0.05$. The means and standard deviations were reported.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Preliminary study of rice bran composition and fiber concentrate preparation

4.1.1 Composition of rice bran

The chemical composition of the bran samples is presented in **Table 4.1**. Native rice bran (NRB) from Bunge Milling is the bran obtained from milling process without any treatment while stabilized rice bran (SRB) from RiceBran Technologies is heated in order to inactivate lipid-degrading enzymes such as lipases and lipoxygenases, thus preventing the bran rancidity. For this current study, commercial defatted rice bran (C-DRB) from Riceland Foods was the bran stabilized by extrusion cooking and defatted by hexane. Generally, the starch content in the three bran samples was statistically consistent at around 20% (w/w). This level was in accordance with the range (10-55% w/w) reported by Saunders (1990) where bran levels depended on the type of milling and amounts of endosperm present. Commercial rice bran contains significant amount of starch mainly located in the germ and aleurone layers (Luh, 1991). The protein content of NRB (17.7% w/w) was not different from that of SRB, but slightly lower than that of C-DRB (19.3% w/w), which was slightly higher than the reported results (12-15.6% w/w) by Luh (1991). Due to fat removal, C-DRB had the lowest content of crude lipid (5.5% w/w), whereas NRB and SRB had high levels (20.7 and 22.9% w/w, respectively) within the previous range of 15-22% w/w by Orthoefer (1996). Regarding the dietary fiber profile, SRB had the highest total dietary fiber (TDF, 44% w/w) as opposed to the C-DRB (36.9% w/w) and NRB (35.4% w/w). In the present study, the TDF content of the three samples was in agreement with the previously reported values (20-55%,(Saunders, 1990)). Most dietary fiber in rice bran is water-insoluble because the insoluble dietary fiber (IDF) contents of three bran samples were considerably high (35.4% w/w in NRB, 39.9% in SRB, and 32.5% in C-DRB). However, the soluble dietary fiber (SDF) contents of the three bran samples were low (4% w/w), and were not significantly different among the samples. The pentosan content (11.3% w/w) in C-DRB was slightly higher than the other two counterparts (8.9% w/w each). The NRB showed the consistent levels of phytate (7.9% w/w) and phosphorus (2.2%) with those of SRB, while the C-DRB had the lower values (5.1% phytate and 1.4% phosphorus). In previous study (Luh, 1991), the ash content of rice bran was reported to be in the range of 6.6-9.9%. In the present study, the ash value ranged from 8.1% for NRB and SRB, and up to 15.6% for C-DRB.

Table 4.1 The % composition of native rice bran samples (dry basis)

Raw material	Source	Starch	Protein	Lipid	IDF[(2)]	SDF[(3)]	TDF[(4)]	Pentosan	Phytate	Total P[(5)]	Ash
Native rice bran (NRB)[(1)]	**Bunge Milling**	19.5±2.1[a]	17.7±0.04[b]	20.7±0.3[b]	33.7±3.7[ab]	4.1±1.1[a]	37.8±1.4[b]	8.9±0.6[a]	7.9±0.9[a]	2.2±0.3[a]	8.1±0.05[b]
Stabilized rice bran (SRB)[(1)]	**RiceBran Technologies**	19.9±2.0[a]	17.8±0.03[b]	22.9±0.1[a]	39.9±0.7[a]	4.1±0.3[a]	44.0±0.3[a]	8.9±1.4[a]	7.0±0.3[a]	1.9±0.1[a]	8.2±0.04[b]
Commercial defatted rice bran (C-DRB)[(1)]	**Riceland Foods**	20.2±0.5[a]	19.3±0.31[a]	5.5±0.01[c]	32.5±0.06[b]	4.5±0.9[a]	36.9±0.9[b]	11.3±0.3[a]	5.1±0.1[b]	1.4±0.03[b]	15.6±0.1[a]

All data represent the mean ± standard deviation of two replicate measurements. Means within a column with different letters are significantly different ($p<0.05$).

[(1)]All rice bran samples were obtained commercially.

[(2)]IDF = Insoluble dietary fiber

[(3)]SDF = Soluble dietary fiber

[(4)]TDF = Total dietary fiber = IDF + SDF

[(5)]Total P = Total phosphorous = 28.2% of phytic acid.

4.1.2 Water washing of rice bran to produce water-washed rice bran fiber concentrate

The yields of rice bran fiber concentrates and rice bran starch concentrates are summarized in **Table 4.2**. In general, water washing reduced the levels of starch, protein, fat, soluble dietary fiber (SDF), phytic acid, phosphorus, and ash; but increased the levels of insoluble dietary fiber (IDF), total dietary fiber (TDF), and pentosan. There were minor differences in terms of yield between washing with a 63 μm or 75 μm sieve. The rice starch granule sizes are small, and range from 3 to 5 μm (Fitzgerald, 2004), thus they could go through the openings easily. When washed with 5:1x1 ratio (water:bran, v/w, single washing), the yields of fiber concentrates of the four bran samples ranged from 39.9% to 48.8% . The yields decreased gradually over the second washing, ranging from 24.79% to 34.92%. The third and fourth washings did not show much difference in fiber yields. Three water washes was thus chosen as the most appropriate water washing protocol for further experiments in the present study.

The chemical composition of water-washed rice bran fiber concentrates (5:1 x 3 ratio) is presented in **Table 4.3**. There is no published data available for comparison of chemical compositions of washed rice bran, since this is the first study to determine those component contents of washed rice bran. In general, the washing of rice bran removed a significant amount of grain components from dietary fiber. After three washings with water, the starch content in the bran samples was noticeably reduced by 49-80%. The highest reduction rate of starch level was found in the native rice bran (NRB, from 19.5% to 3.9%), while the lowest rate was seen in defatted rice bran (C-DRB, from 20.2% to 10.3%) and in stabilized rice bran (SRB, from 19.9% to 9.1%). It is important to understand that C-DRB was stabilized by extrusion cooking while defatting by hexane, and SRB was stabilized by traditional heating. Both of these stabilization techniques were based completely or partially on heat. It is possible that heat-based stabilizations affected the adherence of starch with fiber and other components in rice bran. It is assumed that the starch granules were gelatinized during heat-based stabilization, becoming more adherent to fiber and other components, and less able to be washed away from rice bran. The same trend of decreased content levels with water washing was observed for protein, lipid, fiber, ash, phosphorus and phytate. On the other hand, the total pentosan content increased by approximately 189% in rice bran samples after water washing. The TDF water-washed rice bran

samples increased to approximately 75%; only 1-2% SDF remained in these samples. The increase in IDF and pentosan contents in the washed bran samples was mainly due to a loss of starch, water-soluble protein, water-soluble ash, and other soluble polysaccharides during washing.

A lab-scale defatted rice bran (L-DRB) was also washed with distilled water and the compositions of its washed fiber concentrate were determined. The defatting process was conducted using anhydrous ethanol at room temperature. As compared to commercial defatted rice bran (C-DRB), the washed L-DRB had significantly higher contents of IDF, TDF, and protein. This was mainly due to its significantly lower contents of starch, ash, lipid, and phytate. This may indicate that the solvents of fat extraction may influence the adhesion of complex matrix in rice bran. Further research would be needed to confirm this assumption.

Washing also changed the morphology of rice bran samples. The SEM micrographs (**Figure 4.1**) showed differences among rice bran samples before and after water washing. Due to a high fat content, the SEM images of samples before washing failed to show clearly the cell wall structure. After washing, the images showed better structural detail and organization, with honeycomb-like cell walls being apparent. Most of the residue after water washing was fiber, and the majority of other components were washed away.

Although washed NRB and SRB contained higher contents of IDF, TDF and pentosan, they were not selected for the consequent experiments in this study. NRB contained full fat and lipid-degrading enzymes, therefore would have been very difficult to preserve from rancidity and spoilage. SRB had a high content of oil, and although was stabilized from becoming rancid, the lipid would have caused some problems in further treatments and compositional determination. For example, a high lipid content may cause certain difficulties for rice bran water washing process by preventing particles (e.g starch, protein, etc.) from going through the sieve. Lipids may also hinder enzymes accessing starch and protein, resulting in incomplete enzymatic hydrolysis of these components; consequently the outcomes of starch and dietary fiber analyses may be less accurate. Therefore, C-DRB and L-DRB were selected for enzyme treatment due to their low fat (~5%) and high fiber content (65% and 72%).

Table 4.2 The effect of water washing of rice bran on the yield (%) of starch and fiber concentrates

Raw material	Water washing	Screen size (microns)	Water:bran ratio (v/w)	Yield %	
				Fiber concentrate	Starch concentrate
Native rice bran	YES	63	5:1 x1*	39.90	25.07
		63	5:1 x2	24.79	30.51
		63	5:1 x3	20.22	31.84
		63	5:1 x4	17.42	33.00
		75	5:1 x1	37.43	25.53
		75	5:1 x2	23.15	34.78
		75	5:1 x3	19.79	35.19
		75	5:1 x4	17.57	33.75
Stabilized rice bran	YES	63	5:1 x1	40.93	32.95
		63	5:1 x2	25.16	45.30
		63	5:1 x3	18.69	47.11
		63	5:1 x4	17.92	43.82
		75	5:1 x1	40.18	35.01
		75	5:1 x2	24.52	42.87
		75	5:1 x3	20.69	46.45
		75	5:1 x4	16.00	45.17
Commercial defatted rice bran	YES	63	5:1 x1	47.71	30.74
		63	5:1 x2	32.87	43.38
		63	5:1 x3	28.96	47.36
		63	5:1 x4	24.93	47.33
		75	5:1 x1	48.83	30.58
		75	5:1 x2	34.92	42.65
		75	5:1 x3	26.03	48.65
		75	5:1 x4	24.35	47.70
Lab-defatted rice bran	YES	63	5:1 x1	46.47	34.67
		63	5:1 x2	28.01	50.45
		63	5:1 x3	23.15	53.39
		63	5:1 x4	19.95	54.85
		75	5:1 x1	46.88	35.07
		75	5:1 x2	28.88	51.77
		75	5:1 x3	22.76	53.48
		75	5:1 x4	18.98	54.52

*water (v) : rice bran (w) x number of washings.

Table 4.3 The % composition of water-washed rice bran fiber concentrates (dry basis)

Fiber concentrate	Starch	Protein	Lipid	IDF [5]	SDF [6]	TDF [7]	Pentosan	Phytate	Total P[8]	Ash
Washed NRB[1]	3.96±0.5^{c}	14.5±0.2^{c}	10.1±0.1^{b}	74.0±1.2^{a}	0.8±0.8^{a}	74.8±1.9^{a}	25.7±1.9^{a}	1.4±0.1^{d}	0.39±0.02^{d}	3.0±0.02^{bc}
Washed SRB[2]	9.1±0.4^{a}	16.5±0.1^{b}	10.8±0.1^{a}	70.0±3.4^{ab}	1.6±1.5^{a}	71.5±4.9^{ab}	21.6±1.0^{b}	1.7±0.1^{c}	0.46±0.02^{c}	2.6±0.05^{c}
Washed C-DRB[3]	10.3±0.9^{a}	16.4±0.2^{b}	5.0±0.2^{c}	63.8±2.5^{b}	1.7±0.2^{a}	65.5±2.2^{b}	21.9±0.6^{b}	2.4±0.1^{a}	0.7±0.02^{a}	8.6±0.23^{a}
Washed L-DSRB[4]	6.4±0.1^{b}	17.7±0.1^{a}	4.3±0.04^{d}	71.2±2.7^{a}	2.0±0.3^{a}	73.2±2.9^{ab}	23.5±0.7^{ab}	1.9±0.03^{b}	0.53±0.01^{b}	3.1±0.04^{b}

All data represent the mean ± standard deviation of at least two replicate measurements. Means within a column with different letters are significantly different ($p<0.05$).

[1]NRB washed fiber = Water-washed native rice bran fiber

[2]SRB washed fiber = Water-washed stabilized rice bran fiber

[3]C-DRB washed fiber = Water-washed commercial-defatted rice bran fiber

[4]L-DSRB washed fiber = Water-washed lab-defatted stabilized rice bran fiber

[5]IDF = Insoluble dietary fiber

[6]SDF = Soluble dietary fiber

[7]TDF = Total dietary fiber

[8]Total P = Total phosphorus = 28.2% of phytic acid.

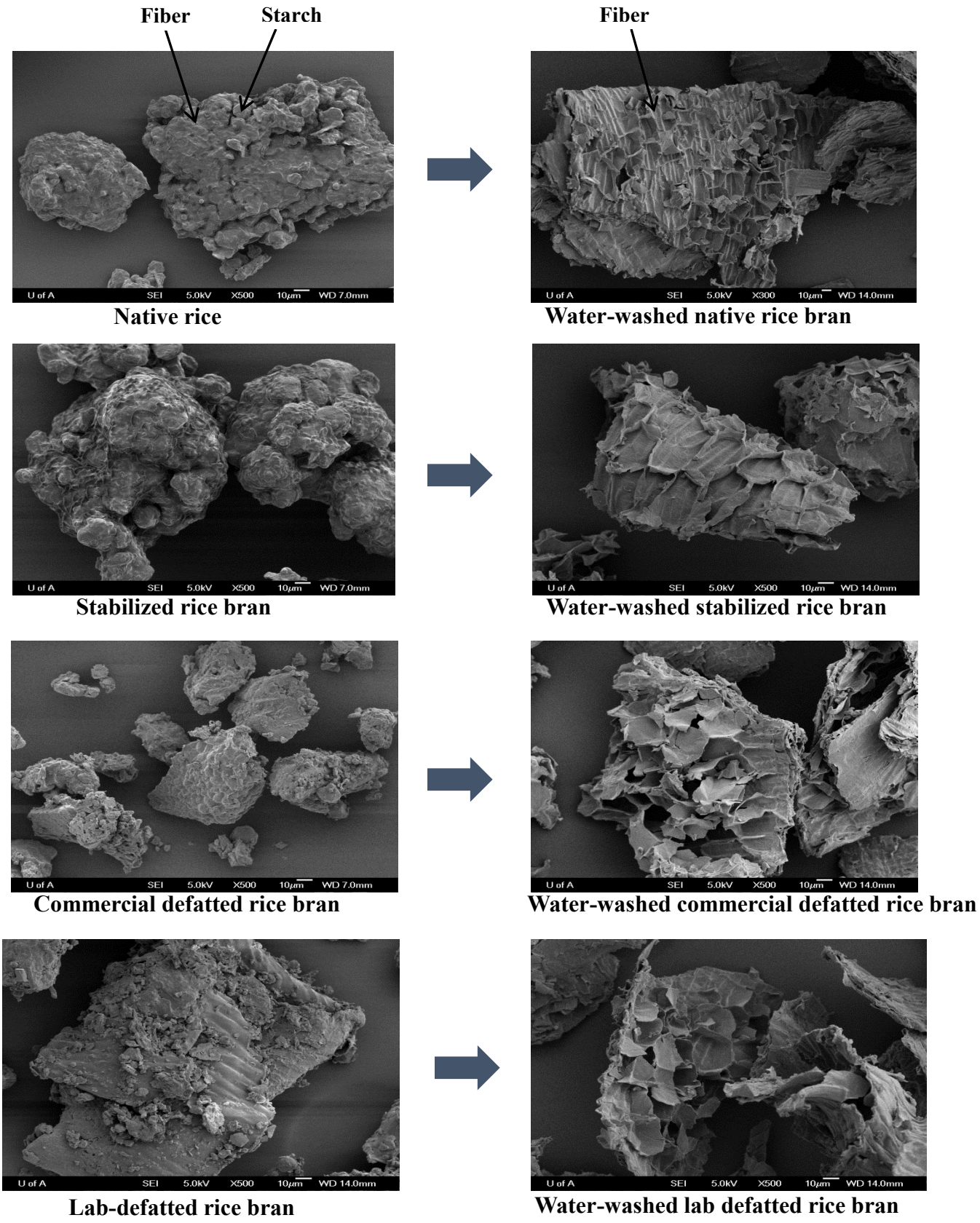


Figure 4.1 Scanning electron micrographs of raw rice bran and water washed fiber concentrates

4.2 Enzyme treatments to water-washed rice bran

The improvement of water-soluble components (especially pentosan) using enzymatic methods has been studied extensively on wheat, oat, and rye bran. However, rice bran has received less attention using this method, particularly regarding chemical composition. The information obtained during the present investigation is some of the first data published for rice bran solubilization with an enzymatic method, so it cannot be compared directly with results by other researchers.

Table 4.4 shows the effect of different enzyme treatments to commercial-defatted rice bran (C-DRB) and lab-defatted rice bran (L-DRB) washed concentrates on their hot-water soluble compositions. The solubles will include soluble pentosan, pentose, oligosaccharides, soluble sugars, soluble protein, soluble ash, phytate and phosphorus. In general, all enzyme treatments increased the total soluble content of washed rice bran. The greatest increase was seen in samples treated with ALI Xylanase (~17%) and BIO-CAT Xylanase (~17%). Multifect 720 Xylanase was less effective on rice bran materials, and released less solubles. Enzeco Xylanase hydrolyzed the washed bran samples relatively well, but less efficiently compared to the ALI and BIO-CAT counterparts. L-DRB washed concentrate had higher soluble content than C-DRB. The soluble pentosan content was represented as the same pattern of other solubles, of which ALI Xylanase and BIO-CAT Xylanase gave the greatest content of approximately 9.5% in L-DRB, and Multifect 720 Xylanase gave the lowest content of 6%. These enzymes acted more efficiently on L-DRB than on C-DRB in terms of xylanolytic hydrolysis. Noticeably, the L-DRB treated with ALI Xylanase or BIO-CAT xylanase released the greatest soluble pentosan content (9.19% each), and the greatest hot-water solubles (17.75% and 17.26% respectively). The degree of degradation of insoluble pentosan from C-DRB and L-DRB in ALI and BIO-CAT Xylanase were approximately the same.

The pentose content increased slightly after enzymatic treatment, and was approximately the same (~ 1.5%) for both L-DRB and C-DRB. The pentoses were mostly likely to be xylose and arabinose (Mod et al., 1978), the products of hydrolysis of endo-xylanase or exo-xylanase present in the enzyme preparations. The commercial xylanase enzymes were not pure due to little or insufficient purification after fungal fermentation production. They contain a specific

amount of cellulase and amylase capable of hydrolyzing cellulose and residual starch into the sugars. This also explains the significant increase of starch content in treated rice bran.

The total starch content in ethanol solubles was slightly lower than that in the hot-water solubles (roughly 3% compared to 4%). This can be explained that there was a tiny amount of high molecular maltodextrins which precipitated in 50% ethanol. Glucose, maltose and low molecular maltrodextrins occupied the major proportion in the total starch content, and were likely produced from the combined amylase and cellulase activities of commercial xylanase enzymes.

One % protein in hot-water solubles was mainly albumin which is solubilized in water. In comparison with the water-washed untreated rice bran samples, the enzyme-treated samples contained only a small content of soluble protein vs total protein (1% compared to 16%). This implies that rice bran proteins were mostly in a bound form with other components, and the commercial xylanase enzymes contained very little active proteolytic enzymes. Interestingly, the protein content in washed C-DRB increased from 0.4% to approximately 1% when hydrolyzed with the four enzymes, whereas no changes were seen in L-DRB. This suggests that the association between protein and fiber components in C-DRB may be looser or less complicated than that in L-DRB.

The total amount of pentosan, starch, protein, phytate, phosphorous, and ash in samples, which were soluble in hot water, accounted for approximately 14% out of 17% hot-water solubles. This may indicate that 3% difference was non-starch oligosaccharides rather than soluble pentosan. These oligosaccharides could originate from the enzymatic hydrolysis of xyloglucan which is a predominantly co-existing hemicellulose with arabinoxylan in rice bran (Shibuya & Iwasaki, 1985). Mod et al. (1979) also found 28-31% galactose, 2-3% glucose, 1-2% mannose in pure water-soluble rice bran hemicellulose depending on rice varieties. These observations suggest that aside from major pentoses, a large quantity of hexoses contribute to the composition of water soluble hemicellulose in rice bran. In this present study, the hexose-containing oligosaccharides may have not been measured as “soluble pentosans” in the method used. Further characterization of those treated samples is required to confirm this assumption. A sugar profile could be performed using chromatographic techniques (e.g. HPLC, GLC, etc.) in

order to identify the content of not only pentoses (xylose and arabinose) but also hexoses (glucose, galactose, and mannose) occurring in the soluble fraction of rice bran.

A profile of soluble compositions of enzyme treated washed rice bran in warm water (37^{o}C, body temperature) is also presented in **Table 4.5**. Generally, all soluble compositions of treated rice bran were lower at warm water (37^{o}C) than at hot water (100^{o}C) although they were extracted for a longer time (60 minutes as opposed to 10 minutes extracted hot water solubles). There may be thermal hydrolysis of polysaccharides of enzyme-treated rice bran during boiling in hot water, thus releasing some additional soluble oligosaccharides. However, hot-water-soluble and warm-water-soluble pentosans (also known as arabinoxylans) have certain differences in molecular weight (Meuser, Abd-Elgawad, & Suckow, 1981) and structure (Hoffmann, Roza, Maat, Kamerling, & Vliegenthart, 1991a; Hoffmann, Roza, Maat, Kamerling, & Vliegenthart, 1991b). The hot-water-soluble arabinoxylans contain higher molecular polymers than the warm-water-soluble counterparts. Hoffmann et al. (1991b) reported that warm-water-soluble arabinoxylans are composed of relatively lower amounts of unbranched xylose and a significantly higher amount of trans-ferulic acid, compared to warm-water-soluble arabinoxylans. They also suggested that ferulic acid plays a role in the formation of water-insoluble polymer clusters.

Although L-DRB had higher overall soluble content, it was not selected to continue extrusion cooking process due to economic, environmental and timing reasons. Defatting L-DRB used large volumes of ethanol, which is associated not only with ethanol cost but a recovery or disposal waste cost as well. Moreover, lab-defatting processes take critical time for fat extraction (1 hour), filtration (varies in time-consuming aspect depending on the quantity of defatting rice bran), and drying (overnight) and make the protocol unpractical for research and development. For these reasons, C-DRB was the best choice for further experiments.

Table 4.4 The % composition of hot-water (100^{o}C) solubles in xylanase-treated washed rice bran concentrates (dry basis)

Samples		Solubles	Pentosan	Pentose	Starch	Protein	Ash	Phytate	Phosphorus	Ethanol&hot water Solubles	Ethanol&hot water soluble Starch
Untreated washed rice bran	**C-DRB[(1)]**	7.29±0.71^{g}	2.41±0.61^{d}	1.24±0.08^{b}	2.59±0.02^{e}	0.44±0.03^{b}	1.23±0.56^{c}	0.29±0.01^{e}	0.08±0.00^{e}	7.24±0.31^{e}	0.50±0.12^{f}
	L-DRB[(2)]	8.93±0.67^{f}	2.29±0.53^{d}	1.26±0.08^{b}	1.98±0.00^{f}	0.44±0.02^{b}	1.15±0.28^{c}	0.36±0.02^{e}	0.10±0.01^{e}	7.93±0.61^{e}	0.57±0.09^{e}
Enzeco Xylanase	**C-DRB**	14.28±0.25^{d}	6.17±1.23^{bc}	1.48±0.02^{a}	2.57±0.01^{e}	0.91±0.07^{a}	2.51±0.03^{ab}	0.84±0.01^{b}	0.24±0.00^{b}	13.23±0.06^{c}	2.17±0.35^{cde}
	L-DRB	15.57±0.28^{cd}	6.37±0.37^{bc}	1.44±0.03^{a}	3.44±0.19^{d}	0.49±0.14^{b}	1.27±0.03^{c}	0.99±0.09^{a}	0.28±0.03^{a}	15.19±0.58^{b}	2.63±0.49^{cbd}
Multifect 720 Xylanase	**C-DRB**	10.35±0.36^{e}	5.39±0.50^{c}	1.45±0.02^{a}	2.72±0.23^{e}	0.95±0.08^{a}	2.75±0.55^{ab}	0.18±0.05^{f}	0.05±0.01^{f}	10.84±0.83^{d}	2.02±0.17^{ed}
	L-DRB	10.64±0.40^{e}	6.09±0.25^{bc}	1.41±0.05^{a}	1.85±0.07^{f}	0.56±0.11^{b}	1.22±0.21^{c}	0.52±0.01^{d}	0.15±0.00^{d}	10.91±0.59^{d}	3.16±0.38^{ab}
ALI Xylanase	**C-DRB**	16.39±0.14^{bc}	6.79±0.54^{bc}	1.47±0.06^{a}	4.11±0.05^{ab}	0.96±0.09^{a}	2.99±0.18^{a}	0.30±0.01^{e}	0.08±0.00^{e}	14.30±0.20^{b}	2.80±0.22^{abc}
	L-DRB	17.75±0.82^{a}	9.19±1.62^{a}	1.43±0.08^{a}	3.81±0.31^{bc}	0.55±0.11^{b}	2.27±0.14^{b}	0.75±0.02^{c}	0.21±0.01^{c}	17.52±0.14^{a}	1.65±0.19^{e}
BIO-CAT Xylanase	**C-DRB**	16.26±1.15^{bc}	7.32±0.82^{b}	1.46±0.04^{a}	4.25±0.16^{a}	1.13±0.29^{a}	3.03±0.09^{a}	0.53±0.02^{d}	0.15±0.01^{d}	14.25±0.29^{bc}	2.60±0.37^{cbd}
	L-DRB	17.26±0.26^{ab}	9.19±1.06^{a}	1.42±0.07^{a}	3.60±0.20^{cd}	0.59±0.06^{b}	1.54±0.13^{c}	0.53±0.01^{d}	0.15±0.00^{d}	16.84±0.44^{a}	3.40±0.21^{a}

All data represent the mean ± standard deviation of two replicate measurements. Means with different letters within a column are significantly different ($p<0.05$).

[(1)]C-DRB = Commercial defatted rice bran

[(2)]L-DRB = Lab-defatted rice bran

Table 4.5 The % composition of warm-water (37°C) solubles in xylanase-treated washed rice bran fiber concentrates (dry basis)

Samples		Solubles	Pentosan	Pentose	Starch	Protein	Ash	Phytate	Phosphorus	Ethanol&hot water Solubles	Ethanol&hot water soluble Starch
Untreated washed rice bran	**C-DRB[(1)]**	6.99±0.48^{e}	2.09±0.30^{e}	1.04±0.01^{f}	0.60±0.09^{e}	0.55±0.03^{b}	0.90±0.01^{cd}	0.11±0.02^{c}	0.05±0.01^{c}	4.33±0.88^{g}	0.50±0.12^{f}
	L-DRB[(2)]	6.03±0.95^{e}	2.45±0.31^{e}	1.08±0.01^{ef}	0.67±0.02^{e}	0.73±0.01^{a}	1.64±0.15^{a}	0.23±0.08^{c}	0.07±0.02^{c}	5.82±0.36^{f}	0.57±0.09^{f}
Enzeco Xylanase	**C-DRB**	13.90±0.68^{c}	3.34±0.31^{cde}	1.20±0.03^{a}	1.73±0.43^{d}	0.63±0.15^{ab}	1.18±0.10^{bc}	0.74±0.26^{a}	0.21±0.07^{a}	12.97±0.59^{c}	1.79±0.02^{d}
	L-DRB	15.64±0.87^{b}	3.85±0.30^{cd}	1.17±0.01^{ab}	2.59±0.43^{bc}	0.22±0.09^{c}	0.93±0.29^{cd}	0.22±0.02^{c}	0.06±001^{c}	13.93±1.61^{bc}	2.21±0.12^{cd}
Multifect 720 Xylanase	**C-DRB**	9.83±0.95^{d}	2.89±0.70^{de}	1.12±0.01^{cd}	1.50±0.14^{d}	0.66±0.03^{ab}	0.69±0.13^{d}	0.50±0.09^{b}	0.14±0.02^{b}	8.59±0.61^{e}	1.24±0.11^{e}
	L-DRB	10.85±0.39^{d}	3.86±0.17^{cd}	1.12±0.02^{cde}	1.24±0.01^{d}	0.26±0.01^{c}	1.34±0.09^{ab}	0.07±0.02^{c}	0.02±0.01^{c}	10.06±1.09^{d}	0.97±0.13^{ef}
ALI Xylanase	**C-DRB**	15.51±2.54^{bc}	4.21±0.48^{bc}	1.10±0.02^{ed}	2.46±0.07^{c}	0.64±0.04^{ab}	1.05±0.20^{bc}	0.13±0.09^{c}	0.04±0.02^{c}	14.23±0.67^{b}	2.55±0.19^{bc}
	L-DRB	19.30±1.38^{a}	5.23±0.29^{b}	1.17±0.04^{ab}	3.27±0.20^{a}	0.33±0.08^{c}	1.61±0.26^{a}	0.08±0.03^{c}	0.02±0.01^{c}	17.03±0.49^{a}	3.03±0.12^{ab}
BIO-CAT Xylanase	**C-DRB**	14.51±0.36^{bc}	4.32±0.56^{bc}	1.15±0.01^{bc}	2.95±0.48^{abc}	0.69±0.01^{ab}	0.92±0.03^{cd}	0.19±0.03^{c}	0.05±0.01^{c}	14.30±0.98^{b}	2.77±0.41^{ab}
	L-DRB	18.38±1.14^{a}	6.79±1.29^{a}	1.19±0.00^{ab}	3.00±0.05^{ab}	0.35±0.01^{c}	1.53±0.39^{a}	0.08±0.00^{c}	0.02±0.00^{c}	17.19±0.65^{a}	3.18±0.51^{a}

All data represent the mean of two replicate measurements. Means with different letters within a column are significantly different ($p<0.05$).

[(1)]C-DRB = Commercial defatted rice bran

[(2)]L-DRB = Lab-defatted stabilized rice bran

4.3 Extrusion cooking to water-washed rice bran

The water-soluble composition of untreated washed rice bran and extruded washed rice bran is presented in **Table 4.6**. In general, the solubility of extruded washed rice bran (EWRB) was significantly ($p<0.05$) higher than that of untreated washed rice bran (UWRB). A two-way statistical analysis showed there was a significant reduction in solubles from 6.8% to 5.9% when added moisture increased from 25% to 40%, but a dramatic increase in solubles to 8.4% occurred when added moisture was increased between 45% and 60%. The screw speed was also an influential factor in the present study. A significant increase of 12% in solubles was observed at a screw speed of 100 rpm compared to 50 rpm. The extruded rice bran with 60% added water and 100 rpm screw speed demonstrated the highest content of soluble materials (10.29%), due mainly to an increase in both soluble starch to 5.93% and soluble pentosan to 4.96%. Larrea et al. (2005) suggested that extrusion would result in the breakage of covalent and non-covalent linkages between carbohydrates and proteins associated with the fiber to produce smaller and more soluble molecular fragments. As seen from SEM images (**Figure 4.2**), the honeycomb-like cell walls were disrupted after extrusion cooking, indicating the complex matrix of cell wall was dissociated to a great extent.

The increases in feed moisture content and screw speed were correlated to increases in warm-water-soluble pentosan and starch, and thus contributing to increases in overall solubles content. Soluble starch content (4-6%) of extruded samples with 45% and 60% added water was noticeably higher than that of samples with less water addition. The increase in starch solubility may be due to the occurrence of dextrinization which degrades starch granules during extrusion cooking (Gui, Gil, & Ryu, 2012). High shear force created from high screw speed in the extruder may have led to starch degradation (Anderson, Conway, & Peplinsk, 1970). However, contrary to the results of the current study, previous authors cited here reported that the water solubility of extruded products was higher with lower moisture content.

The soluble pentosan content of EWRB (3.5-5%) was significantly higher than that of UWRB (2%). It is difficult to compare these pentosan figures from washed rice bran with those in previously published data since they focused mainly on non-washed rice bran and dietary fiber determined by an enzymatic-gravimetric method. Numerous studies have been in agreement that

extrusion generally increases soluble fiber content (Bjorck et al., 1984; Jing & Chi, 2013; Ralet et al., 1990; Vasanthan et al., 2002). In the present study, there were no variations in solubles between levels of water addition, whereas high screw speed solubilized more pentosan than lower screw speed. However, one study on extruded corn fiber that was pretreated with sodium hydroxide observed a decrease in arabinoxylan solubilization with an increased feed moisture content. Here the maximum soluble arabinoxylan content was obtained at 30% and 40% feed moisture content (Jeon, Singkhornart, & Ryu, 2014; Lamsal, Yoo, Brijwani, & Alavi, 2010; Singkhornart, Lee, & Ryu, 2013). Arabinoxylans consist predominantly of the pentoses arabinose and xylose, and are therefore often classified as pentosans. The current study showed that soluble arabinoxylan content increased with the increase of screw speed. The higher shear stress with increased screw speed likely caused the sugar content to increase due to furfural formation, secondary to dehydration of hexoses and pentoses at high temperature and in acidic solution (Saha, Iten, Cotta, & Wu, 2005). After available reducing sugars convert into furfurals, the soluble arabinoxylan content decreases as a consequence. The increase in soluble pentosan content after extrusion cooking was also likely due to the molecular degradation of arabinoxylan chains into smaller molecules easily solubilized in water. Also, extrusion may lead to an increase in soluble fiber values due to the redistribution of insoluble fiber into soluble fractions (Camire, Camire, & Krumhar, 1990; Gualberto et al., 1997). Ralet at al. (1990) suggested that this mechanical transformation - rather than a thermal effect related to extrusion temperature changes could better explain the increase in soluble fiber content associated with extrusion. The extrusion could also break the chemical bonds between phenolics, particularly ferulic acid and arabinoxylans, thus releasing more soluble arabinoxylans and increasing phenolic availability (Hole et al., 2013).

Although the soluble protein content was low (<1%) in unextruded and extruded rice bran, it showed a trend of significant decrease of approximately 50% in extruded samples as compared to the unextruded. It is possible that during extrusion some proteins complexed with starch, dietary fiber and lipid, preventing them from being solubilized in water. During extrusion processing, peptides can be associated together due to the formation of new intermolecular disulfide bonds and noncovalent linkages, thus reducing the solubility of proteins (Pham & Rosario, 1984). Similarly, Li and Lee (1996) suggested that the decrease in protein solubility of wheat extrudates was due primarily to aggregation via hydrophobic interactions and disulfide

bond formation. The protein aggregation may result in their molecular weight increase and subsequently a decrease in solubility. Additionally, Cheftel at al. (1985) reported that the decreased solubility of protein during extrusion was related to its denaturation in which hydrophobic groups are uncovered causing a decrease in water solubility.

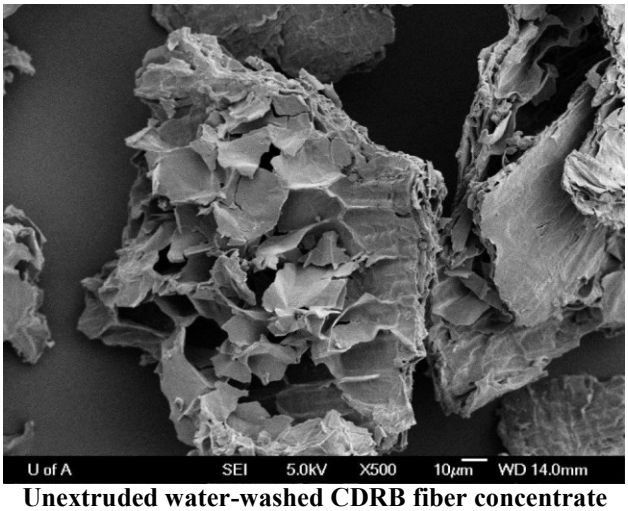
In the current study, generally there were no clear trends on the effects of extrusion cooking on soluble ash content at 50 rpm screw speed, but a significant increase was seen upon 100 rpm screw speed combined with high water content (35-60%). This indicates that high shear stress generated from a high screw speed played a role in releasing soluble minerals from insoluble forms which are naturally entrapped in the fiber matrix A high water content likely decreased the association between minerals and fiber, and subsequently assisting their dissociation under higher shear force.

Table 4.6 The % composition (water soluble at 37^{o}C) of extruded CDRB fiber concentrates without enzyme addition (dry basis)

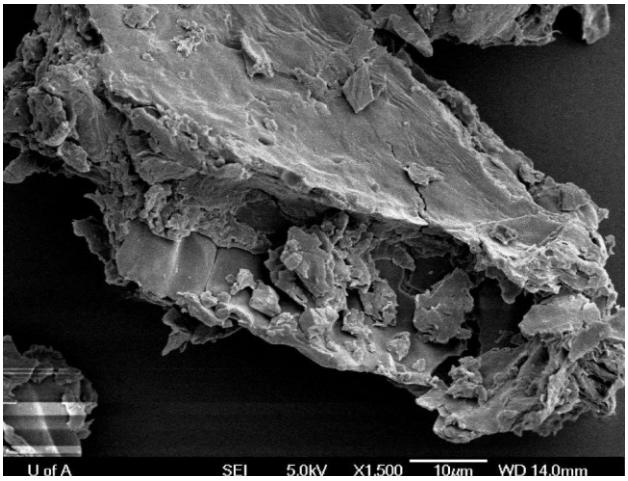
Sample	Solubles	Soluble Pentosan	Soluble starch	Soluble protein	Soluble ash	Pentose
Untreated	6.99±0.48^{c}	2.09±0.30^{e}	0.60±0.09^{h}	0.55±0.03^{a}	0.90±0.01^{cd}	1.04±0.01^{g}
25%/50rpm*	6.63±0.30^{cd}	3.52±0.01^{d}	2.42±0.12^{defg}	0.38±0.01^{bcd}	0.27±0.1^{d}	1.57±0.01^{ab}
30%/50rpm	6.05±0.20^{de}	3.98±0.00^{cd}	2.49±0.05^{defg}	0.35±0.02^{cde}	0.63±0.13^{d}	1.54±0.01^{bc}
35%/50rpm	5.72±0.63^{e}	4.18±0.08^{bcd}	2.87±0.00^{d}	0.37±0.00^{bcde}	0.89±0.25^{cd}	1.50±0.01^{cde}
40%/50rpm	5.65±0.19^{e}	4.36±0.08^{abc}	2.17±0.34^{fg}	0.32±0.00^{de}	0.00±0.00^{d}	1.52±0.03^{cde}
45%/50rpm	8.76±0.24^{b}	4.12±0.09^{cd}	4.64±0.22^{b}	0.32±0.03^{e}	0.45±0.13^{d}	1.52±0.00^{cde}
60%/50rpm	6.10±0.15^{de}	4.41±0.13^{abc}	2.29±0.01^{efg}	0.32±0.04^{e}	0.36±0.00^{d}	1.48±0.02^{e}
25%/100rpm	6.99±0.30^{c}	4.46±0.59^{abc}	2.74±0.25^{de}	0.42±0.00^{b}	0.00±0.00^{d}	1.53±0.01^{bc}
30%/100rpm	6.27±0.23^{cde}	4.20±0.20^{bc}	2.68±0.19^{def}	0.41±0.04^{bc}	0.54±0.26^{d}	1.53±0.02^{bcd}
35%/100rpm	5.85±0.01^{de}	4.89±0.81^{a}	2.72±0.60^{de}	0.37±0.06^{bcde}	1.78±0.01^{bc}	1.58±0.00^{a}
40%/100rpm	6.23±0.29^{cde}	4.91±0.20^{a}	1.99±0.03^{g}	0.34±0.02^{cde}	1.88±0.12^{b}	1.57±0.03^{ab}
45%/100rpm	8.11±0.06^{b}	4.85±0.11^{ab}	4.05±0.20^{c}	0.36±0.05^{bcde}	3.17±1.42^{a}	1.49±0.02^{de}
60%/100rpm	10.29±0.9^{a}	4.96±0.02^{a}	5.93±0.27^{a}	0.40±0.03^{bc}	1.62±0.25^{bc}	1.51±0.01^{cde}

All data represent the mean of two replicate measurements. Means within a column with different letters are significantly different ($p<0.05$)

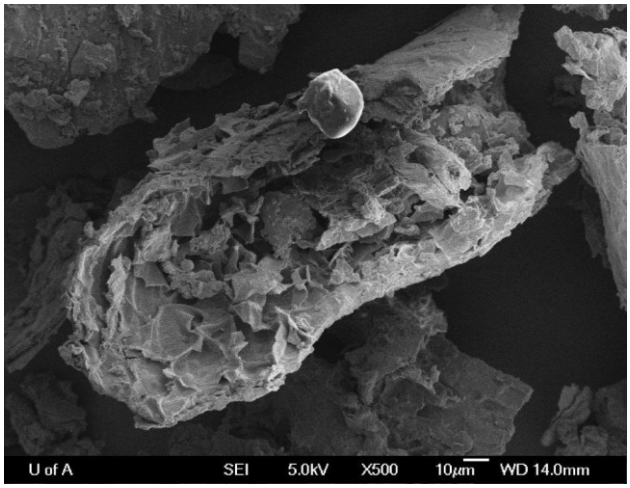
* % water addition (w/w)/screw speed (rpm)



Unextruded water-washed CDRB fiber concentrate



Extruded 45% added water/100rpm CDRB fiber concentrate



Extruded 60% added water/100rpm CDRB fiber concentrate

Figure 4.2 Scanning electron micrographs of un-extruded and extruded CDRB fiber concentrates

4.4 Effect of extrusion cooking and enzyme treatment in combination to water-soluble compositions of water-washed rice bran

4.4.1 A parallel combination of extrusion processing and enzyme treatment to water-washed rice bran

In this study, a twin-screw extruder was used as a continuous reactor for a combination of thermo-mechanical and enzymatic treatment of water-washed rice bran. **Figure 4.3** shows the overall trends of all soluble compositions in relation to the treatments of rice bran. Generally, changes in the compositions showed the same patterns when extruded with and without enzyme treatment. For instance, the overall soluble composition gradually increased with water content, and reached a peak at 45% and 60% water addition. Particularly, **Figure 4.4A** demonstrates that the parallel treatment combination slightly increased the content of total solubles at 37^{o}C in comparison with extrusion alone by approximately 10%, but did not change the content of soluble pentosan (**Figure 4.4B**). Here, there was a corresponding increase in soluble starch from 2-4% to 3-8%, suggesting that parallel mild extrusion and xylanase hydrolysis broke down more glucans (starch and cellulose) than xylans and arabinoxylans. The constancy in soluble pentosan levels demonstrated that BIO-CAT xylanase enzyme (1% and 2% w/w of dry matter) did not act functionally in the extruder. This may be due to the short residence time (around 2 minutes and 1.5 minutes) in the barrel. The enzyme may have required more reaction time to start its xylan hydrolytic function. Some research indicates to increase the residence time of materials in extruders, namely (1) using a long barrel extruder, (2) reducing the screw speed and feed rate, and (3) applying a more aggressive screw configuration (e.g. reverse screw segments) (Cheftel, Kitagawa, & Queguiner, 1992). Extruder residence time has an inversely proportional relationship to screw speed, where a lower screw speed allows materials to reside and react longer in the extruder. This present study investigated two relatively low screw speeds (100 rpm and 50 rpm) corresponding to two residence times (around 1.5 minutes and 2 minutes), but still resulting in no differences in soluble pentosan content. Since screw speed is directly proportional to shear stress which mechanically shortens biomass fibers, a very low screw speed may not be able to provide the sought advantages of extrusion cooking with the current laboratory extruder used for this study. Future research could more fully investigate optimizing

both extruder exposure and shear without compromising adequate reaction time, by increasing extruder barrel length and changing screw configuration as suggested by Cheftel et al. (1992).

The low water content may also be a contributing factor of unchanged soluble pentosan values. Previous studies suggest that enzyme action is reduced at a low water content due to the rheological properties and physical nature of the biomass and its polymers (Roberts, McCarthy, Jeoh, Lavenson, & Tozzi, 2011; Viamajala, McMillan, Schell, & Elander, 2009). Viamajala et al. (2009) demonstrated that at a water content of 60-70%, the biomass water absorption process may result in the absence of a continuous free water phase, causing the bulk to behave like a wet granular material. In this case, they propose portions of the void volume contain air rather than liquid, causing difficulties for shearing and mixing materials. In the Roberts et al. (2011) study, water content was shown as a critical factor to the enzymatic hydrolysis to permit the mass transfer of enzyme and products. Adequate water is needed as a medium through which both the enzymes can diffuse into the biomass and the reaction products to diffuse away from the reaction site. Additionally, the water itself is a reactant in the hydrolysis of glycosidic bonds within polysaccharides. When water content is limited in the biomass, both the mass transfer and water as a reactant can be reaction constraints that ultimately decrease enzyme efficiency. However, a recent study conducted on coarse and fine wheat bran investigated the impact of extrusion and blade-mixing on xylanase action at different moisture contents (Santala, Nordlund, & Poutanen, 2013). They found that extrusion enabled efficient enzyme action at a low moisture content (less than 54%) due to the enhanced diffusion from the formation of continuous mass in the extruder, without the requirement of increasing the water content. The continuous form increased enzyme action by supporting enzyme diffusion through material bed, thus enhancing the enzyme reaction rate at high solid concentrations. In the current study, the results likely suggest that hydrophilic components (e.g. xylan, arabinoxylan) readily absorbed water, and free water was not available or very limited. The xylanase enzyme likely did not have adequate acess to the rice bran due to inadequate diffusivity and limited water as a reactant.

The present observation showed that there was a significant increase in soluble starch content of extrusion-enzyme treated rice bran as compared to extruded rice bran. This increase must be from xylanase action. It is possible that this impure commercial enzyme had high activity of amylase and cellulase, acting quickly at 60^{o}C (barrel temperatures of the extruder) to

hydrolyze the starch and cellulose in washed rice bran, thus producing a certain amount of soluble fragments and being measured as soluble starch. Additionally, the figures of soluble starch jumped to 6-8% when feed moisture content increased to 45% and 60% as the starch hydrolyzing enzymes acted better in excess water conditions.

Changes in the minor compositions (soluble protein, soluble ash, and free pentose) of parallel extrusion-xylanase-treated rice bran with different levels of water addition and screw speeds followed the same trends as those of individually extruded rice bran as described in section 4.3. Therefore, these minor changes are not discussed again in this section.

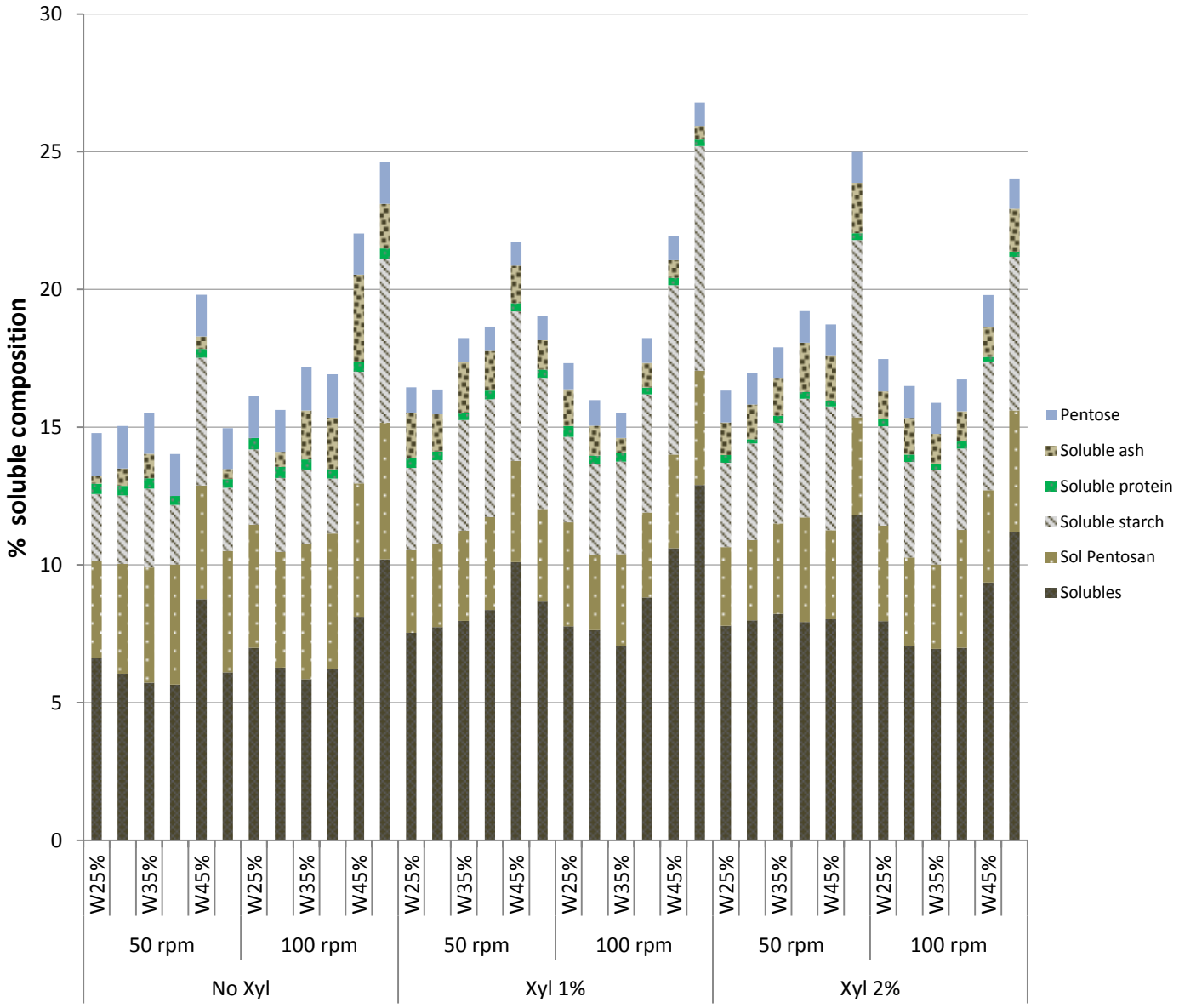


Figure 4.3 Effect of parallel combination of extrusion and enzyme hydrolysis on warm-water (37^{o}C) soluble composition of water-washed CDRB fiber concentrates

Xyl = Xylanase

W25% = 25% water addition

Sol pentosan = soluble pentosan

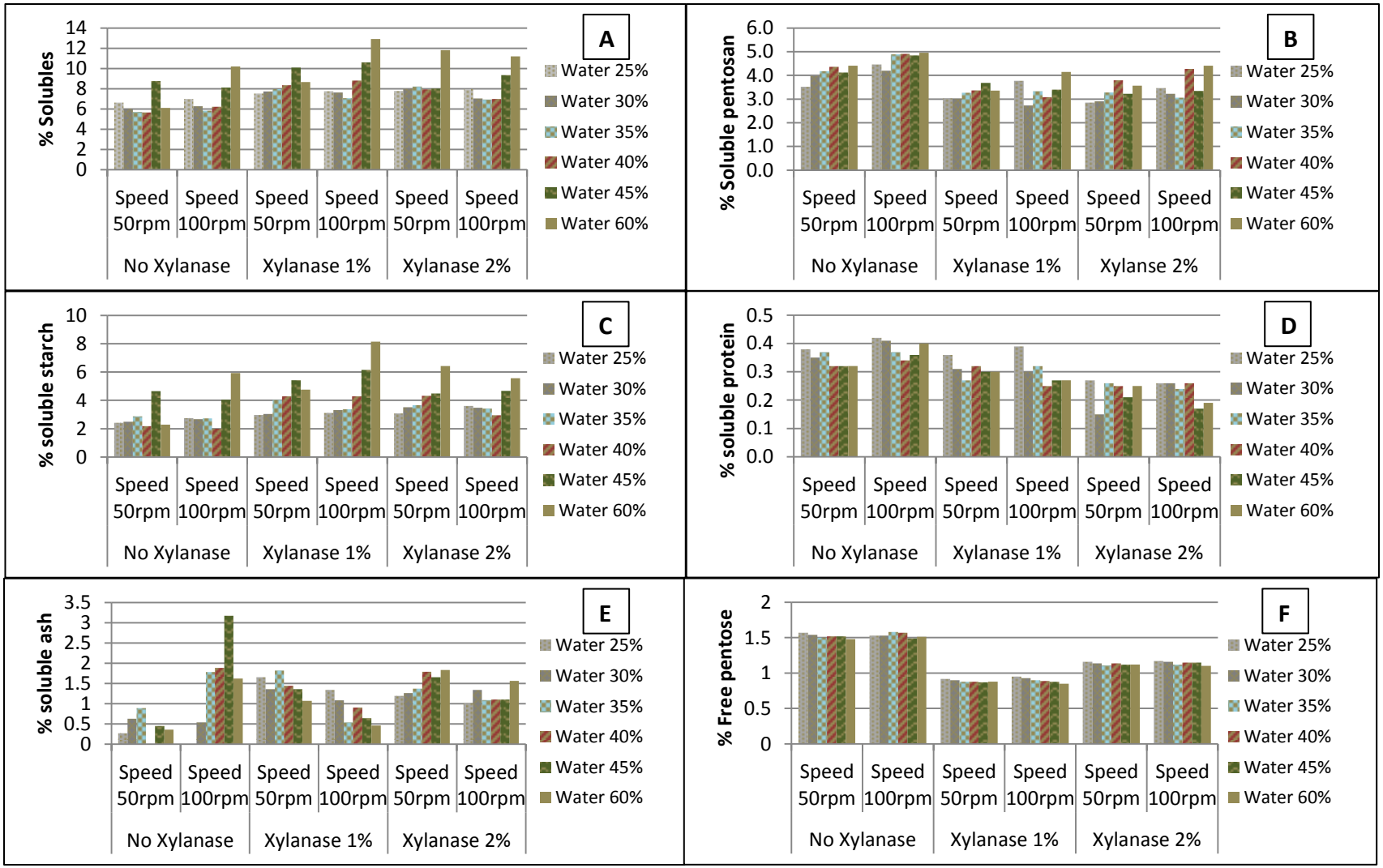


Figure 4.4 Effect of parallel combination of extrusion and enzyme hydrolysis on individual warm-water (37°C) soluble composition of water-washed CDRB fiber concentrates

Means and standard deviations of all the composition are reported in Table A of Appendix.

4.4.2 Extrusion and subsequent treatment of water-washed rice bran with xylanase enzyme

Figure 4.5 presents changes in the contents of solubles and soluble pentosans of extruded rice bran subsequently treated with BIO-CAT xylanase in comparison with treatments with individual enzyme and extrusion. The sequential combination of physical (extrusion) and enzymatic (xylanase) treatments significantly improved the content of soluble fractions of water-washed rice bran. These findings are in agreement with previous studies which also demonstrate that extrusion cooking of water-washed rice bran improves the efficiency of xylanase action on the bran, an effect particularly notable for solubilizing pentosan molecules (Figueroa-Espinoza, Poulsen, Soe, Zargahi, & Rouau, 2004; Santala et al., 2013). Similarly, pre-treatment with extrusion disintegrates the rigid structure of bran cell walls, allowing the cell wall hydrolyzing enzymes (e.g. xylanase) to more easily penetrate the cell wall structure (Hwang, Park, & Yun, 2003). SEM images in **Figure 4.6** are evidence for the cell wall disintegration. Compared with the honeycomb-like structure before treatment (**Figure 4.6A**), the sequential extrusion-enzyme treated structure was dramatically disrupted (**Figure 4.6E&F**), cell walls were collapsed and disintegrated compared to the originally observed state. These morphological changes were also seen in individually extruded (**Figure 4.6B**) and simultaneous extrusion-enzyme treated samples (**Figure 4.6C&D**) to a lesser extent. The combination of the two methods in this study, extrusion and xylanase treatments, regardless of sequence or in parallel, increased the total solubility of final products, compared with each individual process. Sequential combinations of extrusion and enzymatic were superior to a parallel approach in solubilizing pentosans.

While there were no significant changes in total solubles between sequential extrusion-enzyme treated samples solubilized in warm water compared to hot water, the content of hot water soluble pentosan was approximately twice its warm water counterpart. Thus extraction temperature is a considerably influential factor in pentosan extraction. At the higher temperature of 100^{o}C, even a shorter extraction time (10 minutes) could generate significantly more soluble pentosans. Approximately 11% content of hot-water soluble pentosan was obtained in the washed bran sample extruded with 45% added water and 100 rpm screw speed and subsequently hydrolyzed by 2% BIO-CAT xylanase. In comparison to a previous study, the hot-water soluble pentosan content in the present study was lower (11% opposed to 16.2% reported by Hwang,

Park, and Yun (2001)). This difference may be due to the differences in determination method for soluble pentosan, extrusion conditions, and the commercial enzyme used. The present study applied a spectrophotometric technique (instead of chromatography in the former study) to estimate the amount of soluble pentosan, extrusion conditions of 60^{o}C and 50 or 100 rpm (instead of 150^{o}C and 300 rpm) to water-washed rice bran, BIO-CAT xylanase (instead of a cell wall hydrolyzing enzyme cocktail) to hydrolyze the extruded product. A previous study reported the yields of soluble arabinoxylan of rice bran (16%), wheat bran (15.2%), rye bran (13.5%), corn bran (13.5%), barley bran (11.9%), oat bran (15.2%) (Hwang et al., 2001). The values of soluble pentosan of rice bran in both the present study and Hwang et al. (2001) were lower than that of other cereal brans. One explanation for this is that the molecular structure of arabinoxylan (a pentosan) chains from rice bran is more complex than those from wheat, rye, and barley. The rice bran arabinoxylan side chains contain not only arabinose residues but also xylopyranose, galactopyranose and α-D-glucuronic acid or 4-O-methyl-α-D-glucuronic residues (Izydorczyk & Biliaderis, 2007).

There was an increase of approximately 10% in average total warm- and hot-water solubles when the enzyme level increased from 1% to 2%, however, there was no effect on soluble pentosan content. This is explained by the proportional increase of soluble starch values (**Table 4.7**). As for the effect of screw speed (50 and 100 rpm) or for the two water addition levels (45 and 60%), there were no differences in the amount of total solubles or soluble pentosan produced. As for soluble protein (**Table 4.7**), there was a significant increase from 0.3% to 0.5% in soluble protein content when rice bran treated with both extrusion and enzyme in sequence. Enzymatic hydrolysis may have improved protein solubilization by releasing some protein molecules which are naturally associated with arabinoxylans, where were freed into soluble form when the arabinoxylans were hydrolyzed by xylanase. The similar trend was seen in soluble ash content which increased over extrusion and subsequent xylanase processing, except for the sample treated with 45% water addition and 100 rpm screw speed which decreased the ash solubilization after enzyme hydrolysis. In rice bran, phosphorus is the major constituent of ash, and 82% of phosphorus exists in bound form as phytate-phosphorus which is tightly associated to arabinoxylans (Goufo & Trindade, 2014). When xylanase hydrolyzed insoluble arabinoxylan into soluble fragments, a greater content of soluble fractions would increase the

quantity of entrapped phosphorus and thus provide an increase of soluble ash in most subsequent extrusion-enzyme aided samples.

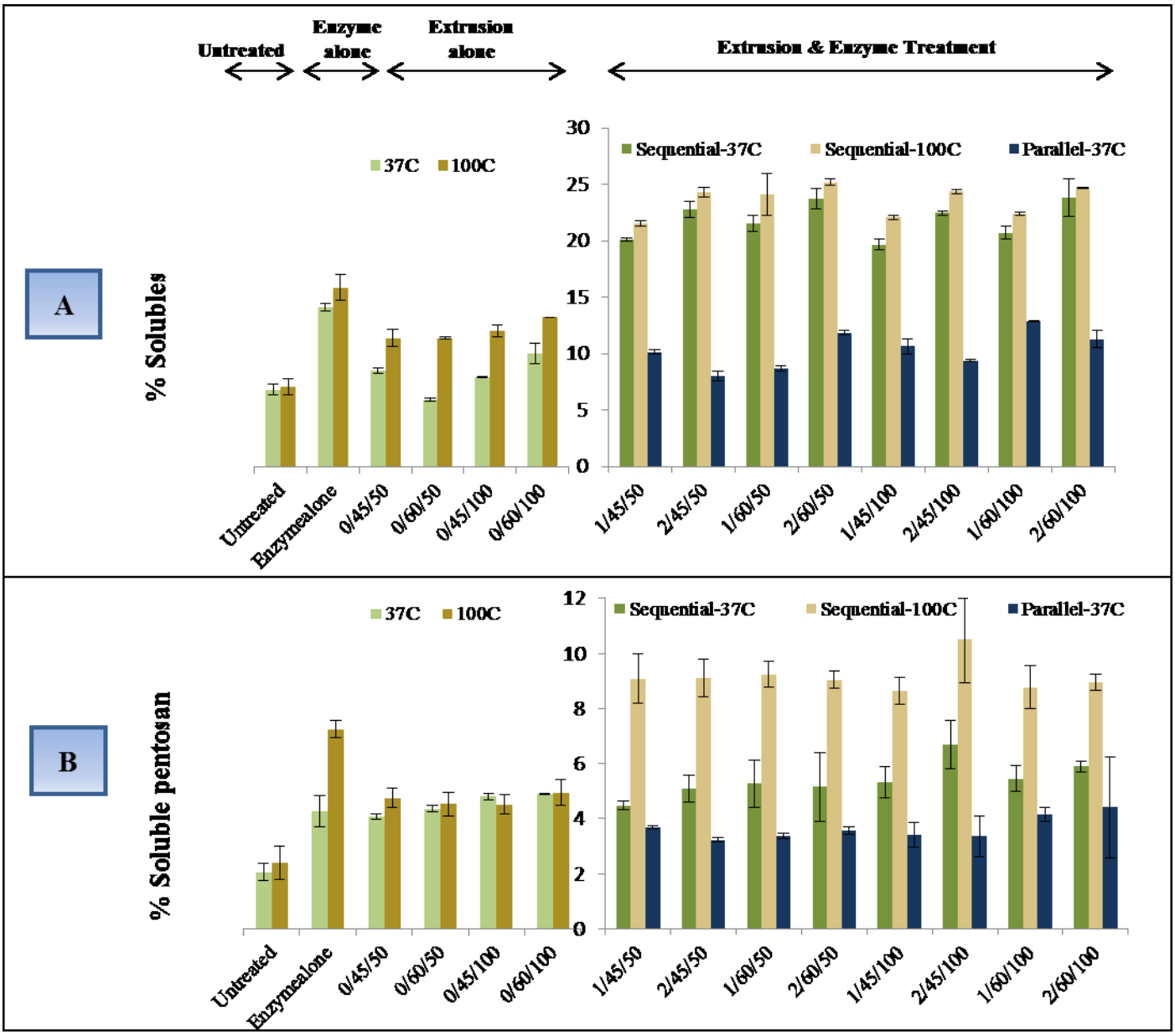


Figure 4.5 Changes in (A) solubles and (B) soluble pentosan of untreated, extruded, xylanase-treated, parallel extruded-xylanase, and sequential extruded-xylanase CDRB fiber concentrates

* % Xylanase (w/w)/ % water addition (w/w)/ screw speed (rpm)

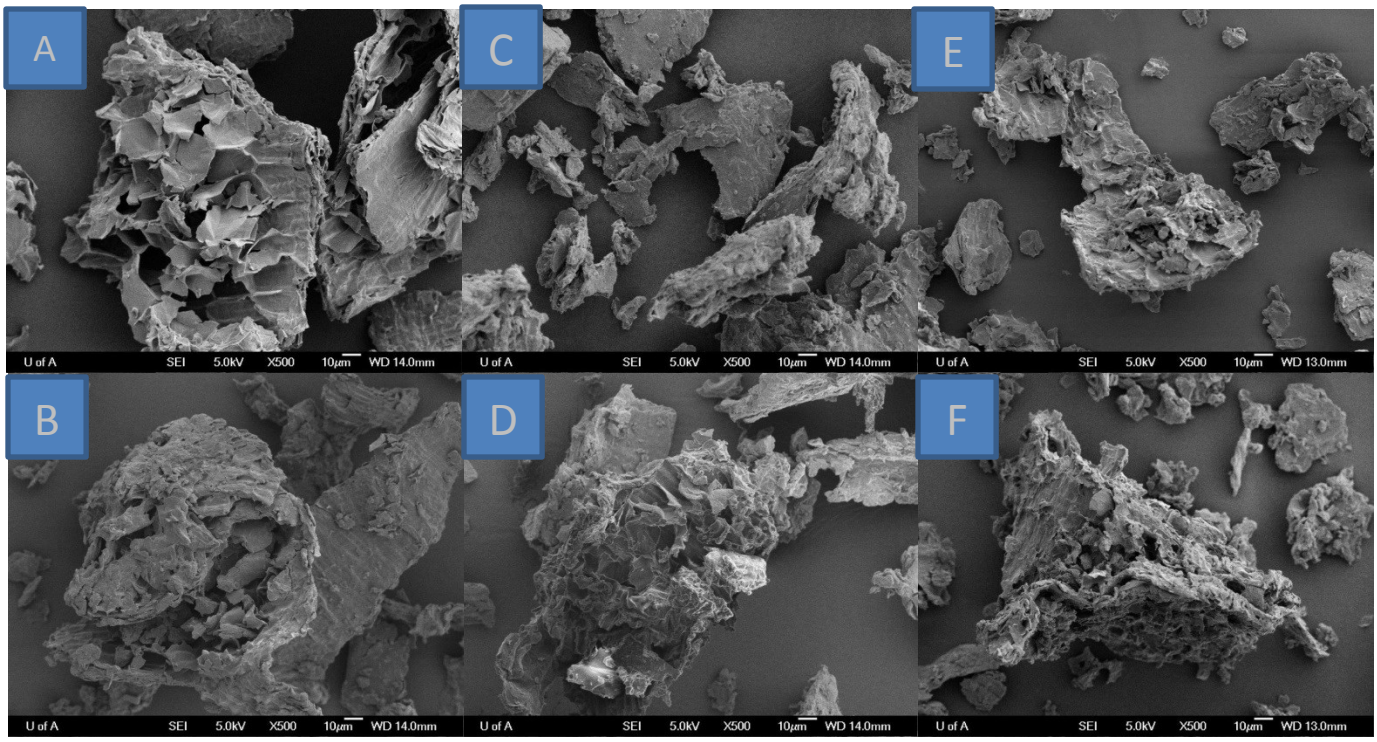


Figure 4.6 Scanning electron micrographs of (A) washed fiber concentrate, (B) extruded fiber concentrate, (C) parallel extrusion-1% xylanase treated fiber concentrate, (D) parallel extrusion-2% xylanase treated fiber concentrate, (E) sequential extrusion-1% xylanase treated fiber concentrate, and (F) sequential extrusion-2% xylanase treated fiber concentrates

Table 4.7 The % composition (water soluble at 37°C) of sequentially extruded & xylanase-treated rice bran fiber concentrates (dry basis)

Sample	Solubles	Soluble pentosan	Soluble starch	Soluble protein	Soluble ash	Pentose
0%/45%/50rpm	8.76±0.24^{g}	4.12±0.09^{d}	4.64±0.22^{f}	0.32±0.03^{d}	0.45±0.13^{d}	1.52±0.00^{a}
0%/60%/50rpm	6.10±0.15^{h}	4.41±0.13^{cd}	2.29±0.01^{g}	0.32±0.04^{d}	0.36±0.00^{d}	1.48±0.02^{b}
0%/45%/100rpm	8.11±0.06^{g}	4.85±0.11^{bcd}	4.05±0.20^{f}	0.36±0.05^{cd}	3.17±1.42^{a}	1.49±0.02^{ab}
0%/60%/100rpm	10.29±0.91^{f}	4.96±0.02^{bcd}	5.93±0.27^{e}	0.40±0.03^{c}	1.62±0.25^{bc}	1.51±0.01^{a}
1%/45%/50rpm	20.09±0.16e	4.48±0.17^{cd}	10.29±0.33^{cd}	0.51±0.02^{b}	1.01±0.13^{cd}	1.04±0.01^{c}
2%/45%/50rpm	22.80±0.70^{ab}	5.09±0.50^{bcd}	11.07±0.28^{ab}	0.54±0.02^{ab}	1.70±0.16^{bc}	1.04±0.01^{c}
1%/60%/50rpm	21.52±0.71^{cd}	5.27±0.85^{bcd}	10.44±0.23^{bcd}	0.55±0.03^{ab}	1.79±0.15^{bc}	1.04±0.01^{c}
2%/60%/50rpm	23.72±0.89^{a}	5.16±1.25^{bcd}	11.24±0.28^{a}	0.58±0.05^{a}	1.54±0.24^{bc}	1.04±0.01^{c}
1%/45%/100rpm	19.67±0.45^{e}	5.31±0.56^{bcd}	9.82±0.43^{d}	0.51±0.02^{b}	1.62±0.24^{bc}	1.03±0.01^{c}
2%/45%/100rpm	22.46±0.19^{bc}	6.68±0.89^{a}	10.85±0.64^{abc}	0.55±0.03^{ab}	1.53±0.48^{bc}	1.02±0.01^{c}
1%/60%/100rpm	20.71±0.59^{de}	5.45±0.46^{bc}	10.42±0.60^{cd}	0.54±0.03^{ab}	1.85±0.28^{bc}	1.04±0.01^{c}
2%/60%/100rpm	23.86±1.67^{a}	5.89±0.21^{ab}	11.27±0.47^{a}	0.57±0.02^{a}	2.21±0.37^{b}	1.03±0.02^{c}

All data represent the mean of two replicate measurements in duplicate treatments. Means within a column with different letters are significantly different ($p<0.05$).

* % Xylanase (w/w)/% water addition (w/w)/screw speed (rpm).

CHAPTER 5. SUMMARY AND CONCLUSIONS

A significant by-product of rice milling industry, rice bran is the most nutritional fraction of rice kernel since it contains equivalently high levels of protein, dietary fiber, starch, nutritious oils (highly unsaturated and unsaponifiable), and many bioactive phytochemicals. Nevertheless, rice bran is currently underutilized, mainly sold as animal feed, rarely used as a human food ingredient. Two major challenges the food industry needs to efficiently address to fully take advantage of rice bran's potential, is its inferior mouth-feel and tendency to become rancid. This texture sensory problem is partly because rice bran contains a large proportion of insoluble dietary fiber (IDF), whereas the rancidity factor is due to the high levels of unsaturated oils (82% out of rice bran oil) and the presence of active lipase enzymes. Currently, most rice bran producers stabilize their bran right after it comes out from the milling process using techniques such as extrusion cooking, heating, and chemical treatment. Increasing the proportion of soluble dietary fiber (SDF) in rice bran would add more value to potential products from rice bran processing by facilitating its incorporation into food formulations. High SDF rice bran would better promote human health benefits to the digestive system directly, and for metabolism generally regarding diabetes and high cholesterol management. Several current technologies are available that allow dietary fiber to be converted from insoluble to soluble forms. This study explored the use of both physical (extrusion) and enzymatic (xylanase) strategies to improve the SDF content by increasing soluble pentosan levels. Since extrusion cooking is a common, non-chemical method used by industry to heat-stabilize rice bran, and it can also promote solubilization of IDF, its use here in combination with xylanase represents the initial development of an efficient one-step method for rice bran stabilization that improves mouth feel characteristics.

The rice bran contains a pericarp, a seed coat, an aleurone, a germ and adherent part of starchy endosperm. It is technically impossible to obtain rice bran free of starchy endosperm during debranning and polishing, the two steps in a rice milling process. The adherent starchy endosperm bran fraction possesses a certain amount of starch and is influenced by the degree of milling. A larger proportion of starch proportionally lowers the dietary fiber level in the total composition of rice bran. Most previous research studying the effects of physical, chemical and enzymatic processing on compositional, nutritional, physicochemical and physiological

properties of rice bran have been conducted using non-washed or amylase-treated rice bran. The industry's pearling standards that produce bran with excess starchy endosperm and the necessary use of amylase to remove this excess starch from rice bran, motivated the author to investigate a more natural and economically viable approaches to gain higher level of dietary fiber in the resulting bran. The novel strategy in this current study was to wash rice bran with water. Washing rice bran with water not only was able to separate the majority of starch from the bran effectively and cost-efficiently, but at the same time it removed a considerable amount of lipid, some water-soluble protein, most soluble dietary fiber, and some phenolics. By having partially washed away these components, the remaining material contained more insoluble dietary fiber, which facilitated the conversion of insoluble fiber into a soluble form. From a practical industrial perspective, washing rice bran with water is advantageous because the residue collected from filterate (co-product) has a high starch content and other nutritionally valuable components (e.g. protein, lipid, fiber, phenolics). For these reasons, the present study developed a washing method that removed most of starch adherent in different rice bran forms (native, stabilized, and defatted) obtained from different sources.

The results demonstrated that water washing removed approximately 80% starch from native rice bran (NRB), 55% starch from stabilized rice bran (SRB), and 50% starch from commercial defatted and stabilized rice bran (C-DRB). A relatively low content of protein was washed away, namely 18% from NRB, 8% from SRB, and 9% from C-DRB. The lipid content of the three washed brans were also reduced by over 51% for NRB, 52% for SRB, and 9% for C-DRB., The washing method noticeably increased insoluble dietary fiber (IDF) by 120%, 76%, and 97%, respectively, for the three brans sources. Corresponding to the increase in the IDF, the pentosan content proportionally increased from 8.9 to 25.7% in NRB, 8.9 to 21.6% in SRB, and 11.3 to 21.9% in C-DRB. Unwashed rice brans naturally contain a small level (~4%) of soluble dietary fiber (SDF), and the washing significantly lowered the soluble dietary fiber level (1-2%). Although more than half of soluble dietary fiber was inevitably washed away during the process, the wasting amount (2-3%) was too small to consider as a problem as compared to the enormous increase of total dietary fiber. The majority of phytate, phosphorous and total ash was removed by washing as well. The lab-defatted rice bran (L-DRB) was obtained by defatting SRB using anhydrous ethanol, and was compositionally determined after washing. This L-DRB was a control. It enabled a comparison with C-DRB regarding the possible effects of different defatting

solvents (ethanol vs hexane) and stabilization methods (heating vs extrusion) on washed fiber yield and composition, as well as further fiber solubility upon treatment application (enzyme hydrolysis). This washed L-DRB contained significantly lower amounts of starch, protein, lipid, phytate, phosphorus and ash but higher levels of IDF and the same level of pentosan compared to washed C-DRB. It is possible that a very non-polar solvent like hexane was utilized to defat rice bran, and/or a technique like extrusion cooking that was used to stabilize the C-DRB rice bran, had particular impacts that resulted less components available for water washing in this study.

After washing, the efficiencies of four different commerical xylanase enzyme sources were analyzed. Here, the hydrolysis of insoluble dietary fiber in washed and xylanase-treated C-DRB and L-DRB was monitored by measuring soluble components in hot water (100^{o}C) and warm water (37^{o}C) extracts. ALI and BIO-CAT xylanases had equivalent pentosan-hydrolyzing efficiencies, and were greater than the ENZECO and Multifect 720 xylanases. ALI and BIO-CAT xylanases released the greatest content of total solubles which included soluble pentosan, soluble starch, soluble protein, soluble ash, free pentose, phytate and a minor amount of phosphorus. Hot-water-soluble and warm-water-soluble pentosan contents were the greatest for C-DRB (7.32% and 4.32%, respectively), and for L-DRB (9.19% and 6.79%, respectively). The soluble starch content significantly increased in all xylanase-treated samples, suggesting that the commercial xylanase preparations contained also amylase, cellulose, and/or β-glucanase contamination to produce soluble hexose sugars from the IDF.

Even though the washed L-DRB showed better solubility than washed C-DRB after enzyme treatment, the washed C-DRB was selected to proceed further with experiments due to economic, environmental and timing reasons. BIO-CAT xylanase was selected over other enzymes (ENZECO and Multifect 720) due to its higher hydrolysis efficacy in rice bran. Although BIO-CAT xylanase was relatively equivalent to ALI xylanase, BIO-CAT was selected randomly since only one enzyme was necessary for further experimentation.

Washed rice bran contained a high level of insoluble dietary fiber (>63%) consisting of macromolecules such as cellulose, hemicellulose, lignin, phytic acid, all bound strongly to each other to form a complex matrix. The extrusion process is able to efficiently dissociate the matrix because of the synergistic combination of high temperature, pressure, and shear force. When the matrix is disintegrated, some components are better solubilized in water due to the dissociation

forces at play. The effect of extrusion cooking on the subsequent solubility of washed rice bran was observed in this study. Generally, the solubility of extruded washed rice bran (EWRB) was significantly higher than that of untreated washed rice bran (UWRB). The increase in soluble pentosan and soluble starch primarily contributed to the increase in overall solubility. There were only minor changes in the solubility of other components. The solubility of EWRB slightly increased with screw speed; particularly the overall solubles content which increased by 12% when the screw speed was increased from 50 rpm to 100 rpm. The same pattern was observed for soluble pentosan and soluble starch. Regarding the effect of water addition, there were no significant differences in the soluble pentosan content of EWRB over the range of water addition levels studied (25-60%). However, a significant increase by 67% in overall solubles and by 730% in soluble starch was observed at 45% and 60% water addition. EWRB contained very little soluble protein (<1%), soluble ash (0-3%) or free pentose (~1%). A water addition level of 60% combined with a 100 rpm screw speed represented the highest level of total solubles (10.29%), soluble pentosan (4.96%), soluble starch (5.93%), soluble protein (0.4%), soluble ash (1.62%) and free pentose (1.51%).

Since both extrusion cooking and enzyme treatment can each contribute toward the solubility of dietary fiber in washed rice bran, combinations of the two approaches were evaluated to optimize their effects. Following a commonly used combination by previous authors for other cereal brans, the present study investigated enzyme treatment after extrusion in sequence. Also, the current study investigated a concurrent and parallel combination of extrusion and enzyme treatment. This would test the hypothesis that the combined conditions of high pressure, shear stress, low water, mild temperature and xylanase activity could efficiently increase the soluble composition in one single step. This combination would make the extruder into a bioreactor for enzyme hydrolysis as well, and was intended to benefit the processing industry by eliminating the need for separate incubators or reactors, by reducing the energy used for mixing the enzyme-bran mixture and for inactivating enzymes at the end, and by reducing the water added to the enzyme-bran mixture and thus minimize the drying requirement after enzyme treatment.

A parallel reaction combination approach did not change the solubility of pentosan as compared to extrusion alone. The author proposes that there were inadequate residency time

and/or water content. The average residence time of bran-enzyme mixture in the extruder was around 2 minutes, probably too short for xylanase to initiate its function. Similarly, water addition levels (25, 30, 35, 40, 45, and 60%) appeared to be only sufficient for fiber absorption, leaving limited free water for the enzyme to diffuse into the matrix, to react, and to permit the products to diffuse away from enzyme reaction site to allow more substrate catalysis. Overall, under the conditions studied, there was inadequate enzyme access to hydrolyze the insoluble fiber. Future research to take advantage of parallel reaction should focus on prolonging the residency time of the mixture in barrel by using a larger extruder with long L/D ratio barrel. Another approach could be to use a more aggressive screw configuration to increase the shear forces. A greater water content, lower feed rate, and greater enzyme addition could also be concurrently investigated in the future.

As expected, the sequential combination of extrusion and enzyme techniques dramatically improved the solubility of the rice bran compared to untreated, individually extruded, or xylanase-treated washed rice bran options. Hot-water solubles accounted for approximately 23% of sequential extrusion-xylanase treated rice bran (EXTRB) of which approximately 9% was soluble pentosan, whereas accordingly, the warm-water solubles accounted for approximately 22% solubles and 5.5% soluble pentosan. The warm-water soluble starch levels were noticeably high (~10%), suggesting that the sequentially physical and enzymatic combination had a dramatic impact not only on arabinoxylan, but also on glucans to greater extent. The glucans could be starch, cellulose, and heteropolysaccharides such as galactoglucan, xyloglucan, galactoxyloglucan, etc. from complex hemicellulose sources. A sugar profile in future research would be necessary to clarify these assumptions. Other components (soluble protein, soluble ash, and free pentose) constituted only a small amount in the EXTRB (~ 0.5% soluble protein, and ~1% each soluble ash and pentose), and experienced minor changes during the combined process.

Overall, washing rice bran with water was shown to be an efficient method to remove non-dietary fiber components. This study will likely represent the first published example for rice bran demonstrating an alternative to enzymatic methods used conventionally (e.g. amylase, protease, lipase) to hydrolyze non-dietary fiber compounds for further fiber processing. This current study has established the foundation to develop an efficient one step process. However,

the washing process produced a significant amount of by-product (rice bran wash) which contained water, starch, protein, lipid, and phenolics. Future research could focus on this by-product together with washed fiber concentrates in order to account for the materials, optimize the process, and to seek opportunities to co-utilize all components in the process. Additionally, future research could seek to optimize the hydrolysis conditions for the xylanase enzyme (temperature, time, pH, water addition, etc.). The next step is to optimize extruder conditions that would both increase residency time for enzyme exposure, and shear forces to improve dissociation of components from the matrix. To achieve this end, optimizing the parallel combination of extrusion and enzyme reaction should focus on a larger and longer barrel, higher water addition, lower feed rate, and higher enzyme levels. Furthermore, characterization of soluble fractions of extruded, enzyme-treated, simultaneous extrusion-enzyme treated, and sequential extrusion-enzyme treated rice bran would provide greater insight and understanding of the soluble dietary fiber profile (monosaccharides, disaccharides, and oligosaccharides). Lastly, future research should explore the physico-chemical, nutritional, and sensory properties of the treated rice bran “as is” and after inclusion into food products so that its utilization can be extended in the food industry.

REFERENCES

Abdul-Hamid, A., & Luan, Y. S. (2000). Functional Properties of Dietary Fibre Prepared from Defatted Rice Bran. *Food Chemistry, 68*(1), 15-19.

Adebiyi, A. P., Adebiyi, A. O., Hasegawa, Y., Ogawa, T., & Muramoto, K. (2009). Isolation and Characterization of Protein Fractions from Deoiled Rice Bran. *European Food Research & Technology, 228*(3), 391-401.

Adebiyi, A. P., Adebiyi, A. O., Yamashita, J., Ogawa, T., & Muramoto, K. (2009). Purification and Characterization of Antioxidative Peptides Derived from Rice Bran Protein Hydrolysates. *European Food Research and Technology, 228*(4), 553-563.

Akihisa, T., Yasukawa, K., Yamaura, M., Ukiya, M., Kimura, Y., Shimizu, N., & Arai, K. (2000). Triterpene Alcohol and Sterol Ferulates from Rice Bran and Their Anti-Inflammatory Effects. *Journal of Agricultural and Food Chemistry, 48*(6), 2313–2319.

Alonso, R., Rubio, L. A., Muzquiz, M., & Marzo, F. (2001). The Effect of Extrusion Cooking on Mineral Bioavailability in Pea and Kidney Bean Seed Meals. *Animal Feed Science and Technology, 94*(1-2), 1-13.

Anderson, J. W., Deakins, D. A., Floore, T. L., Smith, B. M., & Whitis, S. E. (1990). Dietary Fiber and Coronary Heart Disease. *Critical Reviews In Food Science And Nutrition, 29*(2), 95-147.

Anderson, R. A., Conway, H. F., & Peplinsk, A. J. (1970). Gelatinization of Corn Grits by Roll Cooking, Extrusion Cooking and Steaming. *Starch-Starke, 22*(4), 130-135.

Andersson, Y., Hedlund, B., Jonsson, L., & Svensson, S. (1981). Extrusion Cooking of a High-Fiber Cereal Product with Crispbread Character. *Cereal Chemistry, 58*(5), 370-374.

Aoe, S., Oda, T., Tatsumi, K., Yamauchi, M., & Ayano, Y. (1993). Extraction of Soluble Dietary Fibers from Defatted Rice Bran. *Cereal Chemistry, 70*(4), 423-425.

Aoe, S., Oda, T., Tojima, T., Tanaka, M., Tatsumi, K., & Mizutani, T. (1993). Effects of Rice Bran Hemicellulose on 1,2-Dimethylhydrazine-Induced Intestinal Carcinogenesis in Fischer 344 Rats. *Nutrition and Cancer, 20*(1), 41-49.

Arendt, E. K., & Zannini, E. (2013). Rice. In E. K. Arendt & E. Zannini (Eds.), *Cereal Grains for the Food and Beverage Industries* (pp. 114-154). Cambridge: Woodhead Publishing.

Aryusuk, K., Puengtham, J., Lilitchan, S., Jeyashoke, N., & Krisnangkura, K. (2008). Effects of Crude Rice Bran Oil Components on Alkali-Refining Loss. *Journal of the American Oil Chemists Society, 85*(5), 475-479.

Asano, H., Hirano, F., Isobe, K., & Sakurai, H. (2000). Effect of Harvest Time on the Protein Composition (Glutelin, Prolamin, Albumin) and Amylose Content in Paddy Rice Cultivated by Aigamo Duck Farming System. *Japanese Journal of Crop Science, 69*(3), 320-323.

Aspinall, G. O. (1959). Structural Chemistry of the Hemicelluloses. *Advances in Carbohydrate Chemistry, 14*, 429-468.

Bhattacharya, K. R. (2004). Parboiling of Rice. In E. T. Champagne (Ed.), *Rice: Chemistry and Technology* (3rd ed., pp. 329-404). St. Paul, Minnesota: American Association of Cereal Chemists.

Bhattacharya, K. R., & Rao, P. V. S. (1966a). Processing Conditions and Milling Yield in Parboiling of Rice. *Journal of Agricultural and Food Chemistry, 14*(5), 473–475.

Bhattacharya, K. R., & Rao, P. V. S. (1966b). Effect of Processing Conditions on Quality of Parboiled Rice. *Journal of Agricultural and Food Chemistry, 14*(5), 476-&.

Bhattacharya, K. R., & Swamy, Y. M. I. (1967). Conditions of Drying Parboiled Paddy for Optimum Milling Quality. *Cereal Chemistry, 44*(6), 592-600.

Bhattacharyya, A. C., Majumdar, S., & Bhattacharyya, D. K. (1986). Edible Quality Rice Bran Oil from High Ffa Rice Bran Oil by Miscella Refining. *Journal of the American Oil Chemists Society, 63*(9), 1189-1191.

Bhattacharyya, A. C., Majumdar, S., & Bhattacharyya, D. K. (1987). Refining of High Ffa Rice Bran Oil by Isopropanol Extraction and Alkali Neutralization. *Oleagineux, 42*(11), 431-433.

Bhattacharyya, D. K., Chakrabarty, M. M., Vaidyanathan, R. S., & Bhattacharyya, A. C. (1983). A Critical-Study of the Refining of Rice Bran Oil. *Journal of the American Oil Chemists Society, 60*(2), 467-467.

Bhattacharyya, S., & Bhattacharyya, D. K. (1989). Biorefining of High Acid Rice Bran Oil. *Journal of the American Oil Chemists Society, 66*(12), 1809-1811.

Bianchi, G., Lupotto, E., & Russo, S. (1979). Composition of Epicuticular Wax of Rice, Oryza-Sativa. *Experientia, 35*(11), 1417.

Björck, I., & Asp, N. G. (1983). The Effects of Extrusion Cooking on Nutritional Value - a Literature Review. *Journal of Food Engineering, 2*(4), 281-308.

Bjorck, I., Nyman, M., & Asp, N. G. (1984). Extrusion Cooking and Dietary Fiber - Effects on Dietary Fiber Content and on Degradation in the Rat Intestinal Tract. *Cereal Chemistry, 61*(2), 174-179.

Bond, N. (2004). Rice Milling. In E. T. Champagne (Ed.), *Rice: Chemistry and Technology* (3rd ed., pp. 283-300). St. Paul, Minnesota: American Association of Cereal Chemists.

Borresen, E. C., & Ryan, E. P. (2014). Rice Bran: A Food Ingredient with Global Public Health Opportunities. In R. R. Watson, V. Preedy & S. Zibadi (Eds.), *Wheat and Rice in Disease Prevention and Health* (pp. 301-310). San Diego: Academic Press.

Brown, L., Rosner, B., Willett, W. W., & Sacks, F. M. (1999). Cholesterol-Lowering Effects of Dietary Fiber: A Meta-Analysis. *American Journal of Clinical Nutrition, 69*(1), 30-42.

Bucci, R., Magri, A. D., Magri, A. L., & Marini, F. (2003). Comparison of Three Spectrophotometric Methods for the Determination of Gamma-Oryzanol in Rice Bran Oil. *Analytical and Bioanalytical Chemistry, 375*(8), 1254-1259.

Cai, H., Hudson, E. A., Mann, P., Verschoyle, R. D., Greaves, P., Manson, M. M., Steward, W. P., & Gescher, A. J. (2004). Growth-Inhibitory and Cell Cycle-Arresting Properties of the Rice Bran Constituent Tricin in Human-Derived Breast Cancer Cells in Vitro and in Nude Mice in Vivo. *British Journal of Cancer, 91*(7), 1364-1371.

Camire, M. E., Camire, A., & Krumhar, K. (1990). Chemical and Nutritional Changes in Foods During Extrusion. *Critical Reviews in Food Science and Nutrition, 29*(1), 35-57.

Carroll, L. E. (1990). Functional Properties and Applications of Stabilized Rice Bran in Bakery Products. *Food Technology, 44*(4), 74-76.

Cartano, A. V., & Juliano, B. O. (1970). Hemicelluloses of Milled Rice. *Journal of Agricultural and Food Chemistry, 18*(1), 40-42.

Cavanagh, G. C. (1976). Miscella Refining. *Journal of the American Oil Chemists Society, 53*(6), 361-363.

Champagne, E. T., Wood, D. F., Juliano, B. O., & Bechtel, D. B. (2004). Rice Grain and Its Gross Composition. In E. T. Champagne (Ed.), *Rice: Chemistry and Technology* (pp. 77-107). St. Paul, Minnesota: American Association of Cereal Chemists.

Chandrashekar, P., Kumar, P. K. P., Ramesh, H. P., Lokesh, B. R., & Krishna, A. G. G. (2014). Hypolipidemic Effect of Oryzanol Concentrate and Low Temperature Extracted Crude Rice Bran Oil in Experimental Male Wistar Rats. *Journal of Food Science and Technology-Mysore, 51*(7), 1278-1285.

Chang, N. W., & Huang, P. C. (1998). Effects of the Ratio of Polyunsaturated and Monounsaturated Fatty Acid to Saturated Fatty Acid on Rat Plasma and Liver Lipid Concentrations. *Lipids, 33*(5), 481-487.

Chanput, W., Theerakulkait, C., & Nakai, S. (2009). Antioxidative Properties of Partially Purified Barley Hordein, Rice Bran Protein Fractions and Their Hydrolysates. *Journal of Cereal Science, 49*(3), 422-428.

Cheftel, J. C., Kitagawa, M., & Queguiner, C. (1992). New-Protein Texturization Processes by Extrusion Cooking at High Moisture Levels. *Food Reviews International, 8*(2), 235-275.

Chotimarkorn, C., & Silalai, N. (2008). Addition of Rice Bran Oil to Soybean Oil During Frying Increases the Oxidative Stability of the Fried Dough from Rice Flour During Storage. *Food Research International, 41*(3), 308-317.

Choudhury, N. H., & Juliano, B. O. (1980). Effect of Amylose Content on the Lipids of Mature Rice Grain. *Phytochemistry, 19*(7), 1385-1389.

Cicero, A. F. G., & Gaddi, A. (2001). Rice Bran Oil and Gamma-Oryzanol in the Treatment of Hyperlipoproteinaemias and Other Conditions. *Phytotherapy Research, 15*(4), 277-289.

Claye, S. S., Idouraine, A., & Weber, C. W. (1996). Extraction and Fractionation of Insoluble Fiber from Five Fiber Sources. *Food Chemistry, 57*(2), 305-310.

Cousins, E. R., Prachankadee, R., & Bhodhiprasart, S. (1955). Ethanolamines and Other Amino-Containing and Hydroxyl-Containing Compounds in the Refining of Rice Oil. *Journal of the American Oil Chemists Society, 32*(11), 561-564.

Cowan, J. C. (1976). Degumming, Refining, Bleaching, and Deodorization Theory. *Journal of the American Oil Chemists Society, 53*(6), 344-346.

Daou, C., & Zhang, H. (2012). Study on Functional Properties of Physically Modified Dietary Fibres Derived from Defatted Rice Bran. *Journal of Agricultural Science, 4*(9), p85.

Dawe, D., Robertson, R., & Unnevehr, L. (2002). Golden Rice: What Role Could It Play in Alleviation of Vitamin a Deficiency? *Food Policy, 27*(5-6), 541-560.

De, B. K., & Bhattacharyya, D. K. (1998). Physical Refining of Rice Bran Oil in Relation to Degumming and Dewaxing. *Journal of the American Oil Chemists Society, 75*(11), 1683-1686.

de Delahaye, E. P., Jimenez, P., & Perez, E. (2005). Effect of Enrichment with High Content Dietary Fiber Stabilized Rice Bran Flour on Chemical and Functional Properties of Storage Frozen Pizzas. *Journal of Food Engineering, 68*(1), 1-7.

deDeckere, E. A. M., & Korver, O. (1996). Minor Constituents of Rice Bran Oil as Functional Foods. *Nutrition Reviews, 54*(11), S120-S126.

Devi, R. R., & Arumughan, C. (2007). Phytochemical Characterization of Defatted Rice Bran and Optimization of a Process for Their Extraction and Enrichment. *Bioresource Technology, 98*(16), 3037-3043.

Dijkstra, A. J., & Opstal, M. V. (1989). The Total Degumming Process. *Journal of the American Oil Chemists Society, 66*(7), 1002-1009.

Dikeman, C. L., Murphy, M. R., & Fahey, G. C. (2006). Dietary Fibers Affect Viscosity of Solutions and Simulated Human Gastric and Small Intestinal Digesta. *Journal of Nutrition, 136*(4), 913-919.

Dornez, E., Gebruers, K., Delcour, J. A., & Courtin, C. A. (2009). Grain-Associated Xylanases: Occurrence, Variability, and Implications for Cereal Processing. *Trends in Food Science & Technology, 20*(11-12), 495-510.

Dreher, M. L. (2001). Dietary Fiber in Health and Disease. In S. S. Cho & M. L. Dreher (Eds.), *Handbook of Dietary Fiber* (pp. 1-16). New York Marcel Dekker.

Elbert, G., Tolaba, M. P., & Suarez, C. (2001). Model Application: Hydration and Gelatinization During Rice Parboiling. *Drying Technology, 19*(3-4), 571-581.

Esa, N. M., Ling, T. B., & Peng, L.S. (2013). By-Products of Rice Processing: An Overview of Health Benefits and Applications. *Journal of Rice Research, 1*(1), 107.

Fabian, C., & Ju, Y. H. (2011). A Review on Rice Bran Protein: Its Properties and Extraction Methods. *Critical Reviews in Food Science and Nutrition, 51*(9), 816-827.

Fairweathertait, S. J., Portwood, D. E., Symss, L. L., Eagles, J., & Minski, M. J. (1989). Iron and Zinc-Absorption in Human-Subjects from a Mixed Meal of Extruded and Nonextruded Wheat Bran and Flour. *American Journal of Clinical Nutrition, 49*(1), 151-155.

Fan, H., Morioka, T., & Ito, E. (2000). Induction of Apoptosis and Growth Inhibition of Cultured Human Endometrial Adenocarcinoma Cells (Sawano) by an Antitumor Lipoprotein Fraction of Rice Bran. *Gynecologic Oncology, 76*, 170-175.

Faraj, A., Vasanthan, T., & Hoover, R. (2004). The Effect of Extrusion Cooking on Resistant Starch Formation in Waxy and Regular Barley Flours. *Food Research International, 37*(5), 517-525.

Figueroa-Espinoza, M. C., Poulsen, C., Soe, J. B., Zargahi, M. R., & Rouau, X. (2004). Enzymatic Solubilization of Arabinoxylans from Native, Extruded, and High-Shear-Treated Rye Bran by Different Endo-Xylanases and Other Hydrolyzing Enzymes. *Journal of Agricultural and Food Chemistry, 52*(13), 4240-4249.

Fitzgerald, M. (2004). Starch. In E. T. Champagne (Ed.), *Rice: Chemistry and Technology* (3rd ed.). St Paul, Minnesota: American Association of Cereal Chemists.

Fujino, Y. (1978). Rice Lipids. *Cereal Chemistry, 55*(5), 559-571.

Gastrock, E. A., Vix, H. L. E, Aquin, D. E. L., Graci, A. V., & Spadaro, J. J. (1955). Rice Bran Oil Extraction Process: Google Patents.

Ghatak, S. B., & Panchal, S. J. (2012). Investigation of the Immunomodulatory Potential of Oryzanol Isolated from Crude Rice Bran Oil in Experimental Animal Models. *Phytotherapy Research, 26*(11), 1701-1708.

Ghatak, S. B., & Panchal, S. S. (2012). Protective Effect of Oryzanol Isolated from Crude Rice Bran Oil in Experimental Model of Diabetic Neuropathy. *Revista Brasileira De Farmacognosia-Brazilian Journal of Pharmacognosy, 22*(5), 1092-1103.

Ghoneum, M. (1998). Anti-Hiv Activity in Vitro of Mgn-3, an Activated Arabinoxylane from Rice Bran. *Biochemical and Biophysical Research Communications, 243*(1), 25-29.

Ghoneum, M., & Gollapudi, S. (2005). Synergistic Role of Arabinoxylan Rice Bran (Mgn-3/Biobran) in S. Cerevisiae-Induced Apoptosis of Monolayer Breast Cancer Mcf-7 Cells. *Anticancer Research, 25*(6B), 4187-4196.

Ghosh, M. (2007). Review on Recent Trends in Rice Bran Oil Processing. *Journal of the American Oil Chemists Society, 84*(4), 315-324.

Goufo, P., & Trindade, H. (2014). Rice Antioxidants: Phenolic Acids, Flavonoids, Anthocyanins, Proanthocyanidins, Tocopherols, Tocotrienols, Γ-Oryzanol, and Phytic Acid. *Food Science & Nutrition, 2*(2), 75-104.

Gremli, H., & Juliano, B. O. (1970). Studies on Alkali-Soluble, Rice-Bran Hemicelluloses. *Carbohydrate Research, 12*(2), 273-276.

Gualberto, D. G., Bergman, C. J., Kazemzadeh, M., & Weber, C. W. (1997). Effect of Extrusion Processing on the Soluble and Insoluble Fiber, and Phytic Acid Contents of Cereal Brans. *Plant Foods for Human Nutrition, 51*(3), 187-198.

Gui, Y., Gil, S. K., & Ryu, G. H. (2012). Effects of Extrusion Conditions on the Physicochemical Properties of Extruded Red Ginseng. *Preventive Nutrition and Food Science, 17*(3), 203-209.

Hamada, J. S. (1997). Characterization of Protein Fractions of Rice Bran to Devise Effective Methods of Protein Solubilization. *Cereal Chemistry, 74*(5), 662-668.

Haraldsson, G. (1983). Degumming, Dewaxing and Refining. *Journal of the American Oil Chemists' Society, 60*(2), 251-256.

Hartman, L., & Dosreis, M. I. J. (1976). Study of Rice Bran Oil Refining. *Journal of the American Oil Chemists Society, 53*(4), 149-151.

Hashimoto, S., Shogren, M. D., & Pomeranz, Y. (1987). Cereal Pentosans - Their Estimation and Significance .1. Pentosans in Wheat and Milled Wheat Products. *Cereal Chemistry, 64*(1), 30-34.

Herfel, T., Jacobi, S., Lin, X., van Heugten, E., Fellner, V., & Odle, J. (2013). Stabilized Rice Bran Improves Weaning Pig Performance Via a Prebiotic Mechanism. *Journal of Animal Science, 91*(2), 907-913.

Hoffmann, R. A., Roza, M., Maat, J., Kamerling, J. P., & Vliegenthart, J. F. G. (1991a). Structural Characteristics of the Cold-Water-Soluble Arabinoxylans from the White Flour of the Soft Wheat Variety Kadet. *Carbohydrate Polymers, 15*(4), 415-430.

Hoffmann, R. A., Roza, M., Maat, J., Kamerling, J., & Vliegenthart, J. F. G. (1991b). Structural Characteristics of the Warm-Water-Soluble Arabinoxylans from the Tailings of the Soft Wheat Variety Kadet. *Carbohydrate Polymers, 16*(3), 275-289.

Houston, D. F., Iwasaki, T., Mohammad, A., & Chen, L. (1968). Radial Distribution of Protein by Solubility Classes in Milled Rice Kernel. *Journal of Agricultural and Food Chemistry, 16*(5), 720-724.

Hu, G. H., Huang, S. H., Cao, S. W., & Ma, Z. Z. (2009). Effect of Enrichment with Hemicellulose from Rice Bran on Chemical and Functional Properties of Bread. *Food Chemistry, 115*(3), 839-842.

Hu, G. H., & Yu, W. J. (2013). Binding of Cholesterol and Bile Acid to Hemicelluloses from Rice Bran. *International Journal of Food Sciences and Nutrition, 64*(4), 461-466.

Hwang, J., Park, B., & Yun, J. (2001). Biologically Active Materials from Cereals and Process for Preparation Thereof: Google Patents.

Hwang, J., Park, B., & Yun, J. (2003). Extrusion and Subsequent Treatment of Cereal Bran with Cell Wall Hydrolyzing Enzymes: Google Patents.

Ikeda, I., Nakashimayoshida, K., & Sugano, M. (1985). Effects of Cycloartenol on Absorption and Serum Levels of Cholesterol in Rats. *Journal of Nutritional Science and Vitaminology, 31*(3), 375-384.

Itani, T., Tamaki, M., Arai, E., & Horino, T. (2002). Distribution of Amylose, Nitrogen, and Minerals in Rice Kernels with Various Characters. *Journal of Agricultural and Food Chemistry, 50*(19), 5326-5332.

Itoh, T., Tamura, T., & Matsumot, T. (1973). Sterol Composition of 19 Vegetable Oils. *Journal of the American Oil Chemists Society, 50*(4), 122-125.

Iwasaki, T., Shibuya, N., Suzuki, T., & Chikubu, S. (1982). Gel-Filtration and Electrophoresis of Soluble Rice Proteins Extracted from Long, Medium, and Short Grain Varieties. *Cereal Chemistry, 59*(3), 192-195.

Izydorczyk, M. S., & Biliaderis, C. G. (2007). Arabinoxylans: Technologically and Nutritionally Functional Plant Polysaccharides *Functional Food Carbohydrates* (pp. 249-290). Boca Raton, FL: CRC Press.

Jeon, S. J., Singkhornart, S., & Ryu, G. H. (2014). The Effect of Extrusion Conditions on Water-Extractable Arabinoxylans from Corn Fiber. *Preventive Nutrition and Food Science, 19*(2), 124-127.

Jing, Y., & Chi, Y. J. (2013). Effects of Twin-Screw Extrusion on Soluble Dietary Fibre and Physicochemical Properties of Soybean Residue. *Food Chemistry, 138*(2-3), 884-889.

Juliano, B. O. (1971). A Simplified Assay for Milled-Rice Amylose. *Cereal Science Today, 16*(10), 334.

Juliano, B. O. (1985a). Rice Bran. In B. O. Juliano (Ed.), *Rice Chemistry and Technology* (pp. 647-687). St. Paul, Minnesota: American Association of Cereal Chemists.

Juliano, B. O. (1985b). *Rice: Chemistry and Technology* (2nd ed.). St. Paul, Minnesota: American Association of Cereal Chemists.

Juliano, B. O. (1993). *Rice in Human Nutrition*. Rome: International Rice Research Institute & Food and Agriculture Organization of the United Nations.

Juliano, B. O., & Villareal, C. P. (1993). *Grain Quality Evaluation of World Rices*. Manila, Philippines: IRRI.

Kahlon, T. S. (2009). Rice Bran. In S. S. Cho & P. Samuel (Eds.), *Fiber Ingredients* (pp. 305-321). Boca Raton, FL: CRC Press.

Kahlon, T. S., Chow, F. I., Chiu, M. M., Hudson, C. A., & Sayre, R. N. (1996). Cholesterol-Lowering by Rice Bran and Rice Bran Oil Unsaponifiable Matter in Hamsters. *Cereal Chemistry, 73*(1), 69-74.

Kanematsu, H., Ushigusa, T., Maruyama, T., Niiya, I., Fumoto, D., Toyoda, T., Kawaguchi, Y., & Matsumoto, T. (1983). Comparison of Tocopherol Contents in Crude and Refined Edible Vegetable Oils and Fats by High Performance Liquid Chromatography. *Journal of Japan Oil Chemists' Society, 32*(2), 122-126.

Kataoka, K., Ogasa, S., Kuwahara, T., Bando, Y., Hagiwara, M., Arimochi, H., Nakanishi, S., Iwasaki, T., & Ohnishi, Y. (2008). Inhibitory Effects of Fermented Brown Rice on Induction of Acute Colitis by Dextran Sulfate Sodium in Rats. *Digestive Diseases and Sciences, 53*(6), 1601-1608.

Katayama, M., Sugie, S., Yoshimi, N., Yamada, Y., Sakata, K., Qiao, Z., Iwasaki, T., Kobayashi, H., & Mori, H. (2003). Preventive Effect of Fermented Brown Rice and Rice Bran on Diethylnitrosoamine and Phenobarbital-Induced Hepatocarcinogenesis in Male F344 Rats. *Oncology Reports, 10*(4), 875-880.

Kawamura, Y., & Ishikawa, M. (1993). Anti-Tumorigenic and Immunoactive Protein and Peptide Factors in Foodstuffs. 2. Antitumorigenic Factors in Rice Bran. In K. W. Waldron, I. T. Johnson & G. R. Fenwick (Eds.), *Food and Cancer Prevention: Chemical and Biological Aspects* (pp. 327-330). Cambridge, England: Woodhead Publishing Ltd.

Kehrer, J. P. (1993). Free-Radicals as Mediators of Tissue-Injury and Disease. *Critical Reviews in Toxicology, 23*(1), 21-48.

Kestin, M., Moss, R., Clifton, P. M., & Nestel, P. J. (1990). Comparative Effects of Three Cereal Brans on Plasma Lipids, Blood Pressure, and Glucose Metabolism in Mildly Hypercholesterolemic Men. *American Journal of Clinical Nutrition, 52*(4), 661-666.

Khan, S. H., Butt, M. S., Anjum, F. M., & Sameen, A. (2011). Quality Evaluation of Rice Bran Protein Isolate-Based Weaning Food for Preschoolers. *International Journal of Food Sciences and Nutrition, 62*(3), 280-288.

Kim, J. S., & Godber, J. S. (2001). Oxidative Stability and Vitamin E Levels Increased in Restructured Beef Roasts with Added Rice Bran Oil. *Journal of Food Quality, 24*(1), 17-26.

Kim, J. S., Suh, M. H., Yang, C. B., & Lee, H. G. (2003). Effect of Gamma-Oryzanol on the Flavor and Oxidative Stability of Refrigerated Cooked Beef. *Journal of Food Science, 68*(8), 2423-2429.

Kim, S. K., Kim, C. J., Cheigh, H. S., & Yoon, S. H. (1985). Effect of Caustic Refining, Solvent Refining and Steam Refining on the Deacidification and Color of Rice Bran Oil. *Journal of the American Oil Chemists Society, 62*(10), 1492-1495.

Kim, S. P., Kang, M. Y., Nam, S. H., & Friedman, M. (2012). Dietary Rice Bran Component Gamma-Oryzanol Inhibits Tumor Growth in Tumor-Bearing Mice. *Molecular Nutrition & Food Research, 56*(6), 935-944.

Komiyama, Y., Andoh, A., Fujiwara, D., Ohmae, H., Araki, Y., Fujiyama, Y., Mitsuyama, K., & Kanauchi, O. (2011). New Prebiotics from Rice Bran Ameliorate Inflammation in Murine Colitis Models through the Modulation of Intestinal Homeostasis and the Mucosal Immune System. *Scandinavian Journal of Gastroenterology, 46*(1), 40-52.

Kong, C. K., Lam, W. S., Chiu, L. C., Ooi, V. E., Sun, S. S., & Wong, Y. S. (2009). A Rice Bran Polyphenol, Cycloartenyl Ferulate, Elicits Apoptosis in Human Colorectal Adenocarcinoma Sw480 and Sensitizes Metastatic Sw620 Cells to Trail-Induced Apoptosis. *Biochemical Pharmacology, 77*(9), 1487-1496.

Kormelink, F. J. M., & Voragen, A. G. J. (1993). Degradation of Different ((Glucurono)Arabino)Xylans by a Combination of Purified Xylan-Degrading Enzymes. *Applied microbiology and biotechnology, 38*(5), 688-695.

Krishna, A. G. G., Khatoon, S., & Babylatha, R. (2005). Frying Performance of Processed Rice Bran Oils. *Journal of Food Lipids, 12*(1), 1-11.

Krishna, A. G. G., Khatoon, S., Shiela, P. M., Sarmandal, C. V., Indira, T. N., & Mishra, A. (2001). Effect of Refining of Crude Rice Bran Oil on the Retention of Oryzanol in the Refined Oil. *Journal of the American Oil Chemists Society, 78*(2), 127-131.

Krishnan, H. B., White, J. A., & Pueppke, S. G. (1992). Characterization and Localization of Rice (*Oryza Sativa L.*) Seed Globulins. *Plant Science, 81*(1), 1-11.

Kuk, M. S., & Dowd, M. K. (1998). Supercritical Co2 Extraction of Rice Bran. *Journal of the American Oil Chemists' Society, 75*(5), 623-628.

Kuo, P. C., Rudd, M. A., Nicolosi, R., & Loscalzo, J. (1989). Effect of Dietary Fat Saturation and Cholesterol on Low Density Lipoprotein Degradation by Mononuclear Cells of Cebus Monkeys. *Arteriosclerosis, 9*(6), 919-927.

Lai, V. M. F., Lu, S., He, W. H., & Chen, H. H. (2007). Non-Starch Polysaccharide Compositions of Rice Grains with Respect to Rice Variety and Degree of Milling. *Food Chemistry, 101*(3), 1205-1210.

Lakkakula, N. R., Lima, M., & Walker, T. (2004). Rice Bran Stabilization and Rice Bran Oil Extraction Using Ohmic Heating. *Bioresource Technology, 92*(2), 157-161.

Lamberts, L., Brijs, K., Mohamed, R., Verhelst, N., & Delcour, J. A. (2006). Impact of Browning Reactions and Bran Pigments on Color of Parboiled Rice. *Journal of Agricultural and Food Chemistry, 54*(26), 9924-9929.

Lamsal, B., Yoo, J., Brijwani, K., & Alavi, S. (2010). Extrusion as a Thermo-Mechanical Pre-Treatment for Lignocellulosic Ethanol. *Biomass & Bioenergy, 34*(12), 1703-1710.

Larrea, M. A., Chang, Y. K., & Bustos, F. M. (2005). Effect of Some Operational Extrusion Parameters on the Constituents of Orange Pulp. *Food Chemistry, 89*(2), 301-308.

Lebesi, D. M., & Tzia, C. (2012). Use of Endoxylanase Treated Cereal Brans for Development of Dietary Fiber Enriched Cakes. *Innovative Food Science & Emerging Technologies, 13*, 207-214.

Li, M., & Lee, T. C. (1996). Effect of Extrusion Temperature on Solubility and Molecular Weight Distribution of Wheat Flour Proteins. *Journal of agricultural and food chemistry, 44*(3), 763-768.

Lloyd, B. J., Siebenmorgen, T. J., & Beers, K. W. (2000). Effects of Commercial Processing on Antioxidants in Rice Bran. *Cereal Chemistry Journal, 77*(5), 551-555.

Lombardiboccia, G., Dilullo, G., & Carnovale, E. (1991). Invitro Iron Dialyzability from Legumes - Influence of Phytate and Extrusion Cooking. *Journal of the Science of Food and Agriculture, 55*(4), 599-605.

Luh, B. S. (1991). *Rice--Production and Utilization* (2nd ed.). New York: Van Nostrand Reinhold.

Luo, H. F., Li, Q. L., Yu, S. G., Badger, T. M., & Fang, N. B. (2004). Cytotoxic Hydroxylated Triterpene Alcohol Ferulates from Rice Bran. *Journal of Natural Products, 68*(1), 94-97.

Makynen, K., Chitchumroonchokchai, C., Adisakwattana, S., Failla, M., & Ariyapitipun, T. (2012). Effect of Gamma-Oryzanol on the Bioaccessibility and Synthesis of Cholesterol. *European Review for Medical and Pharmacological Sciences, 16*(1), 49-56.

Manjula, S., & Subramanian, R. (2008). Enriching Oryzanol in Rice Bran Oil Using Membranes. *Applied Biochemistry and Biotechnology, 151*(2-3), 629-637.

Marshall, W. E., & Wadsworth, J. I. (1994). *Rice Science and Technology*. New York: Marcel Dekker.

Mawal, Y. R., Mawal, M. R., & Ranjekar, P. K. (1987). Biochemical and Immunological Characterization of Rice Albumin. *Bioscience Reports, 7*(1), 1-9.

McCaskill, D. R., & Zhang, F. (1999). Use of Rice Bran Oil in Foods. *Food Technology, 53*(2), 50-52.

Minhajuddin, M., Beg, Z. H., & Iqbal, J. (2005). Hypolipidemic and Antioxidant Properties of Tocotrienol Rich Fraction Isolated from Rice Bran Oil in Experimentally Induced Hyperlipidemic Rats. *Food and Chemical Toxicology, 43*(5), 747-753.

Mishra, A., Gopalakrishna, A. G., & Prabhakar, J. V. (1988). Factors Affecting Refining Losses in Rice (*Oryza Sativa L.*) Bran Oil. *Journal of the American Oil Chemists' Society, 65*(10), 1605-1609.

Miyoshi, H., Okuda, T., Oi, Y., & Koishi, H. (1986). Effects of Rice Fiber on Fecal Weight, Apparent Digestibility of Energy, Nitrogen and Fat, and Degradation of Neutral Detergent Fiber in Young Men. *Journal of Nutritional Science and Vitaminology, 32*(6), 581-589.

Miyoshi, N., Koyama, Y., Katsuno, Y., Hayakawa, S., Mita, T., Ohta, T., Kaji, K., & Isemura, M. (2001). Apoptosis Induction Associated with Cell Cycle Dysregulation by Rice Bran Agglutinin. *Journal of Biochemistry, 130*(6), 799-805.

Mod, R. R., Conkerton, E. J., Ory, R. L., & Normand, F. L. (1978). Hemicellulose Composition of Dietary Fiber of Milled Rice and Rice Bran. *Journal of Agricultural and Food Chemistry, 26*(5), 1031-1035.

Mod, R. R., Conkerton, E. J., Ory, R. L., & Normand, F. L. (1979). Composition of Water-Soluble Hemicelluloses in Rice Bran from 4 Growing Areas. *Cereal Chemistry, 56*(4), 356-358.

Nagao, K., Sato, M., Takenaka, M., Ando, M., Iwamoto, M., & Imaizumi, K. (2001). Feeding Unsaponifiable Compounds from Rice Bran Oil Does Not Alter Hepatic Mrna Abundance for Cholesterol Metabolism-Related Proteins in Hypercholesterolemic Rats. *Bioscience Biotechnology and Biochemistry, 65*(2), 371-377.

Nakase, M., Adachi, T., Urisu, A., Miyashita, T., Alvarez, A. M., Nagasaka, S., Aoki, N., Nakamura, R., & Matsuda, T. (1996). Rice (Oryza Sativa L) Alpha-Amylase Inhibitors of 14-16 Kda Are Potential Allergens and Products of a Multigene Family. *Journal of Agricultural and Food Chemistry, 44*(9), 2624-2628.

Nakayama, S., Manabe, A., Suzuki, J., Sakamoto, K., & Inagaki, T. (1987). Comparative Effects of Two Forms of Gamma-Oryzanol in Different Sterol Compositions on Hyperlipidemia Induced by Cholesterol Diet in Rats. *Japanese Journal of Pharmacology, 44*(2), 135-143.

Nam, S. H., Choi, S. P., Kang, M. Y., Koh, H. J., Kozukue, N., & Friedman, M. (2005). Bran Extracts from Pigmented Rice Seeds Inhibit Tumor Promotion in Lymphoblastoid B Cells by Phorbol Ester. *Food and Chemical Toxicology, 43*(5), 741-745.

Nasirullah, Krishnamurthy, M. N., & Nagaraja, K. V. (1989). Effect of Stabilization on the Quality Characteristics of Rice-Bran Oil. *Journal of the American Oil Chemists Society, 66*(5), 661-663.

Nicolosi, R. J., & Rogers, E. J. (1993). Rice Bran Oil and Its Health Benefits. In W. E. Marshall & J. I. Wadsworth (Eds.), *Rice Science and Technology*. New York: Marcel Dekker, Inc.

Nicolosi, R. J., Stucchi, A. F., Kowala, M. C., Hennessy, L. K., Hegsted, D. M., & Schaefer, E. J. (1990). Effect of Dietary-Fat Saturation and Cholesterol on Ldl Composition and Metabolism - Invivo Studies of Receptor and Nonreceptor-Mediated Catabolism of Ldl in Cebus Monkeys. *Arteriosclerosis, 10*(1), 119-128.

Norazalina, S., Norhaizan, M. E., Hairuszah, I., & Norashareena, M. S. (2010). Anticarcinogenic Efficacy of Phytic Acid Extracted from Rice Bran on Azoxymethane-Induced Colon Carcinogenesis in Rats. *Experimental and Toxicologic Pathology, 62*(3), 259-268.

Orthoefer, F. T. (1996). Rice Bran Oil: Healthy Lipid Source. *Food Technology, 50*(12), 62-64.

Orthoefer, F. T., & Eastman, J. (2004). Rice Bran and Oil. In E. T. Champagne (Ed.), *Rice: Chemistry and Technology* (3rd ed., pp. 569-593). St. Paul, Minnesota: American Association of Cereal Chemists.

Ostergard, K., Bjorck, I., & Vainionpaa, J. (1989). Effects of Extrusion Cooking on Starch and Dietary Fiber in Barley. *Food Chemistry, 34*(3), 215-227.

Pan, S. J., & Reeck, G. R. (1988). Isolation and Characterization of Rice Alpha-Globulin. *Cereal Chemistry, 65*(4), 316-319.

Park, J., & Floch, M. H. (2007). Prebiotics, Probiotics, and Dietary Fiber in Gastrointestinal Disease. *Gastroenterology Clinics of North America, 36*(1), 47-63.

Pascual, C. D. C. I., Massaretto, I. L., Kawassaki, F., Barros, R. M. C., Noldin, J. A., & Marquez, U. M. L. (2013). Effects of Parboiling, Storage and Cooking on the Levels of Tocopherols, Tocotrienols and Gamma-Oryzanol in Brown Rice (*Oryza Sativa L.*). *Food Research International, 50*(2), 676-681.

Pomeranz, Y., & Ory, R. L. (1982). Rice Processing and Utilization. In Ivan A. Wolff (Ed.), *Handbook of Processing and Utilization in Agriculture* (Vol. 2). West Palm Beach, FL: CRC press.

Prabhakar, J. V., & Venkatesh, K. V. L. (1986). A Simple Chemical Method for Stabilization of Rice Bran. *Journal of the American Oil Chemists Society, 63*(5), 644-646.

Prasad, N. M. N., Sanjay, K. R. , Khatokar, S. M., Vismaya, M. N., & Swamy, N. S. (2011). Health Benefits of Rice Bran - a Review. *Journal of Nutrition and Food Sciences, 3*(1), 108.

Qureshi, A. A., Mo, H. B., Packer, L., & Peterson, D. M. (2000). Isolation and Identification of Novel Tocotrienols from Rice Bran with Hypocholesterolemic, Antioxidant, and Antitumor Properties. *Journal of Agricultural and Food Chemistry, 48*(8), 3130-3140.

Qureshi, A. A., Sami, S. A., Salser, W. A., & Khan, F. A. (2001). Synergistic Effect of Tocotrienol-Rich Fraction (Trf(25)) of Rice Bran and Lovastatin on Lipid Parameters in Hypercholesterolemic Humans. *J Nutr Biochem, 12*(6), 318-329.

Rajam, L., Soban Kumar, D. R., Sundaresan, A., & Arumughan, C. (2005). A Novel Process for Physically Refining Rice Bran Oil through Simultaneous Degumming and Dewaxing. *Journal of the American Oil Chemists' Society, 82*(3), 213-220.

Ralet, M. C., Thibault, J. F., & Valle, G. D. (1990). Influence of Extrusion-Cooking on the Physicochemical Properties of Wheat Bran. *Journal of Cereal Science, 11*(3), 249-259.

Ramezanzadeh, F. M., Rao, R. M., Windhauser, M., Prinyawiwatkul, W., Tulley, R., & Marshall, W. E. (1999). Prevention of Hydrolytic Rancidity in Rice Bran During Storage. *Journal of Agricultural and Food Chemistry, 47*(8), 3050-3052.

Randall, J. M., Sayre, R. N., Schultz, W. G., Fong, R. Y., Mossman, A. P., Tribelhorn, R. E., & Saunders, R. M. (1985). Rice Bran Stabilization by Extrusion Cooking for Extraction of Edible Oil. *Journal of Food Science, 50*(2), 361-364.

Razavi, S. M. A., & Farahmandfar, R. (2008). Effect of Hulling and Milling on the Physical Properties of Rice Grains. *International Agrophysics, 22*(4), 353-359.

Resurreccion, A. P., Juliano, B. O., & Tanaka, Y. (1979). Nutrient Content and Distribution in Milling Fractions of Rice Grain. *Journal of the Science of Food and Agriculture, 30*(5), 475-481.

Riha, W. E., Hwang, C. F., Karwe, M. V., Hartman, T. G., & Ho, C. T. (1996). Effect of Cysteine Addition on the Volatiles of Extruded Wheat Flour. *Journal of Agricultural and Food Chemistry, 44*(7), 1847-1850.

Rimm, E. B., Ascherio, A., Giovannucci, E., Spiegelman, D., Stampfer, M. J., & Willett, W. C. (1996). Vegetable, Fruit, and Cereal Fiber Intake and Risk of Coronary Heart Disease among Men. *Journal of the American Medical Association, 275*(6), 447-451.

Roberts, K. M., McCarthy, M. J., Jeoh, T., Lavenson, D. M., & Tozzi, E. J. (2011). The Effects of Water Interactions in Cellulose Suspensions on Mass Transfer and Saccharification Efficiency at High Solids Loadings. *Cellulose, 18*(3), 759-773.

Rong, N., Ausman, L. M., & Nicolosi, R. J. (1997). Oryzanol Decreases Cholesterol Absorption and Aortic Fatty Streaks in Hamsters. *Lipids, 32*(3), 303-309.

Roy, S. K., Rao, B. V. S. K., & Prasad, R. B. N. (2002). Enzymatic Degumming of Rice Bran Oil. *Journal of the American Oil Chemists Society, 79*(8), 845-846.

Rukmini, C., & Raghuram, T. C. (1991a). Nutritional and Biochemical Aspects of the Hypolipidemic Action of Rice Bran Oil - a Review. *Journal of the American College of Nutrition, 10*(6), 593-601.

Rukmini, C., & Raghuram, T. C. (1991b). Nutritional and Biochemical Aspects of the Hypolipidemic Action of Rice Bran Oil: A Review. *Journal of the American College of Nutrition, 10*(6), 593-601.

Sadawarte, S. K., Sawate, A. R., Pawar, V. D., & Machewad, G. M. (2007). Enrichment of Bread with Rice Bran Protein Concentrate. *Journal of Food Science and Technology-Mysore, 44*(2), 195-197.

Saha, B. C., Iten, L. B., Cotta, M. A., & Wu, Y. V. (2005). Dilute Acid Pretreatment, Enzymatic Saccharification and Fermentation of Wheat Straw to Ethanol. *Process Biochemistry, 40*(12), 3693-3700.

Sakamoto, K., Tabata, T., Shirasaki, K., Inagaki, T., & Nakayama, S. (1987). Effects of Gamma-Oryzanol and Cycloartenol Ferulic Acid Ester on Cholesterol Diet Induced Hyperlipidemia in Rats. *Japanese Journal of Pharmacology, 45*(4), 559-565.

Santala, O., Nordlund, E., & Poutanen, K. (2013). Use of an Extruder for Pre-Mixing Enhances Xylanase Action on Wheat Bran at Low Water Content. *Bioresource Technology, 149*, 191-199.

Saunders, R. M. (1990). The Properties of Rice Bran as a Foodstuff. *Cereal Foods World, 35*(7), 632-636.

Savitha, Y. S., & Singh, V. (2011). Status of Dietary Fiber Contents in Pigmented and Non-Pigmented Rice Varieties before and after Parboiling. *Lwt-Food Science and Technology, 44*(10), 2180-2184.

Scavariello, E. M. S., & Arellano, D. B. (1998). Gamma-Oryzanol: An Important Component in Rice Bran Oil. *Archivos Latinoamericanos De Nutricion, 48*(1), 7-12.

Seetharamaiah, G. S., & Chandrasekhara, N. (1989). Studies on Hypocholesterolemic Activity of Rice Bran Oil. *Atherosclerosis, 78*(2-3), 219-223.

Seetharamaiah, G. S., Krishnakantha, T. P., & Chandrasekhara, N. (1990). Influence of Oryzanol on Platelet-Aggregation in Rats. *Journal of Nutritional Science and Vitaminology, 36*(3), 291-297.

Sharma, H. K., Kaur, B., Sarkar, B. C., & Singh, C. (2006). Thermal Behavior of Pure Rice Bran Oil, Sunflower Oil and Their Model Blends During Deep Fat Frying. *Grasas Y Aceites, 57*(4), 376-381.

Sharma, R. D., & Rukmini, C. (1986). Rice Bran Oil and Hypocholesterolemia in Rats. *Lipids, 21*(11), 715-717.

Shibuya, N., & Iwasaki, T. (1985). Structural Features of Rice Bran Hemicellulose. *Phytochemistry, 24*(2), 285-289.

Shibuya, N., Nakane, R., Yasui, A., Tanaka, K., & Iwasaki, T. (1985). Comparative Studies on Cell-Wall Preparations from Rice Bran, Germ, and Endosperm. *Cereal Chemistry, 62*(4), 252-258.

Shih, F. F. (2004). Rice Proteins. In E. T. Champagne (Ed.), *Rice: Chemistry and Technology* (pp. 143-157). St. Paul, Minnesota: American Association of Cereal Chemists.

Shoji, Y., Mita, T., Isemura, M., Mega, T., Hase, S., Isemura, S., & Aoyagi, Y. (2001). A Fibronectin-Binding Protein from Rice Bran with Cell Adhesion Activity for Animal Tumor Cells. *Bioscience Biotechnology and Biochemistry, 65*(5), 1181-1186.

Shyur, L. F., Wen, T. N., & Chen, C. S. (1994). Purification and Characterization of Rice Prolamins. *Botanical Bulletin of Academia Sinica, 35*(2), 65-71.

Singh, S., & Singh, R. R. (2009). Deacidification of High Free Fatty Acid-Containing Rice Bran Oil by Non-Conventional Reesterification Process. *Journal of Oleo Science, 58*(2), 53-56.

Singkhornart, S., Lee, S. G., & Ryu, G. H. (2013). Influence of Twin-Screw Extrusion on Soluble Arabinoxylans and Corn Fiber Gum from Corn Fiber. *Journal of the Science of Food and Agriculture, 93*(12), 3046-3054.

Southgate, D. A. T. (1977). Definiton and Analysis of Dietary Fiber. *Nutrition Reviews, 35*(3), 31-37.

Spady, D. K., & Dietschy, J. M. (1985). Dietary Saturated Triacylglycerols Suppress Hepatic Low Density Lipoprotein Receptor Activity in the Hamster. *Proceedings of the National Academy of Sciences of the United States of America, 82*(13), 4526-4530.

Spady, D. K., & Dietschy, J. M. (1988). Interaction of Dietary Cholesterol and Triglycerides in the Regulation of Hepatic Low Density Lipoprotein Transport in the Hamster. *Journal of Clinical Investigation, 81*(2), 300-309.

Sugano, M., & Tsuji, E. (1997). Rice Bran Oil and Cholesterol Metabolism. *Journal of Nutrition, 127*(3), S521-S524.

Suh, M. H., Yoo, S. H., Chang, P. S., & Lee, H. G. (2005). Antioxidative Activity of Microencapsulated Gamma-Oryzanol on High Cholesterol-Fed Rats. *Journal of Agricultural and Food Chemistry, 53*(25), 9747-9750.

Suzuki, S., & Oshima, S. (1970a). Influence of Blending of Edible Fats and Oils on Human Serum Cholesterol Level (Part 1). Blending of Rice Bran Oil and Safflower Oil. *The Japanese Journal of Nutrition and Dietetics, 28*(1), 3-6.

Suzuki, S., & Oshima, S. (1970b). Influence of Blending Oils on Human Serum Cholesterol (Part 2). Rice Bran Oil, Safflower Oil, Sunflower Oil. *The Japanese Journal of Nutrition and Dietetics, 28*(5), 194-198.

Tabatabai, A., & Li, S. (2000). Dietary Fiber and Type 2 Diabetes. *Clinical Excellence for Nurse Practitioners, 4*(5), 272-276.

Tanabe, K., Yamaoka, M., & Kato, A. (1981). High Performance Liquid Chromatography and Mass Spectra of Tocotrienols in Rice Bran Oils. *Journal of Japan Oil Chemists' Society, 30*(2), 116-118.

Tanabe, K., Yamaoka, M., Tanaka, A., Kato, A., & Amemiya, J. (1982). Determination of Tocopherols in Rice Bran Oils by High Performance Liquid Chromatography. *Journal of Japan Oil Chemists' Society, 31*(4), 205-208.

Tao, J. (2001). Method of Stabilization of Rice Bran by Acid Treatment and Composition of the Same: Google Patents.

Tao, J., Rao, R., & Liuzzo, J. (1993). Microwave-Heating for Rice Bran Stabilization. *Journal of Microwave Power and Electromagnetic Energy, 28*(3), 156-164.

Tateoka, T. (1964). Notes on Some Grasses .16. Embryo Structure of Genus *Oryza* in Relation to Systematics. *American Journal of Botany, 51*(5), 539-543.

Tauber, H., & Kleiner, I. S. (1932). A Method for the Determination of Monosaccharides in the Presence of Disaccharides and Its Application to Blood Analysis. *Journal of Biological Chemistry, 99*(1), 249-255.

Thakur, A. K., & Gupta, A. K. (2006). Water Absorption Characteristics of Paddy, Brown Rice and Husk During Soaking. *Journal of Food Engineering, 75*(2), 252-257.

Theuwissen, E., & Mensink, R. P. (2008). Water-Soluble Dietary Fibers and Cardiovascular Disease. *Physiol Behav, 94*(2), 285-292.

Tomlin, J., & Read, N. W. (1988). Comparison of the Effects on Colonic Function Caused by Feeding Rice Bran and Wheat Bran. *European Journal of Clinical Nutrition, 42*(10), 857-861.

Topping, D. L., Illman, R. J., Roach, P. D., Trimble, R. P., Kambouris, A., & Nestel, P. J. (1990). Modulation of the Hypolipidemic Effect of Fish Oils by Dietary Fiber in Rats: Studies with Rice and Wheat Bran. *The Journal of Nutrition (USA)*.

Umemoto, T., Nakamura, Y., Satoh, H., & Terashima, K. (1999). Differences in Amylopectin Structure between Two Rice Varieties in Relation to the Effects of Temperature During Grain-Filling. *Starch-Starke, 51*(2-3), 58-62.

Ummadi, P., Chenoweth, W. L., & Uebersax, M. A. (1995). The Influence of Extrusion Processing on Iron Dialyzability, Phytates and Tannins in Legumes. *Journal of Food Processing and Preservation, 19*(2), 119-131.

Van Hoed, V., Depaemelaere, G., Ayala, J. V., Santiwattana, P., Verhe, R., & De Greyt, W. (2006). Influence of Chemical Refining on the Major and Minor Components of Rice Bran Oil. *Journal of the American Oil Chemists Society, 83*(4), 315-321.

Vandeputte, G. E., Vermeylen, R., Geeroms, J., & Delcour, J. A. (2003). Rice Starches. Iii. Structural Aspects Provide Insight in Amylopectin Retrogradation Properties and Gel Texture. *Journal of Cereal Science, 38*(1), 61-68.

Vasanthan, T., Jiang, G. S., Yeung, J., & Li, J. H. (2002). Dietary Fiber Profile of Barley Flour as Affected by Extrusion Cooking. *Food Chemistry, 77*(1), 35-40.

Veenashri, B. R., & Muralikrishna, G. (2011). In Vitro Anti-Oxidant Activity of Xylo-Oligosaccharides Derived from Cereal and Millet Brans - a Comparative Study. *Food Chemistry*(3), 1475.

Velupillai, L., & Verma, L. R. (1986). Drying and Tempering Effects on Parboiled Rice Quality. *Transactions of the Asae, 29*(1), 312-319.

Verschoyle, R. D., Greaves, P., Cai, H., Edwards, R. E., Steward, W. P., & Gescher, A. J. (2007). Evaluation of the Cancer Chemopreventive Efficacy of Rice Bran in Genetic Mouse Models of Breast, Prostate and Intestinal Carcinogenesis. *British Journal of Cancer, 96*(2), 248-254.

Viamajala, S., McMillan, J. D., Schell, D. J., & Elander, R. T. (2009). Rheology of Corn Stover Slurries at High Solids Concentrations – Effects of Saccharification and Particle Size. *Bioresource Technology, 100*(2), 925-934.

Wan, Y. T., Rodezno, L. A. E., Solval, K. M., Li, J., & Sathivel, S. (2014). Optimization of Soluble Dietary Fiber Extraction from Defatted Rice Bran Using Response Surface Methodology. *Journal of Food Processing and Preservation, 38*(1), 441-448.

Wang, K. M., Wu, J. G., Li, G., Zhang, D. P., Yang, Z. W., & Shi, C. H. (2011). Distribution of Phytic Acid and Mineral Elements in Three Indica Rice (*Oryza Sativa L.*) Cultivars. *Journal of Cereal Science, 54*(1), 116-121.

Wang, M., Hettiarachchy, N. S., Qi, M., Burks, W., & Siebenmorgen, T. (1999). Preparation and Functional Properties of Rice Bran Protein Isolate. *Journal of Agricultural and Food Chemistry, 47*(2), 411-416.

Wei, C. H., Nguyen, S. D., Kim, M. R., & Sok, D. E. (2007). Rice Albumin N-Terminal (Asp-His-His-Gln) Prevents against Copper Ion-Catalyzed Oxidations. *Journal of Agricultural and Food Chemistry, 55*(6), 2149-2154.

Wilson, T. A., Nicolosi, R. J., Woolfrey, B., & Kritchevsky, D. (2007). Rice Bran Oil and Oryzanol Reduce Plasma Lipid and Lipoprotein Cholesterol Concentrations and Aortic Cholesterol Ester Accumulation to a Greater Extent Than Ferulic Acid in Hypercholesterolemic Hamsters. *Journal of Nutritional Biochemistry, 18*(2), 105-112.

Xu, Z. M., & Godber, J. S. (1999). Purification and Identification of Components of Gamma-Oryzanol in Rice Bran Oil. *Journal of Agricultural and Food Chemistry, 47*(7), 2724-2728.

Xu, Z. M., & Godber, J. S. (2000). Comparison of Supercritical Fluid and Solvent Extraction Methods in Extracting Gamma-Oryzanol from Rice Bran. *Journal of the American Oil Chemists Society, 77*(5), 547-551.

Xu, Z. M., Hua, N., & Godber, J. S. (2001). Antioxidant Activity of Tocopherols, Tocotrienols, and Gamma-Oryzanol Components from Rice Bran against Cholesterol Oxidation Accelerated by 2,2 '-Azobis(2-Methylpropionamidine) Dihydrochloride. *Journal of Agricultural and Food Chemistry, 49*(4), 2077-2081.

Yap, S. P., Yuen, K. H., & Wong, J. W. (2001). Pharmacokinetics and Bioavailability of Alpha-, Gamma- and Delta-Tocotrienols under Different Food Status. *Journal of Pharmacy and Pharmacology, 53*(1), 67-71.

Zeng, H., Lazarova, D. L., & Bordonaro, M. (2014). Mechanisms Linking Dietary Fiber, Gut Microbiota and Colon Cancer Prevention. *World Journal of Gastrointestinal Oncology, 6*(2), 41-51.

APPENDIX

Table A: The % composition (water soluble at 37°C) of parallel extruded & xylanase-treated CDRB fiber concentrates (dry basis)

Sample	Solubles	Soluble Pentosan	Soluble starch	Soluble protein	Soluble ash	Pentose
0%/25%/50rpm*	6.63±0.30^{mnop}	3.52±0.01^{defghijklmn}	2.42±0.12^{rstu}	0.38±0.01^{abcd}	0.27±0.13^{ij}	1.57±0.01^{a}
0%/30%/50rpm	6.05±0.20^{pq}	3.98±0.00^{bcdefghijk}	2.49±0.05^{qrst}	0.35±0.02^{cdefgh}	0.63±0.13^{efghij}	1.54±0.01^{bc}
0%/35%/50rpm	5.72±0.63^{q}	4.18±0.08^{abcdefgh}	2.87±0.00^{opqr}	0.37±0.00^{bcd}	0.89±0.25^{defghi}	1.50±0.01^{def}
0%/40%/50rpm	5.65±0.19^{q}	4.36±0.08^{abcde}	2.17±0.34^{tu}	0.32±0.00^{fghijk}	0.00±0.00^{j}	1.52±0.03^{cde}
0%/45%/50rpm	8.76±0.24^{efg}	4.12±0.09^{abcdefghij}	4.64±0.22^{fg}	0.32±0.03^{ghijklm}	0.45±0.13^{ghij}	1.52±0.00^{cde}
0%/60%/50rpm	6.10±0.15^{pq}	4.41±0.13^{abcd}	2.29±0.01^{stu}	0.32±0.04^{fghijk}	0.36±0.00^{hij}	1.48±0.02^{f}
0%/25%/100rpm	6.99±0.30^{lmn}	4.46±0.59^{abc}	2.74±0.25^{pqrs}	0.42±0.00^{a}	0.00±0.00^{j}	1.53±0.01^{bc}
0%/30%/100rpm	6.27±0.23^{nopq}	4.20±0.20^{abcdefg}	2.68±0.19^{pqrs}	0.41±0.04^{ab}	0.54±0.26^{fghij}	1.53±0.02^{cd}
0%/35%/100rpm	5.85±0.01^{q}	4.89±0.81^{ab}	2.72±0.60^{pqrs}	0.37±0.06^{bcde}	1.78±0.01^{bc}	1.58±0.00^{a}
0%/40%/100rpm	6.23±0.29^{opq}	4.91±0.20^{a}	1.99±0.03^{u}	0.34±0.02^{defghi}	1.88±0.12^{b}	1.57±0.03^{ab}
0%/45%/100rpm	8.11±0.06^{fghij}	4.85±0.11^{ab}	4.05±0.20^{hi}	0.36±0.05^{bcdefg}	3.17±1.42^{a}	1.49±0.02^{ef}
0%/60%/100rpm	10.29±0.91^{d}	4.96±0.02^{a}	5.93±0.27^{cd}	0.40±0.03^{ab}	1.62±0.25^{bcd}	1.51±0.01^{cdef}
1%/25%/50rpm	7.53±0.02^{jkl}	3.03±0.07^{lmn}	2.96±0.24^{mnopq}	0.36±0.01^{bcdef}	1.65±0.28^{bcd}	0.92±0.02^{lmn}
1%/30%/50rpm	7.74±0.16^{ijk}	3.02±0.08^{lmn}	3.04±0.01^{lmnop}	0.31±0.02^{ghijklm}	1.36±0.40^{bcde}	0.90±0.02^{mno}
1%/35%/50rpm	7.97±0.12^{hij}	3.27±0.04^{hijklmn}	4.02±0.06^{hi}	0.27±0.03^{mnop}	1.82±0.04^{bc}	0.88±0.01^{op}
1%/40%/50rpm	8.36±0.14^{fgh}i	3.37±0.31^{fghijklmn}	4.28±0.14^{gh}	0.32±0.01^{efghij}	1.44±0.01^{bcd}	0.88±0.01^{op}
1%/45%/50rpm	10.14±0.17^{d}	3.68±0.05^{cdefghijklm}	5.42±0.10^{e}	0.30±0.02^{ijklmno}	1.36±0.40^{bcde}	0.87±0.01^{pq}
1%/60%/50rpm	8.67±0.21^{efgh}	3.36±0.10^{fghijklmn}	4.76±0.02^{f}	0.30±0.04^{hijklmn}	1.07±0.25^{cdefgh}	0.88±0.02^{op}
1%/25%/100rpm	7.77±0.09^{ijk}	3.77±0.33^{cdefghijklm}	3.11±0.12^{jklmno}	0.39±0.01^{abc}	1.34±1.15^{bcdf}	0.95±0.01^{l}
1%/30%/100rpm	7.63±0.58^{ijkl}	2.73±1.00^{n}	3.31±0.19^{jklmno}	0.30±0.00^{hijklmn}	1.08±0.00^{cdefgh}	0.93±0.011^{m}
1%/35%/100rpm	7.06±0.39^{klm}	3.33±0.10^{ghijklmn}	3.36±0.47^{jklmn}	0.32±0.00^{efghij}	0.54±0.51^{fghij}	0.90±0.02^{mno}
1%/40%/100rpm	8.82±0.10^{ef}	3.08±0.06^{klmn}	4.29±0.08^{fgh}	0.25±0.04^{pq}	0.90±0.51^{defghi}	0.89±0.01^{nop}
1%/45%/100rpm	10.63±0.67^{cd}	3.40±0.46^{fghijklmn}	6.15±0.53^{bc}	0.27±0.04^{jklmnop}	0.64±0.39^{efghij}	0.88±0.00^{op}
1%/60%/100rpm	12.90±0.05^{a}	4.15±0.56^{abcdefghi}	8.15±0.02^{a}	0.27±0.03^{lmnop}	0.46±0.14^{ghij}	0.85±0.00^{q}
2%/25%/50rpm	7.79±0.20^{ij}	2.85±0.00^{mn}	3.07±0.28^{klmnop}	0.27±0.00^{klmnop}	1.19±0.15^{bcdefg}	1.16±0.01^{gh}
2%/30%/50rpm	7.99±0.25^{hij}	2.91±0.24^{lmn}	3.51±0.00^{jk}	0.15±0.01^{s}	1.26±0.23^{bcdef}	1.14±0.00^{hij}
2%/35%/50rpm	8.22±0.57^{fghij}	3.28±0.36^{ghijklmn}	3.66±0.10^{ij}	0.26±0.00^{nop}	1.37±0.37^{bcde}	1.11±0.04^{ik}
2%/40%/50rpm	7.93±0.17^{ij}	3.79±0.33^{cdefghijkl}	4.31±0.03^{fgh}	0.25±0.01^{pq}	1.79±0.25^{bc}	1.14±0.00^{hij}
2%/45%/50rpm	8.03±0.40^{ghij}	3.23±0.07^{ijklmn}	4.49±0.28^{fgh}	0.21±0.02^{qr}	1.65±0.01^{bcd}	1.12±0.01^{ijk}
2%/60%/50rpm	11.84±0.17^{b}	3.56±0.14^{cdefghijklmn}	6.43±0.30^{b}	0.25±0.01^{pq}	1.83±0.00^{bc}	1.12±0.01^{ijk}
2%/25%/100rpm	7.96±0.50^{hij}	3.47±0.26^{efghijklmn}	3.60±0.08^{ij}	0.26±0.02^{nop}	1.01±0.14^{defghi}	1.17±0.00^{g}
2%/30%/100rpm	7.05±0.15^{klm}	3.22±0.40^{jklmn}	3.47±0.17^{jkl}	0.26±0.01^{nop}	1.34±0.11^{bcde}	1.16±0.00^{gh}
2%/35%/100rpm	6.95±0.37^{lmno}	3.06±0.13^{klmn}	3.42±0.36^{jklm}	0.24±0.00^{pq}	1.09±0.25^{cdefgh}	1.12±0.01^{ijk}
2%/40%/100rpm	6.99±0.47^{lmn}	4.28±0.73^{abcdef}	2.95±0.02^{nopq}	0.26±0.01^{nop}	1.10±0.00^{cdefgh}	1.15±0.00^{ghi}
2%/45%/100rpm	9.36±0.11^{e}	3.35±0.27^{fghijklmn}	4.67±0.31^{fg}	0.17±0.02^{rs}	1.10±0.01^{cdefgh}	1.15±0.02^{ghi}
2%/60%/100rpm	11.28±0.79^{bc}	4.41±1.84^{abcd}	5.57±0.19^{de}	0.19±0.00^{rs}	1.56±0.39bcd	1.10±0.00^{k}

All data represent the mean of two replicate measurements. Means within a column with different letters are significantly different ($p<0.05$).

* % Xylanase (w/w)/% water addition (w/w)/screw speed (rpm).