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Applying a Material Science Approach to Improve Rice Processing Performance

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Applying a Material Science Approach to Improve Rice Processing Performance

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by

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ABSTRACT

Rice is typically consumed as whole kernel, which makes the economic value of whole kernels twice that of brokens. Macro-structural defects such as fissures and chalkiness have been recognized to weaken kernel mechanical strength, and thereby increase breakage during milling. However, high percentages of brokens are sometimes recorded even though these defects are controlled prior to milling. It was hypothesized that rice milling characteristics are influenced by endosperm porosity from the structural arrangement of chemical components and by bran thickness and composition. Thus, this study aimed to modify chemical components by applying treatments to alter rice porosity and its associated physicochemical properties, and to investigate the factors that influence bran removal during milling. Brown rice kernels were subjected to protein denaturation (PD) via heat treatment and/or lipid removal (LR) via hexane extraction and then characterized for porosity, breaking force, swelling power, water solubility, gelatinization, and pasting properties. The bran thickness of brown rice kernels was measured, and then milled to varying times. Bran fractions were then collected, quantified, and analyzed for their chemical compositions. The results showed that although porosity was generally negatively correlated with kernel hardness, porosity was influenced by the quantity of proteins and lipids, whereas kernel hardness was primarily affected by protein composition. Gelatinization temperatures were increased by PD but decreased by LR. Both PD and LR resulted in reduced pasting viscosities and swelling properties. Milling characteristics were not significantly impacted by bran thickness, but rather by the chemical components of arabinoxylans and proteins. The results demonstrate the impacts of rice physical and chemical properties on rice milling characteristics. Results gained will assist rice breeders to develop cultivars with desired chemical compositions and help rice processors optimize processing conditions to improve rice milling quality.

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DEDICATION

To mum and dad, I love you as much as you love me. Thank you for being everything I could ever ask for.

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I. GENERAL INTRODUCTION

Rice is unique among cereal grains because it is consumed mostly as whole kernels or head rice. Head rice yield is an important index for milling rice quality, which makes the economic value of head rice twice that of broken kernels. The milling process is a mechanical process involving applying a series of abrasive and frictional forces to remove bran layers from the brown rice kernels to produce milled rice (Lu & Siebenmorgen, 1995), and rice kernels that are not able to resist these mechanical stresses break. The tendency of rice kernels to break have been attributed to macro-structural defects such as fissures and chalkiness, which weaken kernel mechanical strength and make them susceptible to breakage during milling (Kunze, 2008; Patindol & Wang, 2003). However, high percentages of brokens are sometimes recorded even though these macro-structural defects are controlled prior to milling. Another factor affecting rice kernel mechanical properties, which has not gained much scientific attention, is porosity. Porosity occurs at the molecular level and is only visible with the aid of a scanning electron microscope. Marousis & Saravacos (1990) have reported the impacts of porosity on mechanical and textural properties of food.

Better mechanical properties of vitreous wheats are attributed to the compact arrangement of the chemical components (starch and proteins) in vitreous wheats than in mealy wheats that have large void spaces between the starch granules and protein matrix (Al-Saleh & Gallant, 1985; Moss, Stenvert, Kingswood, & Pointing, 1980). The degree of starch-protein adhesion has significant impacts on the hardness of wheat kernel endosperm (Glenn & Johnston, 1992). Greffeuille, Abecassis, Lapierre, & Lullien-Pellerin (2006) also reported that the wheat bran mechanical properties are related to the structure and chemical composition of the wheat cell walls. Similar to wheat, starch, proteins, and lipids are the major chemical components in rice endosperm,

and therefore the porosity and mechanical properties of rice kernels are primarily determined by the proportion, structure and interaction of proteins and lipids with starch. The quantity and distribution of the chemical constituents in the bran like proteins, lipids, ash and arabinoxylans may also affect rice milling performance (Hemdane et al., 2015).

Because wheat and rice are both cereals, and found in the same family of poaceae, the factors affecting wheat hardness and milling like endosperm porosity, and bran thickness and composition may also affect rice milling. Thus, it was hypothesized that rice milling characteristics are influenced by endosperm porosity from the structural arrangement of chemical components and by bran thickness and composition. The dissertation is presented in a "published/to-be submitted papers" format wherein each chapter is a stand-alone paper that has been published or is in preparation for submission to a peer-reviewed journal. The objectives of this dissertation are as follows:

1. Chapter 1…. To improve kernel strength by decreasing porosity through protein conformation change by heat treatment and by reducing lipid content.
2. Chapter 2…. To investigate the impacts of protein denaturation and lipid removal and their combined treatments on the swelling power, and water solubility, gelatinization and pasting properties of treated brown rice flours.
3. Chapter 3…. To investigate the relationship between the rice bran layer thickness and composition in relation to their milling properties.

Data gained will assist rice breeders to develop cultivars with desired chemical compositions and help rice processors optimize processing conditions to improve rice milling quality. The long-term goal of this study is to contribute to building a more sustainable rice industry by reducing waste and improving the economic value of rice.

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II. LITERATURE REVIEW

Rice is the most consumed staple food, feeding over 3.5 billion people worldwide (Amagliani, O'Regan, Kelly, & O'Mahony, 2016). It is grown in over 100 countries and on every continent except Antarctica, which is largely due to its adaptation to diverse environments including mountain terraces, tropical lowlands and deep-water swamps (Fairhurst & Dobermann, 2002). There are two cultivated rice species; *Oryza sativa* L., originated from Asia but now grown in many parts of the world, and *Oryza glaberrima* L. mostly cultivated in West Africa (Rosell & Marco, 2008). There are more than 100,000 varieties of rice, and they differ in their shapes, sizes and grain weights (Juliano, 2003). Unlike other cereals, rice is mostly consumed as a whole grain, therefore it is important to understand the impacts of the structural and chemical components on the mechanical properties and head rice yield of rice kernels.

Head rice yield and its importance

Although rice is sometimes consumed in the form of flour, flakes and puffs, the main consumption pattern is in the form of intact kernels, which is referred to as head rice. Head rice is defined as "all the unbroken kernels of rice and broken kernels that are at least three-fourths of an unbroken kernel" (USDA, 1983) as demonstrated in Figure 1. Head rice yield (HRY) is the mass of head rice expressed as a percentage of the original rough rice mass after complete milling (Reid, Siebenmorgen, & Mauromoustakos, 1998). HRY is an important index for milling quality of rice because low HRYs lead to significant economic losses, with the economic value of head rice being twice that of broken rice (Banaszek & Siebenmorgen, 1990; Lu & Siebenmorgen, 1995; Childs, 2006). The percentage of broken kernels is also one of the factors that determines the grade of milled rice in the US, where grade 1 and 6 is given to rice lot with maximum limit of 4 and 50% brokens, respectively (USDA, 2009). Factors such as the moisture content of the rice kernel,

temperature during the milling process, and the duration of milling have been reported to affect HRY (Siebenmorgen & Jindal, 1986). The HRY has also been found to be significantly correlated with the mechanical properties of the rice kernels (Lu & Siebenmorgen, 1995).

Rice mechanical properties

Mechanical properties are described as properties that a material exhibits when it encounters a force/load (Nature, 2019). During the rice milling process, mechanical loads come into contact with rice kernels to remove the bran and the germ (Lu & Siebenmorgen, 1995). These mechanical loads introduce bending or breaking, compressive, shear and frictional forces to the rice kernels, which could lead to breakage and consequently reduced HRY (Shitanda, Nishiyama, & Koide, 2002). The milling quality is a ratio of the HRY to the milled rice yield (MRY), which is the mass of milled rice expressed as a percentage of the original dried rough rice mass (USDA, 2009). The standard method for rice milling quality test requires 150 g of rough rice for each measurement. Pomeranz & Webb (1985) outlined some limitations and drawbacks associated with this method, such as the quantity of rice used, the time and cost involved, and little information on the observed differences in milling quality of different rice varieties. Siebenmorgen (1998) reported that the milling behavior of rice can only be fully understood after the individual kernel property distributions are fully characterized. Thus, in the quest to develop rapid, simple and meaningful tests to measure milling quality, the hardness test was selected because hardness is one of the major mechanical properties affecting the processing qualities of cereals (Pomeranz, 1982; Webb, Pomeranz, Afework, Lai, & Bollich, 1986).

Hardness, in terms of mechanical properties, is defined as the ability of a material to resist deformation or breakage under applied stress (Turnball & Rahman, 2002). Hardness affects the

milling behavior and the end-use quality of rice. Goodman & Rao (1985) researched the effects of rice kernel type and hardness on HRY. They measured the hardness as the maximum amount of force that was used to compress individual rice kernels under a lateral contact-point compression; however, they found a low correlation (r = 0.22) between HRY and rice kernel hardness. Lu & Siebenmorgen (1995) also recorded low and insignificant correlation between kernel hardness and HRY, and thus concluded that the compressive force could not be used to accurately reflect the actual HRY. These low correlations could be resulting from the fact that the compression tests measure the force used to compress the grain in a lateral contact-point (Bamrungwong, Satake, Vargas, & Yoshizaki, 1988) and not specifically to break it.

Solid objects only break when a breaking or bending force is exerted on them, which is a combination of two forces: compressive force to push against objects to result in size reduction and tensile force to pull objects to result in size elongation (Setareh et al., 2011). Siebenmorgen & Qin (2005) adopted a three-point bending test for breaking force to measure the hardness of brown rice kernels and found a significant positive correlation (r = 0.920) between HRY and the average breaking force. They also used models to predict the actual HRY, and this value was highly comparable to the actual HRY after milling of the rice kernels.

Effect of kernel structure on mechanical properties and head rice yield

The Codex Standards for rice classifies brown rice into long-, medium- and short-grain according to their length and width (FAO, 1995). Long-grain rice has a kernel length of 6.0 mm or more and a length/width ratio of 3.0 or greater. Medium-grain rice has a kernel length of 5.2 - 6.0 mm and a length/width ratio of 2.1 - 3.0. Short-grain rice has a kernel length of 5.2 mm or less and a length/width ratio of less than 2.0 (FAO, 1995). The Federal Grain Inspection Service, FGIS,

(1994) gives similar rice classification to that of the Codex standards (Houston, 1972a). The amylose content of the different classes of rice also differs, ranging 19-23% and 16-18% for the U.S. long-grain and medium-grain rice, respectively (Bergman, Chen, Delgado, & Gipson, 2011). The amylose content of the U.S. short-grain rice is similar to that of the medium-grain rice.

Wadsworth, Matthews, & Spadaro (1982) and Wadsworth & Hayes (1991) reported significant differences in the HRYs and processing losses of the three classes of rice. The short-grain was the least susceptible to breakage during milling, and the long-grain rice was the most susceptible. Bamrungwong et al. (1988) reported that the breaking force range of two short-grain japonica varieties (Koshihikari and Nipponbare) were greater (27-68 N) than those of two long-grain indica varieties (KDML105 and IR60) (15-40 N) using the three-point bending test. Bamrungwong et al. (1988) also considered the breaking energy, which measures the amount of energy applied to the rice kernel until it breaks. They found the breaking energy range of Koshihikari and Nipponbare to be greater (0.80-3.02 mJ) than those of KDML105 and IR60 (0.30-1.38 mJ), and the greater the energy, the stronger the kernel. This breaking energy is determined by integrating the area under the load/force-deformation curve produced during the three-point bending test.

Siebenmorgen & Qin (2005) studied how rice kernel size, i.e., length, width, and thickness, impacted its milling quality. They used the breaking force of the brown rice kernels as a measure of the kernel's mechanical strength, where kernels with a breaking force of < 15 N were considered as weak kernels, and those with a breaking force of > 20 N were strong kernels. The strong kernels were regarded to have a greater mechanical strength, and thus high milling quality. They reported very weak correlations between kernel length ($r = 0.08$) or width ($r = 0.10$) with breaking force. There was a much stronger correlation between the kernel thickness ($r = 0.44$) and the breaking

force. Siebenmorgen & Qin (2005) concluded that thicker kernels had a greater breaking force, and that the percentage of the stronger kernels was related to the thickness fractions of the rice cultivars. The results by Siebenmorgen & Qin (2005) support Mathews & Spadaro (1976), who reported that thicker kernels of three different varieties of long-grain brown rice were more resistant to breakage during milling than their thinner counterparts.

Impact of the bran layers on rice mechanical properties

The bran layers envelop the caryopsis and makes up about 10-15% of the rough rice weight (Champagne, Wood, Juliano, & Bechtel, 2004). The bran is a heterogenous structure, comprising the pericarp, seed coat/testa, nucellus and aleurone layers, and all these layers contribute in part to the mechanical properties of the rice bran (Bechtel & Pomeranz, 1977). Rice bran comprises 34.1-52.3% carbohydrates, 15.0-19.7% lipids, 12.0-15.6% proteins and 6.6-9.9% ash (Gohl, 1981; Luh, Barber, & Benedito de Barber, 1991). The carbohydrates in rice bran are composed of 13.8% starch, 9.5-16.9% hemicellulose, 5.9-9.0% cellulose, and 5.5-6.9% free sugars (Houston & Kohler, 1970; Juliano 1972; Houston, 1972b; Robles & Ewan, 1982).

Arabinoxylans (AX) is the predominant rice bran hemicellulose, which constitutes 4.87-7.15% of the rice bran (Hashimoto, Shogren, Bolte, & Pomeranz, 1987). AX has a linear backbone of β-1,4 linked xylose residues with frequent branching of arabinose residues (Izydorczyk & Biliaderis, 1995). Arabinose and xylose are the predominant sugars in rice bran after glucose, containing 27 and 26% of the total rice bran sugar content respectively (Maniñgat & Juliano, 1982; Fincher & Stone, 1986). AX contains ferulic acid, a phenolic compound that is covalently linked via an ester linkage to the arabinose residues (Figure 2). The ferulic acids in adjacent AX chains can connect with each other to form di-ferulic acid bridges, which results in AX cross-linking (Fry,

1979, 1982; Peyron, Chaurand, Rouau, & Abecassis, 2002). The AX cross-linking promotes tissue cohesion and maintain the structural integrity of the bran cell walls (Buanafina, 2009; Chateigner-Boutin et al., 2016). This makes the tissues firm, thus increasing resistance to bending/ breaking forces. Peyron et al. (2002) reported a significant positive correlation between the degree of wheat bran AX cross-linking and the bran mechanical strength.

The thickness of the bran layer also impacts the mechanical properties of rice kernels and is a major factor that determines how easily the bran is removed during milling (Glenn & Johnston, 1992). Wu et al. (2016) reported that rice bran layers increased the hardness of rice kernels. The difference between thicker and thinner rice kernels could be due to the contribution of the bran layers, as reported by Webb (1980) that thicker kernels have a thicker aleurone layer than thinner kernels. Thus, rice millers tend to over-mill thicker brown rice kernels in an attempt to attain a uniform degree of milling for both thick and thin kernels (Grigg & Siebenmorgen, 2013). This continuous removal of the bran layer however decreases the hardness of rice kernels (Mohapatra & Bal, 2006; Wu et al., 2016). The degree of milling (DOM) determines the extent to which the bran layer is removed from the rice kernels by the milling operation (Reid et al., 1998; Sun & Siebenmorgen, 1993). Andrews et al. (1992) reported that as the milling time of brown rice kernels increased, there was a corresponding increase in DOM and a decrease in milling rice yield (MRY) and HRY. Sun & Siebenmorgen (1993) found a linear relationship between the DOM and HRY in all rice kernel thickness fractions, and as high as 2.2-7.8% decrease in HRY when the milling time increased from 15 to 60 sec.

Rice structural defects

Rice structural defects affect the integrity and consequently the mechanical strength of rice kernels. Fissures and chalkiness are two well-known defects that affect the HRY (Kunze & Choudhary, 1972; Counce et al., 2005). The presence of these structural defects on the rice kernel can easily be visualized, and thus are proposed to be macro-structural defects. High percentages of brokens (up to 70%) are sometimes recorded during the milling process even though these macro-structural defects are controlled prior to milling. Therefore, it is proposed that the structural arrangement of rice chemical components (starch granules/protein bodies/lipid droplets/minerals) may form micro-structural defects as void spaces that are not visible but contribute to porosity, thus weakening kernel strength and reducing head rice yield.

Fissures

Fissures are surface or internal fractures that occur in the endosperm of the rice kernels (Kunze, 1979; Kunze & Calderwood, 2004). Fissures can be formed in rice kernels by two distinct ways: adsorption or desorption. Fissuring resulting from adsorption occurs when dried rice kernels are exposed to high relative humidity environments, and the surface of kernels absorb moisture, which causes starch granules to expand (Kunze & Hall, 1965). As the starch granules expand, compressive stresses are formed on the surface of the rice kernel. The tensile strength possessed by the rice kernel is present at its center and is defined as the resistance of the kernel to break under tension (Kunze & Hall, 1965; Kunze & Choudhary, 1972). When the tensile strength at the center is exceeded by the compressive stresses at the surface of the kernel, a fissure develops (Kunze & Hall, 1965; Kunze & Choudhary, 1972). Desorption is caused by the loss of moisture from rice kernels during rapid drying (Kunze & Hall, 1965). This rapid drying creates a steep moisture

gradient inside the rice kernel, which gradually decreases over time. As a way of achieving a uniform moisture distribution in the rice kernel, moisture from the interior of the kernel then begins to migrate to its surface, which in turn causes the interior to contract and the surface to expand. The continuing contraction of the interior results in a gradual failure of the tensile strength, until the kernel completely fails in tension and pulls itself apart from the center, leading to a fissure (Craufurd, 1963; Kunze, 1979; Kunze, 2008). The fissures formed as a result of adsorption are straight and are referred to as transverse/longitudinal fissures; those formed as a result of desorption are irregular and are referred to as 'turtle back' fissures (Figure 3) (Stermer, 1968).

Chalkiness

Chalk is the opaque area that can occupy a very small portion or the entire structure of the rice kernel (Lisle et al., 2000). Chalkiness in rice is attributed to both genetic and environmental factors, such as heat stresses or high temperatures that affect the critical stages of grain development. The high temperatures of exceeding daily minimum temperatures of 23 and 26°C after heading and during grain filling, respectively, cause the incomplete accumulation of starch during the grain filling period (Lisle et al., 2000; Patindol & Wang, 2003; Tsukimori, 2003). This makes the starch content in the chalky regions of rice kernels lower than that in the translucent regions (Xi et al., 2014). Chalky kernels are not only visually undesirable, but also deviate from quality characteristics, hence affecting the milling and head rice yields.

The starch granules in the amyloplasts of chalky rice kernels are rounder and have more air spaces, less amylose and more short branch-chain amylopectin, as opposed to the angular, tightly packed, and translucent structure of non-chalky rice kernels (Lisle et al., 2000; Patindol & Wang, 2003). The loosely packed structure of chalky grains makes chalky kernels break easily

during milling, hence reducing the head rice yield (Figure 4) (Chun, Song, Kim, & Lee, 2009). Leethanapanich, Mauromoustakos, & Wang (2016) reported of a significant decrease in chalkiness and a corresponding increase in head rice yield of brown rice kernels after the kernels were soaked in water at 65 °C. The chalkiness in individual kernels were almost completely eliminated when the soaking temperature was increased to 75 °C for 3 h. They explained that the soaking temperature caused a swelling and rearrangement of the starch granules and protein bodies, respectively, which filled the void spaces in chalky kernels.

Porosity

The porosity of a rice kernel, which is defined as "the ratio of volume of void spaces inside the kernels to the apparent volume of the rice kernels" can significantly affect the mechanical strength, breakage susceptibility and milling yields of rice kernels (Chang, 1988). These void spaces are isolated from the surface of the rice kernels and are only visible with the aid of a scanning electron microscope (Figure 5). The volume of the void spaces is calculated experimentally as the difference between the apparent volume and specific volume of the rice kernels. The porosity has been measured to study the air movement and mass transfer in the bulk paddy, and also the thermal diffusivity and conductivity of rough rice (Sujka & Jamroz, 2007; Ghadge & Prasad, 2012). However, in those studies, they only measured the void spaces in between paddy instead of the void spaces inside the brown rice kernels. Marousis & Saravacos (1990) have reported the correlation between the mechanical and textural properties of food and porosity. Dobraszczyk (1994) reported of an apparent relationship between the milling behavior of hard wheats and their vitreousness (low porosity). Later, Dobraszczyk, Whitworth, Vincent, & Khan (2002) stated that the differences in the rheological properties of soft and hard wheat were

due to the variations in porosity. Moss, Stenvert, Kingswood, & Pointing (1980) and Al-Saleh & Gallant (1985) had reported the better mechanical properties as a result of the compact arrangement of the chemical components (starch and proteins) in vitreous wheats than in mealy wheats, which have large void spaces between the starch granules and protein matrix. There is little information on the role and the impact of void spaces inside the rice kernels, and how their impact on the mechanical properties of brown rice.

Effect of chemical components on porosity and head rice yield

Starch

Starch is composed of two types of alpha-glucans, amylose, and amylopectin. Amylose is essentially linear and joined by α-1,4-D-glucopyranosidic linkages (Figure 6A). Peat, Whelan, & Pirt (1949, 1952) were the first to suggest the presence of branching in amylose when they found that sweet potato β-amylase hydrolyzed only partially (about 70%) potato amyloses. Banks & Greenwood (1967) reported that the β-amylolysis limit of whole amylose was in the range of 70-85% and was related to the amounts and locations of branch linkages, the proportion of linear and branched molecules, and the chain length of internal chains. The α-1, 6 branch linkages are the main barriers that limit the β-amylolysis of amylose (Peat et al., 1952; Hizukuri, Takeda, Yasuda, & Suzuki, 1981), and the addition of debranching enzymes like bacterial pullulanase and yeast isoamylase lead to increased hydrolysis of amylose (Kjølberg & Manners, 1963; Banks & Greenwood, 1966; Hizukuri et al., 1981).

The weight-average degree of polymerization (DPw) and number-average degrees of polymerization (DPn) of amylose are in the range of 2000-7000 and 700-5000 respectively, and the molecular weight and its distribution are characteristic of the origin of the amylose. For

instance, the molecular weight of amylose in glucose units is 3090 for Japonica rice, 3420 for Indica rice, 2500 for maize, 6360 for potato and 6680 for tapioca (Hizukuri & Takagi, 1984; Takeda, Shirasaka, &. Hizukuri, 1984; Takeda, Hizukuri, & Juliano, 1986; Takeda, Shitaozono, & Hizukuri, 1988; Takeda, Maruta, Hizukuri, & Juliano, 1989). Starches with small amylose molecules have less branch linkages and greater β-amylolysis limits, as compared to those with large amylose molecules (Takeda, Maruta, & Hizukuri, 1992). Amylose adopts a left-handed helical structure that can form an inclusion complex with hydrophobic compounds. The amylose helical structure is able to accommodate polyiodide ions in its central tunnel, and this amylose-iodine complex gives a blue color (Rundle & French, 1943; Banks, Greenwood, & Khan, 1971; Teitelbaum, Ruby, & Marks, 1978; Moulik & Gupta, 1986), which is used conventionally to measure the amylose content. Most starches contain 16-28% amylose.

Amylopectin, the major fraction of starches, is highly branched and joined together by α-1,4-D-glucopyranosidic linkages, and α-1,6-D-glucopyranosidic linkages at the branch points (Figure 6B). Amylopectin is characterized by a low β-amylolysis limit (55-61%), a large molecular weight ($4\text{-}5\times10^8$) ($2.5\text{-}3\times10^6$ DP), a A-to-B-chain ratio of 1.0~1.4 and an average chain of 18-24 glucose units (Banks & Greenwood, 1975; Hizukuri, 1996). The molecular structure of amylopectin has been proposed and revised over the years after physical, chemical, and enzymatic analyses (Staudinger & Husemann, 1937; Haworth, 1939; Meyer, Brentano, & Bernfeld, 1940; Gunja-Smith, Marshall, Mercier, Smith, & Whelan, 1970). The cluster model proposed by French (1972) (Figure 7A) and updated by Hizukuri (1986) (Figure 7B) is the widely accepted molecular structure of amylopectin, because the model provides evidence for the crystallinity of starch molecules, the higher viscosities of amylopectin molecules, and the resistance of starch to attack by amylolytic enzymes and acids.

In characterizing the amylopectin structure, Peat et al. (1952 and 1956) classified amylopectin chains into A, B and C. The A chains are linear and uses its reducing residue to bind to the C-6 of the B and C chains. The B chain carries the A or other B chains at its C-6, and the C chain is the only chain that has a free reducing residue. Chain length (CL) distribution analysis involves debranching of amylopectin with pullulanase and/or isoamylase to elucidate the fine structure of amylopectin (Lee, Mercier, & Whelan, 1968; Akai, Yokobayashi, Misaki, & Harada, 1971; MacGregor & Morgan, 1984; Inouchi, Glover, & Fuwa, 1987; Hizukuri, 1996). Hizukuri (1986) used high performance liquid chromatography (HPLC) to investigate into more detail the CL distribution of amylopectin. He further divided the B chains into B1-B4, after he observed a polymodal distribution having five peaks, namely A, B1, B2, B3 and B4 (Figure 8).

Rice starch

For most starches, the granule is synthesized and stored within the internal membrane compartments of amyloplast, and only one starch granule is present in each amyloplast. Rice starch is, however, the only starch source present as compound starch, where many small granules are produced in one amyloplast (Evers, 1971; Eliasson & Gudmundsson, 1996). Rice starch is angular and polygonal in shape and has the smallest size (3-8 μm) among all cereal starches. The amylose content in rice varies greatly with cultivars, ranging from 1-2% for waxy rice to 7-20%, 20-25% and > 25% for low, intermediate, and high amylose content rice, respectively (Juliano, 1985). Rice starch exhibits the A-type diffraction pattern, which is the most stable pattern of all the polymorphs (Slade & Levine, 1988). The crystallinity of non-waxy and waxy rice starches ranges from 25-42% and 37-51%, respectively (Ong & Blanshard, 1995; Qi, Tester, Snape, & Ansell, 2003; Chung, Liu, Lee, & Wei, 2011; Cai et al., 2015; Kong, Zhu, Sui, &. Bao, 2015). The degree of crystallinity significantly affects the mechanical properties, where a higher degree of crystallinity

leads to an increase in mechanical strength (Nielsen, 1954; El-Hadi, Schnabel, Straube, M¨uller, & Henning, 2002; Wang, Ju, Chen, Chen, & Hsieh, 2014).

Rice proteins

An increase in protein content is associated with an increased milled and head rice yield, and a reduced grain breakage during milling (Nangju & De Datta, 1970; Seetanum & De Datta, 1973; Jongkaewwattana, Geng, Hill, & Miller, 1993; Leesawatwong, Jamjod, Kuo, Dell, & Rerkasem, 2005; Song, Choi, Sharma, & Kang, 2012; Kaur et al., 2016). High protein rice kernels are harder, possess greater resistance to milling, and thus produce lower yields of bran and polish after milling (Cagampang, Cruz, Espiritu, Santiago, & Juliano, 1966). The image from a field emission scanning electron microscope (FESEM) shows a condensed packaging of proteins in head rice as compared to abnormal protein filling in broken rice kernels (Virdi, Singh, Pal, Kaur, & Kaur, 2019). Depending on the rice cultivar, the brown rice protein content varies widely from 4.3 to 18.2%, with a mean of 9.5% (Gomez, 1979; Champagne et al., 2004). Rice proteins exist as protein bodies, and they accumulate between the amyloplasts (Figure 9A). The structural arrangement of these protein bodies contributes to the void spaces inside the rice kernels. The protein bodies can either be crystalline (2-3 μm) or spherical (1-2 μm for large and 0.5-0.8 μm for small) depending on their dimensions (Figure 9B) (Tanaka, Sugimoto, Ogawa, & Kasai, 1980; Coffman & Juliano, 1987). The distribution of proteins and starch is not homogenous but varies throughout the endosperm. The concentration of starch granules increases from the periphery to the center of the endosperm. Thus, at the periphery, the starch amyloplasts are small, surrounded by a lot of protein bodies, whereas the starch amyloplasts become bigger and the protein bodies decrease toward the center of the endosperm (Juliano & Bechtel, 1985; Champagne et al., 2004).

All the three types of storage protein bodies (large spherical, small spherical and crystalline) are present in the starchy endosperm, but only the large spherical protein bodies are present at the center of the endosperm.

Rice proteins can be classified into four groups depending on solubility. Glutelin, the predominant protein fraction, constitutes 60-80% of the protein content in the endosperm and is alkali soluble. Albumin (1-5%), globulins (4-15%) and prolamin (2-8%) are the other protein fractions and are water, salt, and alcohol soluble, respectively (Cagampang et al., 1966). The differences in protein content of rice cultivars are primarily due to differences in glutelin content (Cagampang et al., 1966). Globulin and albumin have, however, been reported to be the major proteins in rice bran, constituting approximately 40% and 31%, respectively. The prolamin and glutelin distribution in the bran is 21% and 5%, respectively (Juliano, 1972; Shih, 2004). Thus, the proportion of glutelin is lowest in the outer layers of milled rice and increases toward the center, whereas the proportion of albumin has an inverse distribution (Houston, Iwasaki, Mohammad, & Chen, 1968; Shih, 2004). Balindong et al. (2017) reported of a positive correlation between head rice yield and glutelin ($r = 0.64$), globulin ($r = 0.75$) and prolamin ($r = 0.79$).

The total protein content is affected by environmental and cultural practices as well as by the genotypes (Huebner, Bietz, Webb, & Juliano, 1990; Kim et al., 2013), which cause the protein content of a brown rice variety to vary from 9.0 to 14.7% (IRRI, 1963; Cagampang et al., 1966). Taira (1971) reported that rice grown on an upland culture had higher protein contents than those grown on a lowland culture, even though they were of the same variety. Cultural practices like fertilizer application greatly impact rice protein content. The nitrogen application time has also been reported to affect the protein content of rice kernels, where late nitrogen application results in higher protein contents than early nitrogen application (Perez, Juliano, Liboon, Cassman, &

Alcantara, 1996). Perez et al. (1996) explained that the nitrogen applied in the early stage gets used up in the early grain development stages, and thus there is a low concentration of nitrogen in the leaf canopy during the final stages of the grain development. The increase in protein content resulting from increase in nitrogen led to a corresponding increase in the hardness of the cooked rice (Kaur et al., 2016).

Rice lipids

Lipids are the third most abundant component in rice, after starch and proteins, making up 2.9% of the total weight of brown rice at 14% moisture content (Resurreccion et al., 1979; Champagne et al., 2004). Brown rice contains 1.6-2.8% of lipids, with 15.0-19.7% of this fraction present in the bran (Juliano & Bechtel, 1985; Champagne et al., 2004). The lipids found in the bran are mostly present in the aleurone layer in the form of spherosomes or as lipid bodies (Figure 10) (Bechtel & Pomeranz, 1977; Juliano & Tuaño, 2019). The embryo is constituted of five embryonic tissues, including scutellum, embryonic axis, coleoptile, coleorhiza and the epiblast, all containing lipid bodies, which may either be scattered throughout the tissue body or be present only at their peripheries (Bechtel & Pomeranz, 1978). The lipids present in the endosperm are housed by another aleurone layer found inside the endosperm. The difference between the endosperm aleurone layer and that of the bran is that the endosperm aleurone layer has smaller and fewer lipid bodies and thus has a less densely packed cytoplasm than the bran aleurone layer (Bechtel & Pomeranz, 1977).

Because rice lipid is heavily concentrated in the bran, there is an observed decrease in total and surface lipid during rice milling (Chen, Siebenmorgen, & Du, 1999; Perdon, Siebenmorgen, Mauromoustakos, Griffin, & Johnson, 2001; Siebenmorgen, Matsler, & Earp, 2006). Morrison

(1978, 1988, and 1995) classified lipids present in starch into three basic categories: starch lipids, starch surface lipids and non-starch lipids. The non-starch lipids are the most abundant form of lipids and are present in the embryo and aleurone layers. The starch lipids are lipids found inside the starch granule and represent a relatively small proportion of total lipid content in rice. The starch surface lipids are those that surround the proteinaceous matrix of the endosperm. The starch surface lipids are loosely attached to the surface of starch granules in the endosperm (Morrison, 1995; Hu et al., 2017), and could contribute to the pore spaces inside the rice kernels.

Rice minerals

Minerals constitute 2.9-5.2% of the total rough rice weight. The hulls and bran contain 13.2-21.0% and 6.6-9.9% of the total mineral content, respectively (Juliano & Bechtel, 1985; Champagne et al., 2004). The distribution of minerals in brown rice is 51% in the bran, 10% in the germ, 10% in the polish and 28% in the milled rice (Leonzio, 1967; Resurrección et al., 1979; Champagne et al., 2004). The minerals highly concentrated in the hulls and bran become dissolved and migrate into the endosperm during parboiling, thus improving the nutrional quality of rice (Demont et al., 2012). Phosphorus is the most abundant mineral, of which most of the phosphorus present is phytin phosphorus. Rice seeds uptake phosphorus when they are applied to the soil during the plants flowering stage. Phosphorus is an important constituent, needed for energy transfer and storage within the rice plant. It is also a component of the many compounds that are required for protein synthesis. An increase in phosphorus fertilizer application has been reported to increase grain yield, and there is a positive relationship between grain yield and phosphorus accumulation in rice kernels (Lan, Lin, Wang, Zhang, & Chen, 2012; Zhang, Hua, Li, Chen, & Yang, 2012). Zohoun et al. (2018) reported a positive correlation between grain minerals and head

rice yield and hardness. They explained that the ability of minerals such as phosphorus, potassium and magnesium to form specific linkages with protein bodies and amylopectin of starch granules could be responsible for the observed correlations. Xi et al. (2016) also reported of lower minerals concentrations in weak chalky kernels as compared to strong translucent rice kernels. This could thus imply that rice varieties with high minerals concentration may have improved head rice yield.

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FIGURES

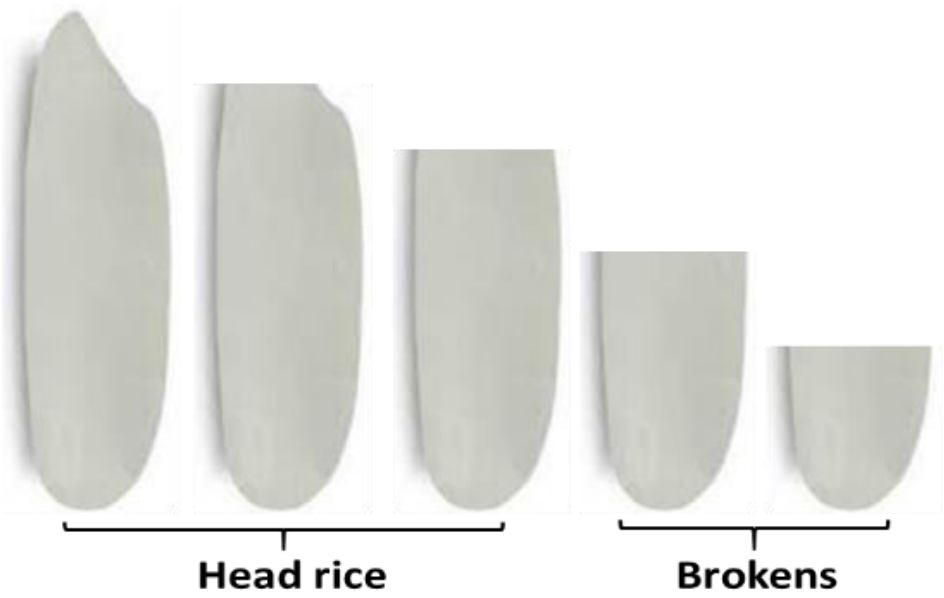

Figure 1. Illustration of head rice and brokens after milling.

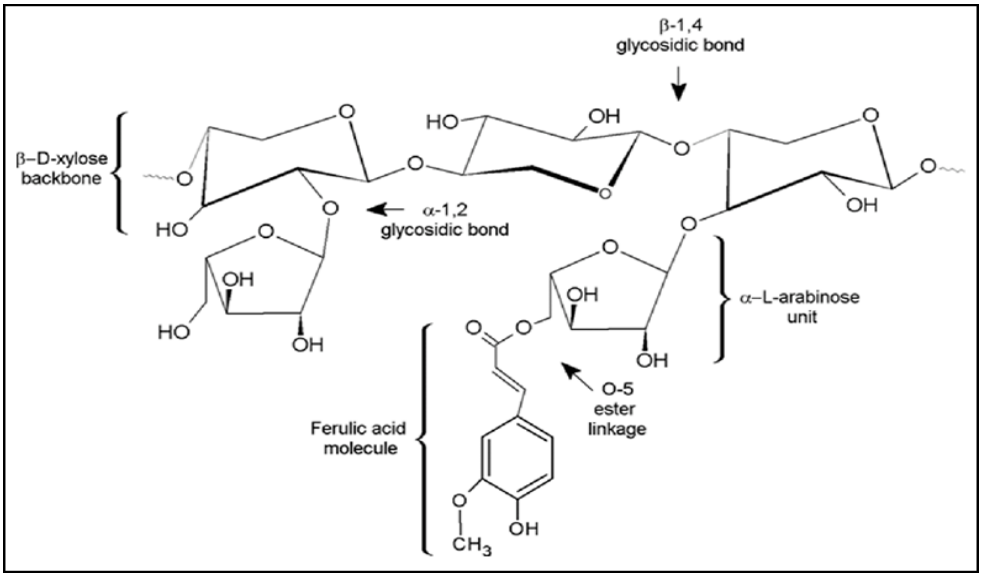

Figure 2. The chemical structure of a representative fraction of ferulated arabinoxylans (Morales-Burgos et al., 2017).

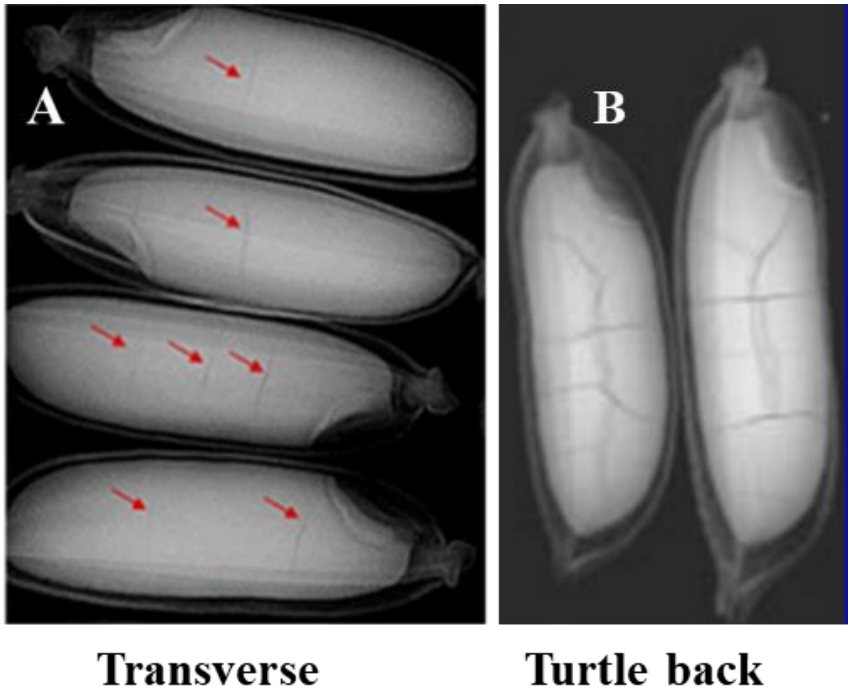

Figure 3. X-ray images of the (A) transverse (Patindol et al., 2017). and (B) fissured rice kernels (Siebenmorgen, Qin, & Jia, 2005).

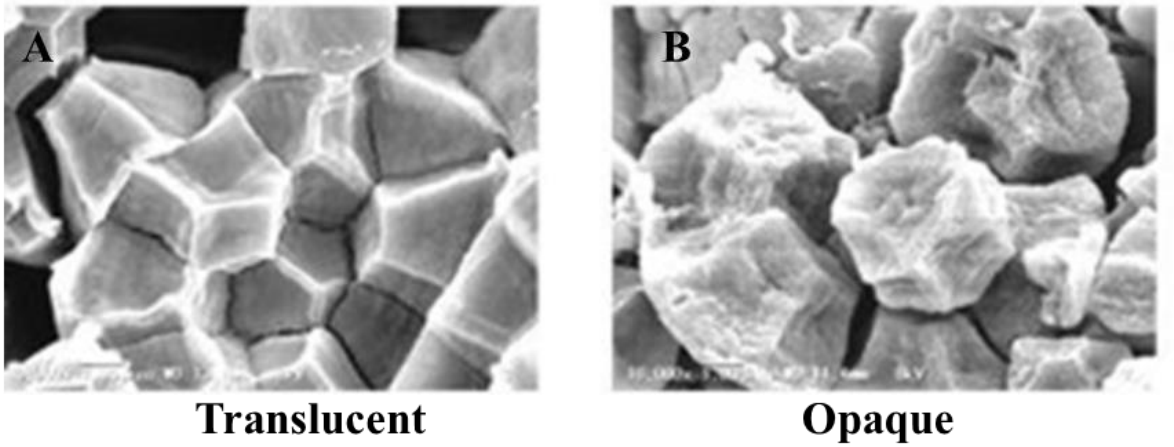

Figure 4. Scanning electron microscopy of (A) perfect kernel with tightly packed structure and (B) chalky kernel with loosely packed structure (Mitsui, Shiraya, Kaneko, & Wada, 2013).

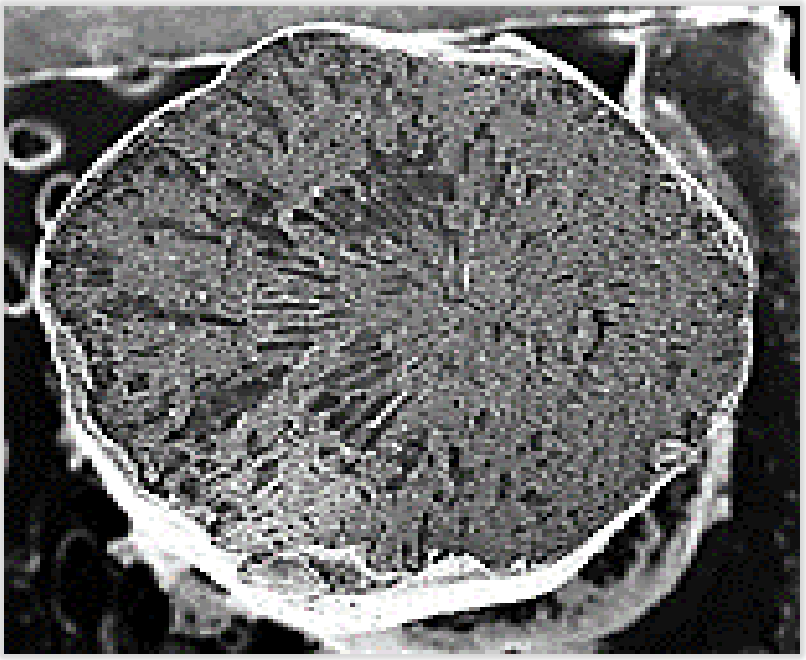

Figure 5. The pore spaces inside the transverse cross-section of a rice kernel as revealed by scanning electron microscopy (Kasem, Waters, Rice, Shapter, & Henry, 2011).

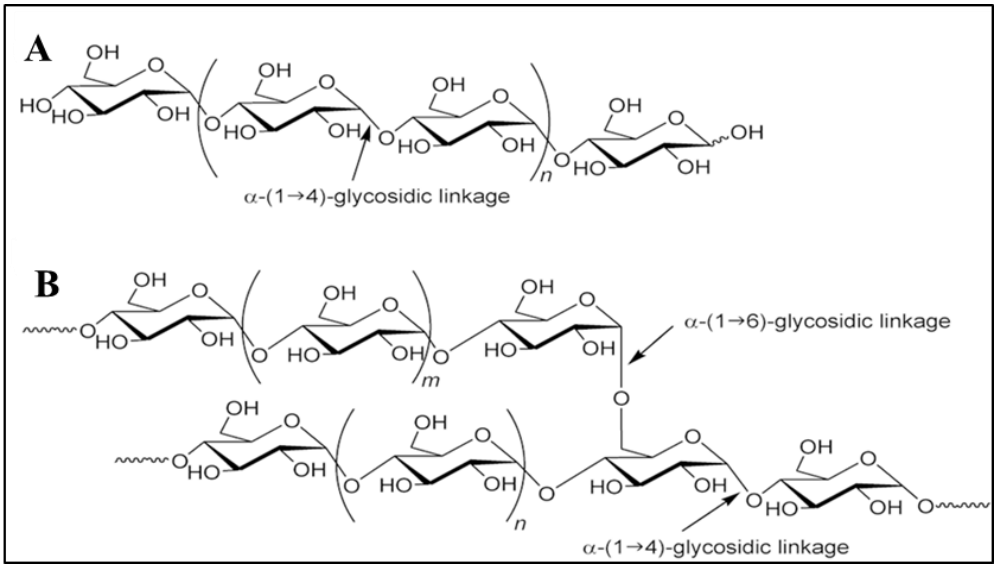

Figure 6. The molecular structure of (A) amylose and (B) amylopectin.

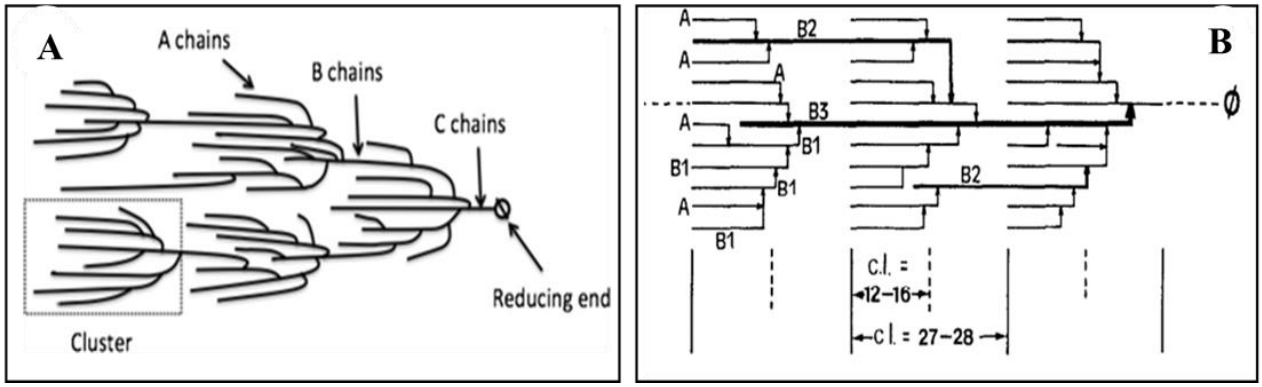

Figure 7. A cluster structure of amylopectin by (A) French (1972) and updated by (B) Hizukuri (1986). A, B and C = chains of amylopectin. Cl = chain length in glucose units.

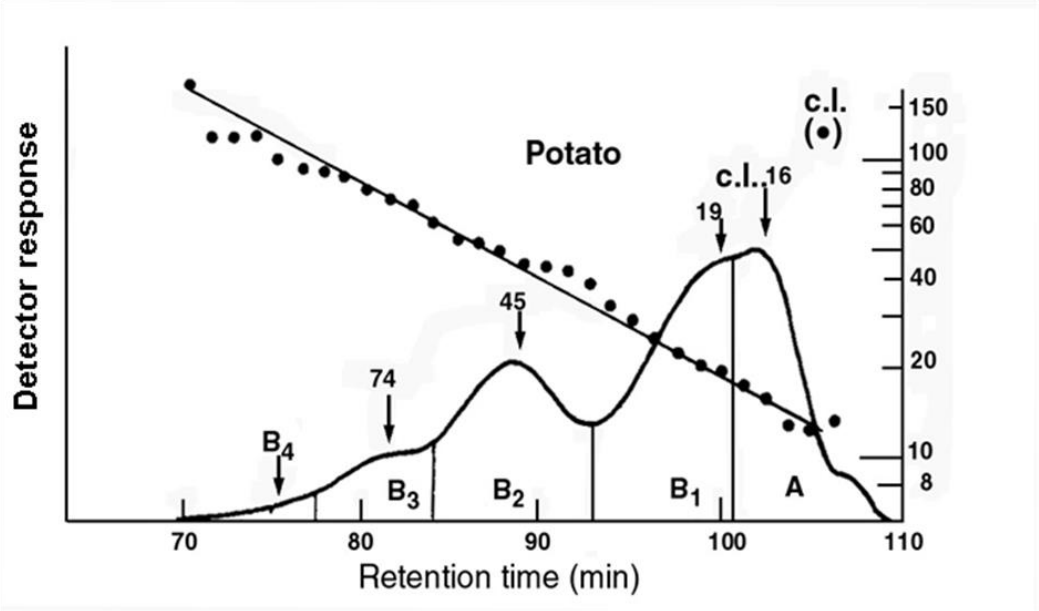

Figure 8. Polymodal chain length distribution of amylopectin (Hizukuri, 1986).

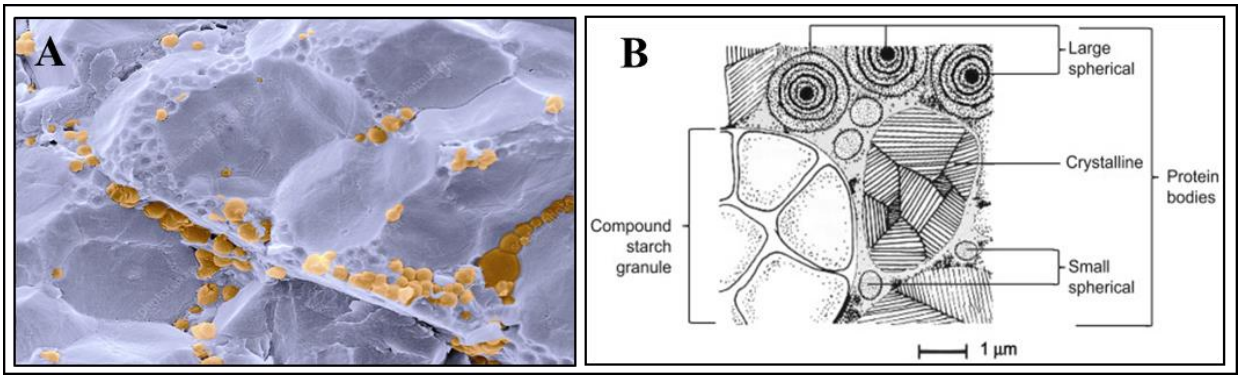

Figure 9. (A) Color-enhanced scanning electron micrograph of long-grain rice fractured to reveal protein bodies (orange color) (Science Photo Library Limited, 2019). (B) Spherical and crystalline protein bodies in rice endosperm (Coffman and Juliano, 1987).

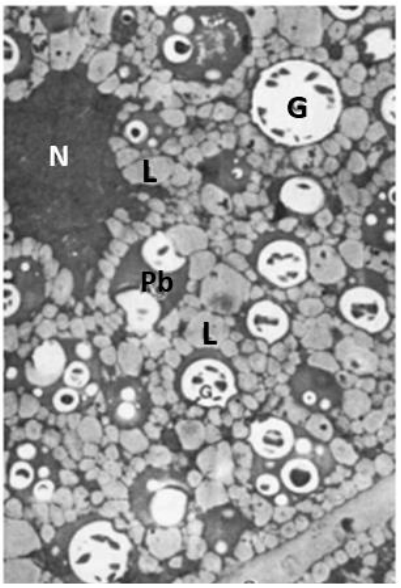

Figure 10. Transmission electron microscopy of aleurone layer of rice, showing protein bodies (Pb) with globoid inclusions (G), lipid droplets (L) and nucleus (N) (Bechtel and Pomeranz, 1977; Juliano and Tuaño, 2019).

III. CHAPTER 1: Porosity and Hardness of Long-Grain Brown Rice Kernels in Relation to their Chemical Compositions

Michelle Oppong Siaw, Ya-Jane Wang, Anna M. McClung, Andy Mauromoustakos

ABSTRACT

Rice kernel hardness is an important characteristic to the rice industry because of the greater economic value of whole kernels over brokens. Rice hardness may be weakened by increased porosity, i.e., void spaces formed from loose interaction between chemical components like starch, proteins, and lipids. The objective of this study was to elucidate the impacts of rice proteins and lipids on porosity and hardness via heat treatment and solvent extraction. Brown rice kernels of similar thickness from four cultivars with varying protein and lipid contents were subjected to protein denaturation by heat treatment and/or lipid removal by hexane extraction and then characterized for protein solubility, residual lipids, porosity, and breaking force. The results show that although porosity is generally negatively correlated with kernel hardness, porosity was primarily influenced by the quantity of proteins and lipids, whereas kernel hardness was primarily affected by their compositions. The continuity of the protein-starch matrix was the dominant factor that governed kernel hardness. This matrix is proposed to be strengthened by an increase in glutelin content and a decrease in non-polar lipids. This study demonstrates the importance of chemical composition on kernel hardness and elucidates the relationship between porosity and breaking force in rice kernels.

Keywords: Rice; Protein denaturation; Lipid removal; Porosity; Breaking force

INTRODUCTION

Unlike other cereals, rice is predominately consumed in the form of whole kernels. Therefore, broken rice kernels are valued at only 50–60% to that of whole rice, which illustrates the importance of reducing rice breakage during processing (Siebenmorgen & Lanning, 2014). Rice breakage is related to kernel hardness, which is the ability of the grain to resist deformation under applied stress and is an important criterion for rice quality because hardness impacts rice milling and end-use (Turnball & Rahman, 2002). Kernel hardness is influenced by various physical and chemical factors such as kernel size, fissures, vitreousness, proteins, lipids, and moisture content (Pasha, Anjum, & Morris, 2010; Turnball & Rahman, 2002).

Fissures and chalkiness are two major structural defects that are visible and affect the integrity and consequently, the hardness or mechanical strength of rice kernels. Odek, Prakash, & Siebenmorgen (2017) reported a strong positive correlation ($r = 0.97$) between the percentage of fissured kernels and the percentage of broken kernels. Chalky kernels tend to be weaker and more susceptible to breaking during processing (Lanning, Siebenmorgen, Counce, Ambardekar, & Mauromoustakos, 2011). There have been reports on the characterization (Cnossen & Siebenmorgen, 2000; Lanning et al., 2011; Odek et al., 2017; Patindol & Wang, 2003; Siebenmorgen, Qin, & Jia, 2005) and control (Ambardekar, Siebenmorgen, Counce, Lanning, & Mauromoustakos, 2011; Schluterman & Siebenmorgen, 2007; Yoshioka, Iwata, Tabata, Ninomiya, & Ohsawa, 2007) of fissures and chalkiness in rice kernels. Schluterman & Siebenmorgen (2007) reported that tempering during paddy drying can help reduce fissuring and increase kernel strength. Chalkiness is genetically controlled by complex quantitative trait loci and by temperature extremes during the late rice grain filling stage (Ambardekar et al., 2011; Yoshioka et al., 2007). However, high percentages of broken kernels are sometimes recorded during the milling process, even though these defects are controlled before milling. Besides fissures and

chalkiness, there are void spaces inside the kernels resulting from the arrangement and interactions of chemical components, which are referred to as porosity and invisible to the human eye.

Up until recently, there was a greater emphasis on research regarding kernel hardness and porosity of wheat. The greater adhesion strength between wheat chemical components and the better continuity of the protein matrix contribute to the lower porosity and greater hardness of hard wheats than soft wheats (Geneix et al., 2019; Pasha et al., 2010). Better mechanical properties in vitreous wheats have been attributed to the compact arrangement of starch and proteins in those wheats in comparison to mealy wheats, which are composed largely of void spaces between the starch granules and protein matrix (Samson, Mabille, Chéret, Abécassis, & Morel, 2005). The presence of a discontinuous protein matrix with many void spaces in soft wheat results in the easy separation of starch granules and protein, which consequently weakens their mechanical properties (Pasha et al., 2010; Turnball & Rahman, 2002).

Apart from the influence of proteins on porosity and kernel hardness, the effects of lipids on wheat kernel hardness have also been studied. Qin et al. (2019) reported of higher lipid contents in soft wheats than in hard wheat throughout grain development. Similarly, Konopka, Rotkiewicz, & Tańska (2005) found that wheat kernel hardness was negatively correlated with the content of starch surface lipids, particularly the non-polar fraction. The lipids on the starch surface may interfere with the interaction of starch and proteins, contributing to increased porosity and decreased kernel hardness.

Because of the importance of rice breakage to the rice industry, the objective of this research was to elucidate the relation of rice chemical components with porosity and hardness. It was hypothesized that rice porosity can be decreased by changing protein conformation through denaturation and by reducing lipid content, which in turn would promote protein-starch

interactions and consequently increase kernel hardness. Brown rice kernels from four rice cultivars of different protein and lipid contents were subjected to heat and/or solvent extraction for varying durations. They were then characterized for protein solubility, residual lipid content, porosity, and breaking force.

MATERIALS AND METHODS

Materials

Rough rice of four long-grain brown rice cultivars, with moisture contents of ~12% and varying protein and lipid contents, were obtained and stored at 65% relative humidity and at 4 ◦C, which is the best storage temperature for grains including rice. Cocodrie from 2009 and Sierra from 2014 were produced by the USDA Dale Bumpers National Rice Research Center, Stuttgart, AR. CL153 and CLXL745 from the 2018 crop year were produced by the University of Arkansas Rice Research and Extension Center in Harrisburg, AR. Rough rice samples were thickness-graded into 1.78–1.88, 1.88–1.98, 1.98–2.03, and >2.03 mm with a dockage tester (Model XT4, Carter-Day, Minneapolis, MN). A kernel thickness range of 1.98–2.03 mm was found to be the predominant fraction of the four cultivars and therefore, was selected for this study to reduce variation because kernel dimension affects rice mechanical properties. Rough rice was dehulled with a Satake rice machine (Model THU, Satake Engineering Co. Ltd. Tokyo, Japan), and broken brown rice kernels were separated from brown head rice using a shaker table (Grainman Machinery Mfg. Corp. Miami, FL).

Physical and chemical properties of brown rice kernels

The dimensions of brown head rice kernels were determined using SeedCount (Next Instruments Pty. Ltd. NSW, Australia). Brown rice was ground into flour using a Cyclone Sample

Mill (Udy Corp. Fort Collins, CO). Flours were analyzed for crude protein using the micro Kjeldahl method according to Approved Method 46–12.01 (AACC, 2000) using a conversion factor of 5.95, for crude lipids following Approved Method 30–20 (AACC, 2000) using hexane as the solvent, and for apparent amylose content using iodine colorimetry (Juliano, 1971).

Treatments to change kernel porosity and hardness

Protein denaturation

Fifteen grams of brown rice kernels were placed in a CLARITY™ vacuum pouch (6" × 8", 3 mm thick, Bunzl Inc. Riverside, MO) and sealed under 95% vacuum to prevent moisture loss during heating. The pouches were placed in a convection oven at 100 °C for 30, 60 and 90 min, and then placed in an equilibrium moisture content (EMC) chamber at 65% relative humidity and at 26 °C overnight to promote a gradual cool down.

Lipid removal

Ten grams of brown rice kernels were immersed in 30 mL hexane in a 50-mL centrifuge tube and rotated on a rotary shaker (Barnstead, model 400110, Dubuque, IA) at room temperature for 30, 90 and 180 min. The hexane and lipid mixture were poured into pre-weighed aluminum pans and the hexane evaporated in a convention oven at 40 °C for 24 h. The recovered rice kernels were desolventized by drying in a hood overnight. The percent residual lipid in the brown rice kernels was the difference between the total lipid content and the extracted lipid content on a dry weight basis.

Combined treatments

The combined treatments were done in two ways: protein denaturation (PD) first followed by lipid removal (LR) (PD-LR) and lipid removal first followed by protein denaturation (LR-PD). The protein denaturation and lipid removal treatments followed the same procedure as described previously, except that a protein denaturation time of 60 min was selected due to a small decrease in protein solubility after 60 min of heat treatment based on preliminary studies.

Characterization of treated rice kernels

Protein solubility

The extent of protein denaturation in treated brown rice samples was expressed by protein solubility, which was determined according to the method by Araba & Dale (1990) but with these modifications. Treated brown rice was ground into flour, and 0.8 g of the flour was mixed with 40 mL of 0.2% KOH in a 50-mL centrifuge tube on a rotary shaker for 20 min. The mixture was then centrifuged at 755×g for 15 min, and the supernatant was filtered through a Whatman™ No. 2 filter paper. Fifteen mL of the aliquot was used for crude protein measurement. Protein solubility was calculated as the ratio of the protein solubilized in 0.2% KOH over the total protein in the treated brown rice sample.

Porosity

The porosity of untreated and treated brown rice kernels was determined according to Chang (1988) using a gas pycnometer (AccuPyc II, Micrometrics Instrument Corp. Norcross, GA). Porosity (P) was defined as the ratio of void spaces inside the kernels to the apparent volume of kernels and calculated by the following equation.

$$P\ (\%) = \frac{V2 - V1}{V2} \times 100$$

V1 = true specific volume (cm^3), V2 = apparent specific volume (cm^3)

Brown rice was ground into flour, and the volume of the flour, measured with the gas pycnometer, was termed as the true specific volume (V1). For the apparent specific volume (V2) measurement, 5 g of rice kernels were individually coated via dipping in heated liquid paraffin wax (CAS No. 8002742, Merck KGaA, Darmstadt, Germany). The amount of wax coated on each sample was determined by the weight difference before and after the coating, which was divided by its density (0.92 g/cm^3) to obtain its volume. The wax-coated rice kernels were then placed in a sample container for weight measurement and volume determination using a gas pycnometer. The apparent specific volume of the sample was obtained by subtracting the volume of wax from the total volume of the wax-coated kernels.

Breaking force

The breaking force of treated and untreated brown rice kernels was used to represent rice hardness and was determined using a texture analyzer (TA-XT2, Texture Technologies Corp. Scarsdale, NY) with a 3- point bending test according to Siebenmorgen & Qin (2005). The distance between the two supporting points was set at 3.4 mm for all the bending tests. The deformation rate was set at 0.5 mm/s, and the loading head had a flat end with a thickness of 1.5 mm and a width of 9.9 mm. A total of 100 sound kernels were tested for each treatment. The breaking force value was the average of all the breaking forces of 100 individual rice kernels. The percentage of strong kernels was calculated by summing the number of kernels with breaking force values > 20 N as a proportion of the total.

Statistical analysis

A completely randomized design (CRD) with full 4 × 4 factorial treatment design with two replications was used to establish the effect of cultivar and treatment duration on porosity and breaking force of rice kernels. Data were analyzed using JMP Pro software (version 15.2, SAS Software Institute, Cary, NC), and the Tukey's honestly significant difference (HSD) test at a significance level of $P < 0.05$ was used to detect significant differences among means. Pearson's correlation coefficient was used to determine the statistical relationship between variables.

RESULTS AND DISCUSSION

Physical and chemical properties of brown rice kernels

The dimensions of brown rice kernels of the four long-grain rice cultivars varied significantly by length, width, and thickness in the range of 7.22–7.73 mm, 2.25–2.54 mm and 1.95–2.02 mm, respectively (Table 1). Khush, Paule, & de la Cruz (1979) classified brown rice kernel length into extra-long (>7.50 mm) and long (6.61–7.50 mm), and thus Sierra, with a mean length of 7.73 ± 0.01 in this study, is classified as an extra-long grain variety. Sierra is a U.S. aromatic long-grain rice that was developed to be like basmati-type rice (Hardke, Moldenhauer, & Sha, 2018). Khush et al. (1979) used the length-to-width (L/W) ratio to characterize the shape of the kernel and further classified rice cultivars into slender (>3), medium (2.1–3.0), bold (1.1–2.0) and round (≤1.0). Most cultivars in this study were classified as slender, except for Cocodrie which was classified as a medium in width. Although all rough rice samples were thickness-graded and the fraction of kernel thickness 1.98–2.03 mm was used to produce brown rice, Cocodrie and Sierra, both purelines, were thinner than CL153, an inbred variety, and CLXL745, a hybrid.

The apparent amylose, protein and lipid contents varied significantly among rice cultivars with Cocodrie and Sierra containing greater amylose, protein, and lipid content than CL153 and

CLXL745. Variation in rice chemical components was primarily attributed to their differences in kernel dimensions, which are affected by genetic and environmental factors (Champagne, Wood, Juliano, & Bechtel, 2004; Saleh, Akash, & Ondier, 2018). For instance, when rice cultivars of different thickness levels were compared, thinner kernels were found to comprise thicker bran layers and greater protein and lipid content (Lanning & Siebenmorgen, 2011). This trend was observed in this study where Cocodrie and Sierra were thinner and contained greater protein and lipid content than CL153 and CLXL745.

Protein denaturation on protein solubility, porosity, and breaking force of brown rice kernels

Native proteins occur as discrete bodies throughout the caryopsis development and in the mature rice kernel. Proteins are classified into four fractions depending on their solubility, which include alkali-soluble glutelin, alcohol-soluble prolamin, salt-soluble globulin, and water-soluble albumin. Glutelin is the predominant protein fraction in rice proteins, accounting for 60–80% of the total protein content (Kim, Lee, Yoon, Lim, & Kim, 2013), followed by prolamins, which account for 20–30% (Chen, Wang, & Ouwerkerk, 2012; Kim et al., 2013).

The protein solubility in 0.2% KOH of the four rice cultivars varied significantly before the heat treatment and ranged from 68.58 to 93.33% (Table 2). Cocodrie and Sierra had significantly lower protein solubility than the other two cultivars although their protein contents were greater. Because protein solubility at 0.2% KOH includes albumin, globulin and glutelin fractions, but excludes prolamin, the lower protein solubility of Cocodrie and Sierra is ascribed to their high prolamin fractions (Chen et al., 2012; Iida, Amano, & Nishio, 1993). Low glutelin content in rice is accompanied by high prolamin content, and vice versa, as revealed by a genetic

analysis on a backcross of the Nihonmasari rice cultivar and its 1433 mutant lines (Iida et al., 1993). Similar results were also reported by Yadav, Khatkar, & Yadav (2013) when they characterized rice protein from six Indian rice cultivars.

The protein solubility of the four rice cultivars decreased significantly ($r = -0.81$, p-value = <0.0001) when the heat treatment duration increased. The applied energy led to conformational changes and a subsequent decrease in the solubility of the proteins. The greatest decrease in protein solubility occurred in the first 30 min of the heat treatment for all four cultivars, which illustrates the sensitivity of rice proteins to heat. There are two types of protein bodies present in rice endosperm with type I rich in prolamins and type II rich in glutelins (Tanaka, Sugimoto, Ogawa, & Kasai, 1980). The type I protein body is more resistant to heat and degradation by proteolytic enzymes, and better retains their conformation during heat treatment (Ogawa et al., 1987; Iida et al., 1993). As previously stated, the decrease in protein solubility was less in Cocodrie and Sierra, likely because of their high prolamin content, which is more stable in response to thermal processing as compared to other protein fractions (Ogawa et al., 1987).

The porosity of untreated kernels was in the range of 12.14–13.85%, with Sierra displaying the lowest porosity, which could be attributed to its greater protein content. During denaturation, the unfolding and expansion of proteins gradually fills the void spaces around and between starch granules and consequently, reduces porosity (Chen, Huang, He, & Fu, 2015; Silva et al., 2017). The porosity gradually decreased with increasing denaturation time, and the greatest decrease in porosity was observed between 60 and 90 min of heat treatment. There was less decrease in porosity in the first 30 min of heat treatment, although the greatest decrease in protein solubility occurred at the same time interval, which explains the moderate positive correlation ($r = 0.67$, p-value = <0.0001) between protein solubility and porosity. The smaller decrease in porosity in the

first 30 min of heat treatment could result from the incomplete unfolding of the rice proteins during that time period.

Dobraszczyk, Whitworth, Vincent, & Khan (2002) found that wheat kernels of greater porosity had lower density and decreased mechanical strength. The present results show that a decrease in protein solubility ($r = -0.73$, p-value = <0.0001) and porosity ($r = -0.86$, p-value = <0.0001) was associated with an increase in breaking force, which measures the maximum force required to break the kernel. An increase in breaking force implies an increase in the hardness of rice kernels. The greatest increase in breaking force was observed after 30 min of heat treatment, which correlates with the greatest decrease in protein solubility but not with the greatest decrease in porosity. These results suggest that although porosity is generally negatively correlated with kernel hardness, the continuity of the protein matrix is the dominant factor affecting the breaking force in rice kernels. The greater breaking forces of CL153 and CLXL745 compared with those of Cocodrie and Sierra, both before and after heat treatment, imply the importance of protein composition, not protein content. The greater proportion of glutelin, as shown by the greater protein solubility in CL153 and CLXL745, is proposed to be responsible for their greater breaking force. Our result is supported by Baxter, Blanchard, & Zhao (2004, 2014), who studied the effect of glutelin and prolamin on textural and pasting properties of rice starch vs. rice flour. They reported a significantly positive correlation between glutelin concentration with the hardness of the rice flour gel. The prolamin concentration however decreased hardness of the rice flour gels.

Lipid removal on porosity and breaking force of brown rice kernels

Hexane was used to remove non-polar lipids to elucidate their impact on brown rice properties. Although non-polar solvents are capable of denaturing proteins, the extent and nature

of denaturation are affected by the type of protein and the hydrophobicity of the solvent, and the denaturing power of solvents increases with the addition of water (Fukushima, 1969). Hexane did not significantly decrease the protein solubility of the four cultivars after varying durations of extraction (data not shown), therefore protein denaturation was limited from the lipid removal process in this study.

The residual lipid content decreased with increasing extraction time, and the greatest amount of lipid extraction occurred in the first 30 min and the least occurred between 90 and 180 min for all cultivars (Table 3). It is known that the distribution of lipids in rice kernels is not uniform, as the greatest concentration is found in the outer bran layer, and it progressively decreases toward the center (Zhou, Robards, Helliwell, & Blanchard, 2002; Godber & Juliano, 2004). Lipids not associated with starch granules, referred to as non-starch lipids, are found in the outer bran layers and are mostly triacylglycerols, which as non-polar molecules are more prone to extraction by nonpolar solvents such as hexane (Morrison, 1995). Thus, the greater percentages of lipids extracted from Cocodrie and CL153 from all extraction times suggest their greater proportions of non-starch lipids. There was a strong positive correlation ($r = 0.93$-0.98, p-value = <0.0001-0.009) between the residual lipids and porosity within each cultivar, but no correlation was noted when considering all cultivars, which was attributed to their inherently different lipid contents. This correlation was greater than that of protein solubility and porosity, which demonstrates the dominant role of lipids in affecting porosity due to its hydrophobic nature. A smaller amount of residual lipids led to better adhesion between rice components, and consequently a lower porosity.

The breaking force significantly increased in the first 30 min of lipid removal, which corresponded to the greatest decrease in residual lipids, and gradually increased with further lipid

removal. CL153 had the greatest decrease in residual lipids and the greatest increase in breaking force after 180 min of lipid extraction. The presence of hydrophobic lipids interrupted the hydrophilic matrix of proteins and starch, and therefore weakened the adhesion strength of rice kernels as shown by the increase in breaking force with decreasing residual lipids. The removal of non-polar lipids therefore, allowed for more protein-starch interaction.

Combined treatments on porosity and breaking force of brown rice kernels

The treatments of protein denaturation and lipid removal were combined to understand their respective effects on brown rice kernel porosity and hardness at the same protein denaturation time of 60 min while varying hexane extraction time to 30, 90 and 180 min. The residual lipid contents decreased with lipid removal time, and the greatest amount of lipids removed followed the pattern of CL153 > Cocodrie > Sierra > CLXL745, which is similar to that observed in the individual treatments (Table 4). There was also a similar trend of a continual significant decrease in porosity and an increase in breaking force with increasing lipid removal as observed in the individual treatments.

The lipid removal first followed by protein denaturation (LR-PD) treatments resulted in a significantly greater amount of lipid removal, lower porosity, and greater breaking force than the PD-LR treatments for all cultivars at all treatment durations, indicating their different roles on the mechanical strength of the rice kernels. In the LR-PD treatment, the removal of lipids first allowed for better starch-starch and starch-protein interactions, and subsequent protein denaturation further strengthened their interactions. In contrast, in the PD-LR treatment, the conformation changes from protein denaturation enclosed lipids so that they could not be extracted and did not interact with proteins, thus, resulting in greater residual lipids, higher porosity, and lower breaking force.

CL153 and CLXL745 consistently exhibited much greater breaking forces than Cocodrie and Sierra for all treatment durations, which agree with the results obtained from the individual treatments. In addition, the greater difference in the breaking force between the treatments of PD-LR and LR-PD for CL153 and CLXL745 support the different roles of glutelin and non-polar lipids in the protein-starch matrix as described previously. The combined treatment resulted in rice kernels with a breaking force significantly greater than their respective breaking force from the individual treatments.

Breaking force distribution of brown rice kernels

Siebenmorgen & Qin (2005) classified rice kernels into "strong" and "weak" groups according to their breaking forces. The weak kernels were those which recorded a force of 15 N or less because they are likely to break, whereas the strong kernels could withstand 20 N force or greater. Therefore, the percentage of strong kernels with a breaking force ≥20 N before and after individual and combined treatments were calculated and presented in Table 5, and their distributions are depicted in Figures 1-2. The frequency of strong kernels (≥20 N) increased with treatment time under all treatments, and the breaking force even exceeded 40 N for the combined treatments with a lipid removal time of 180 min for all cultivars. The percentage of strong kernels was greater in CL153 and CLXL745 than in Sierra and Cocodrie before and after all treatments. The greater residual lipids and prolamin contents of Sierra and Cocodrie are proposed to be partly responsible for their greater occurrence of weak kernels. The percentage of strong kernels increased with treatment time for all four rice cultivars, which reflects the increase in breaking force values with increasing treatment time. The combined treatments followed a similar trend as the individual treatments, which resulted in a significant increase in the percentage of strong

kernels with increasing treatment duration. The treatment LR-PD resulted in a greater increase in the percentage of strong kernels than the PD-LR for all four cultivars, agreeing with their respective breaking force results. These results highlight the importance of chemical composition and processing conditions on rice milling properties, particularly head rice yield.

Statistical summary

The effects of rice cultivars (Cocodrie, Sierra, CL153 and CLXL745), treatments (protein denaturation and lipid removal), treatment durations (0, 30, 60, 90, 180), and their interactions on porosity and breaking force were analyzed. We found that all the main effects along with their 2-way and 3-way interactions significantly affected the porosity and breaking force at $p<0.0001$ (Figure 3). When assessing individual factors, the treatment duration exerted the greatest influence on the variability of the responses as shown in Figure 4. The influence of treatment duration on breaking force was about twice that on porosity, illustrating how increasing treatment durations improve rice kernel strength. Rice cultivars was the second individual factor influencing both responses, which highlights the importance of rice chemical compositions.

CONCLUSIONS

Chemical composition affects porosity and hardness of rice kernels differently. Porosity was more related to the quantity of proteins, whereas kernel hardness was more affected by its composition of proteins and lipids. The protein-starch matrix was responsible for rice kernel hardness and is proposed to be strengthened by an increase in glutelin content and a decrease in non-polar lipids. The protein-starch matrix was improved following protein denaturation via heat treatment because the conformational change of protein reduced void spaces and led to improved continuity of the protein-starch matrix due to enhanced protein-protein and protein-starch

interaction. Non-polar lipids weakened the protein-starch matrix by interfering with the hydrophilic matrix of proteins and starch, and thus reduced lipid content, decreased porosity, and improved the strength of kernels. Porosity does not always correlate with the kernel hardness of rice cultivars because different cultivars vary in the continuity of their starch-protein matrix and differ in the distribution of lipids among chemical components. This study demonstrates the importance of rice chemical components on porosity and kernel hardness. Further studies are needed to elucidate the influence of the distribution of rice protein composition on kernel strength.

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TABLES AND FIGURES

Table 1. Brown rice kernel dimensions and chemical composition of four selected rice cultivars[a].

	Cocodrie	Sierra	CL153	CLXL745
Dimensions				
Length (mm)	7.22±0.01[d]	7.73±0.01[a]	7.50±0.01[b]	7.45±0.00[c]
Width (mm)	2.43±0.00[b]	2.54±0.01[a]	2.25±0.00[d]	2.40±0.00[c]
Length/Width (L/W)	2.97±0.00[d]	3.04±0.01[c]	3.33±0.00[a]	3.10±0.00[b]
Thickness (mm)	1.97±0.00[c]	1.95±0.01[d]	2.02±0.00[a]	2.00±0.00[b]
Chemical composition (%, db)				
Apparent amylose	22.90±0.04[b]	26.62±0.03[a]	21.95±0.04[c]	20.90±0.01[d]
Crude protein	9.47±0.01[b]	11.04±0.01[a]	9.15±0.00[c]	8.56±0.00[d]
Crude lipid	3.33±0.02[a]	2.75±0.04[b]	2.52±0.01[d]	2.62±0.01[c]

[a]Mean values of two replications in the same row followed by the same letter are not significantly different ($P < 0.05$) based on Tukey's HSD test.

Table 2. Protein solubility, porosity and breaking force of four selected rice cultivars subjected to protein denaturation for varying durations[a].

Parameter	Denaturation time (min)	Cocodrie	Sierra	CL153	CLXL745
Protein solubility (%)	0	70.01±0.00[a]	68.58±0.01[a]	90.00±0.02[a]	93.33±0.00[a]
	30	56.52±0.01[b]	55.56±0.03[b]	67.62±0.04[b]	73.08±0.01[b]
	60	52.19±0.00[c]	45.88±0.04[c]	57.85±0.04[c]	55.89±0.01[c]
	90	47.83±0.02[d]	43.33±0.00[d]	55.17±0.03[d]	53.33±0.00[d]
Porosity (%)	0	12.44±0.02[a]	12.14±0.03[a]	13.85±0.14[a]	12.51±0.29[a]
	30	12.07±0.06[b]	11.73±0.04[b]	12.44±0.06[b]	11.02±0.06[b]
	60	11.95±0.06[b]	11.37±0.04[c]	11.47±0.01[c]	9.88±0.16[c]
	90	9.26±0.04[c]	9.24±0.02[d]	8.95±0.04[d]	8.54±0.06[d]
Breaking force (N)	0	13.98±0.02[d]	14.07±0.03[d]	14.28±0.14[d]	15.15±0.02[d]
	30	17.11±0.03[c]	16.94±0.01[c]	19.26±0.06[c]	18.87±0.01[c]
	60	19.76±0.04[b]	20.74±0.03[b]	20.51±0.01[b]	20.76±0.02[b]
	90	21.51±0.03[a]	22.21±0.04[a]	23.20±0.09[a]	23.69±0.07[a]

[a]Mean values of two replications with the same letter in the same column within the same cultivar are not significantly different ($P<0.05$) based on Tukey's HSD test.

Table 3. Residual lipid content, porosity and breaking force of four selected rice cultivars subjected to hexane extraction for varying durations[a].

Parameter	Lipid removal time (min)	Cocodrie	Sierra	CL153	CLXL745
Residual lipid content (%)	0	3.33±0.02[a]	2.75±0.04[a]	2.52±0.01[a]	2.62±0.01[a]
	30	2.80±0.02[b]	2.33±0.02[b]	2.05±0.01[b]	2.30±0.02[b]
	90	2.67±0.01[c]	2.27±0.02[c]	1.85±0.01[c]	2.19±0.02[c]
	180	2.64±0.03[c]	2.22±0.02[c]	1.66±0.02[d]	2.10±0.01[d]
Porosity (%)	0	12.44±0.02[a]	12.14±0.03[a]	13.85±0.14[a]	12.51±0.29[a]
	30	11.62±0.02[b]	10.02±0.00[b]	13.35±0.05[b]	12.13±0.04[b]
	90	11.13±0.01[c]	9.68±0.04[c]	12.54±0.05[c]	11.94±0.02[c]
	180	10.82±0.07[d]	9.00±0.03[d]	12.09±0.04[d]	11.82±0.01[d]
Breaking force (N)	0	13.98±0.02[d]	14.07±0.02[d]	14.28±0.14[d]	15.15±0.02[d]
	30	19.56±0.00[c]	18.99±0.01[c]	21.08±0.02[c]	20.62±0.04[c]
	90	20.74±0.04[b]	19.50±0.01[b]	23.56±0.04[b]	21.21±0.02[b]
	180	21.61±0.07[a]	19.69±0.02[a]	24.30±0.28[a]	22.88±0.04[a]

[a]Mean values of two replications with the same letter in the same column within the same cultivar are not significantly different ($P<0.05$) based on Tukey's HSD test.

Table 4. Porosity and breaking force of four selected rice cultivars subjected to combined treatments of protein denaturation (PD) for 60 min and lipid removal (LR) for varying durations[a].

Parameter	Treatment	Lipid removal time (min)	Cocodrie	Sierra	CL153	CLXL745
Residual lipid content (%)	PD-LR	30	2.97±0.05[a]	2.52±0.01[a]	2.18±0.03[a]	2.40±0.01[a]
	LR-PD		2.78±0.03[b]	2.35±0.04[b]	2.04±0.01[b]	2.35±0.02[b]
	PD-LR	90	2.85±0.02[a]	2.43±0.01[a]	2.04±0.03[a]	2.33±0.04[a]
	LR-PD		2.69±0.01[b]	2.30±0.01[b]	1.87±0.04[b]	2.23±0.02[b]
	PD-LR	180	2.80±0.08[a]	2.35±0.02[a]	1.84±0.01[a]	2.25±0.01[a]
	LR-PD		2.66±0.03[b]	2.24±0.02[b]	1.68±0.03[b]	2.14±0.03[b]
Porosity (%)	PD-LR	30	10.40±0.02[a]	10.71±0.03[a]	11.13±0.01[a]	10.98±0.01[a]
	LR-PD		10.15±0.00[b]	10.44±0.03[b]	10.88±0.01[b]	10.49±0.01[b]
	PD-LR	90	9.60±0.02[a]	9.67±0.02[a]	10.91±0.01[a]	10.46±0.01[a]
	LR-PD		9.00±0.01[b]	9.26±0.01[b]	9.87±0.03[b]	10.01±0.01[b]
	PD-LR	180	8.50±0.01[a]	8.82±0.02[a]	9.84±0.02[a]	9.57±0.06[a]
	LR-PD		8.00±0.01[b]	8.40±0.02[b]	8.53±0.04[b]	9.36±0.06[b]
Breaking force (N)	PD-LR	30	24.53±0.01[b]	24.07±0.02[b]	28.45±0.04[b]	29.33±0.08[b]
	LR-PD		26.63±0.02[a]	25.43±0.03[a]	33.18±0.01[a]	33.27±0.13[a]
	PD-LR	90	26.67±0.01[b]	26.08±0.02[b]	29.85±0.12[b]	30.65±0.05[b]
	LR-PD		28.40±0.01[a]	27.24±0.01[a]	34.15±0.04[a]	34.89±0.08[a]
	PD-LR	180	28.42±0.02[b]	28.17±0.01[b]	32.13±0.05[b]	32.31±0.01[b]
	LR-PD		30.27±0.01[a]	29.27±0.02[a]	35.41±0.06[a]	36.16±0.30[a]

[a]Mean values of two replications with the same letter in the same column within the same cultivar for the same time duration are not significantly different ($P<0.05$) based on Tukey's HSD test.

Table 5. Percentages of strong kernels with breaking force ≥20 N after different treatments.

Treatment	Treatment time (min)	Cocodrie	Sierra	CL153	CLXL745
Control	0	1	3	4	8
PD[a]	30	26	21	38	33
	60	41	37	50	48
	90	56	59	63	64
LR[b]	30	34	22	45	38
	90	49	36	54	52
	180	54	50	60	58
PD-LR	30	57	72	85	83
	90	76	75	87	91
	180	82	87	93	92
LR-PD	30	65	73	89	89
	90	73	77	91	92
	180	84	85	94	95

[a]PD: Protein denaturation
[b]LR: Lipid removal

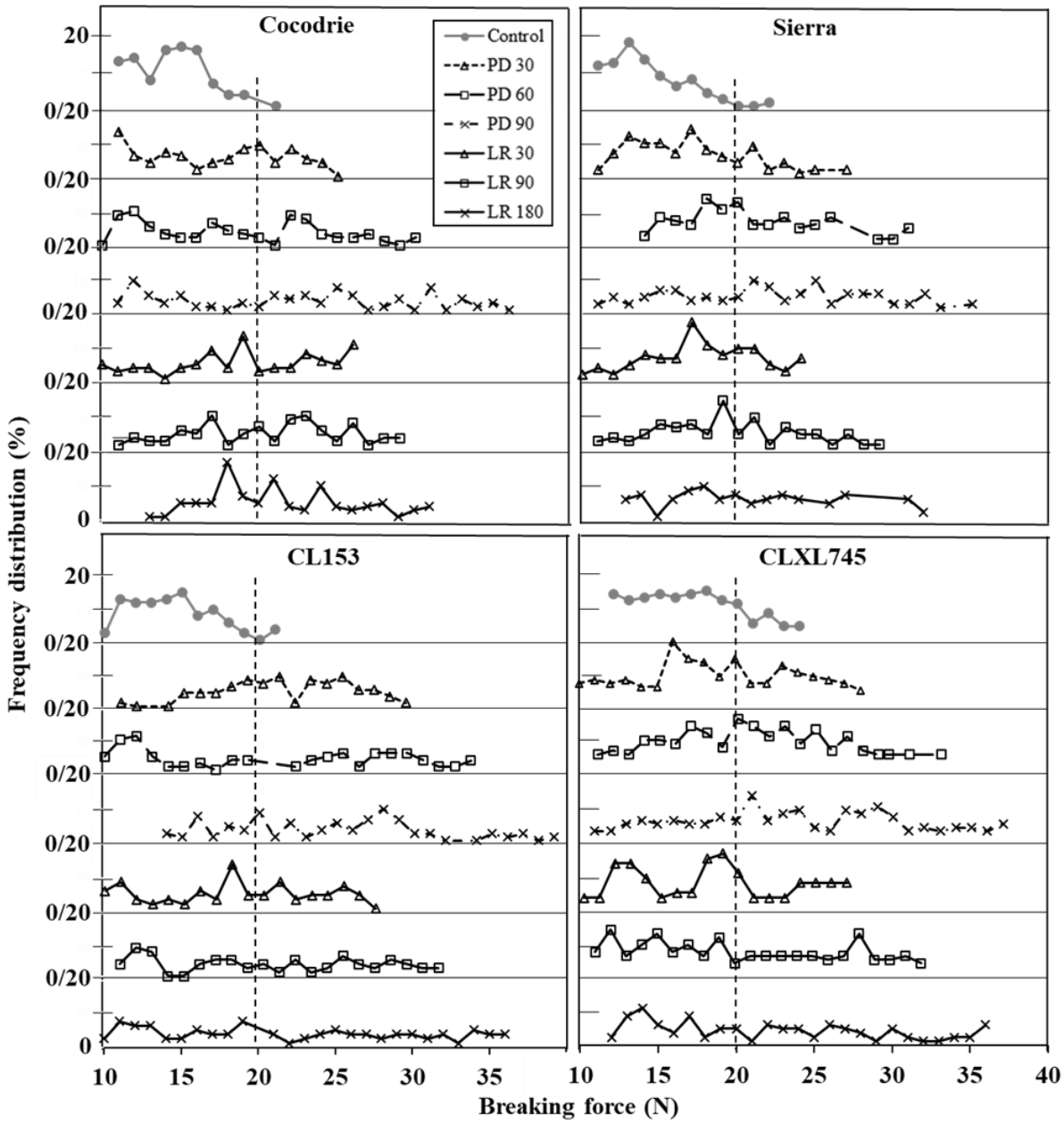


Figure 1. Breaking force distributions of rice kernels. Each curve represents the breaking force values of 100 sound brown rice kernels.
PD, Protein denaturation; LR, Lipid removal; number, treatment time.

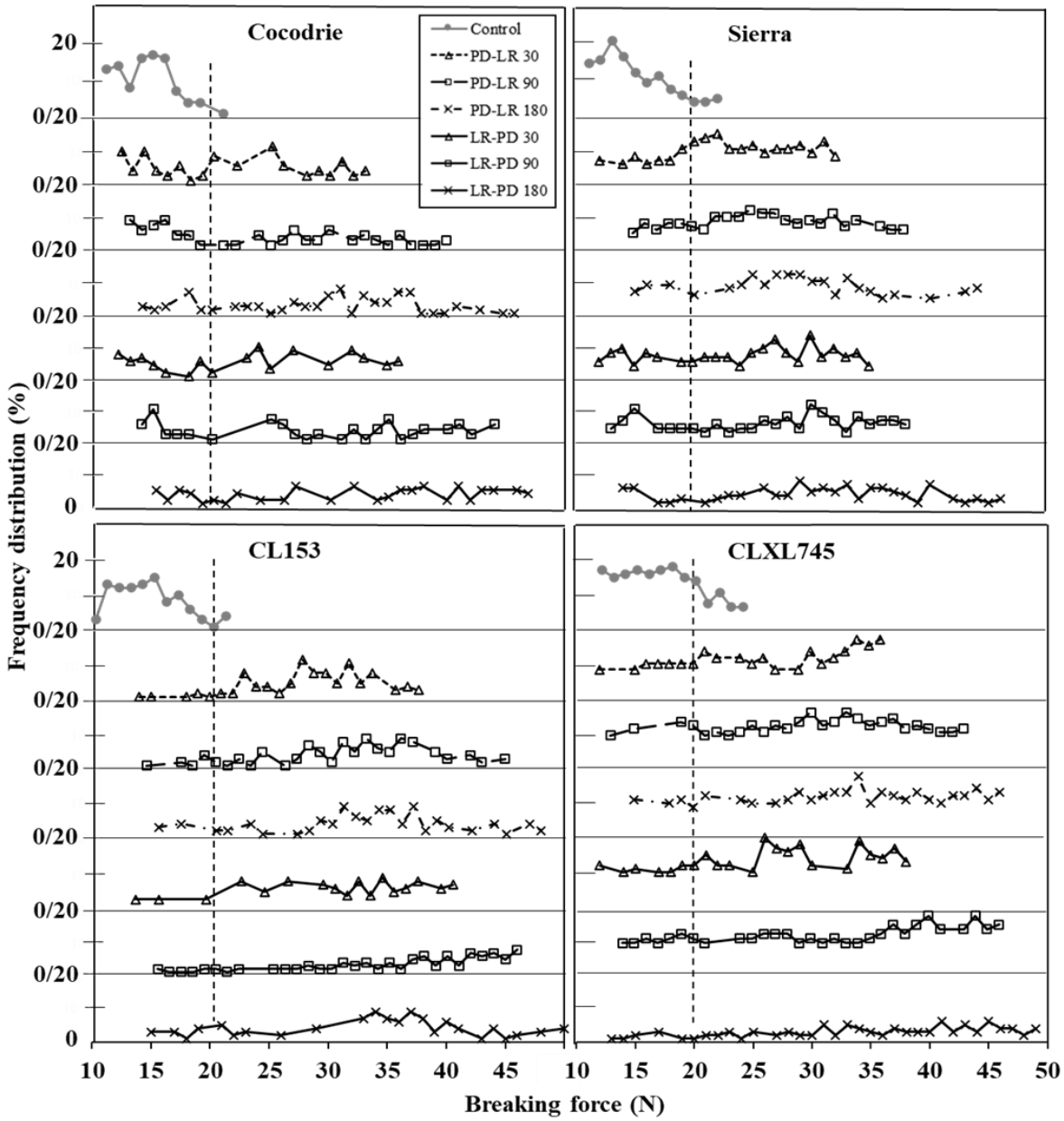


Figure 2. Breaking force distributions of rice kernels. Each curve represents the breaking force values of 100 sound brown rice kernels.
PD-LR, Protein denaturation first for 60 min followed by lipid removal; LR-PD, Lipid removal first followed by protein denaturation for 60 min; number, lipid removal time.

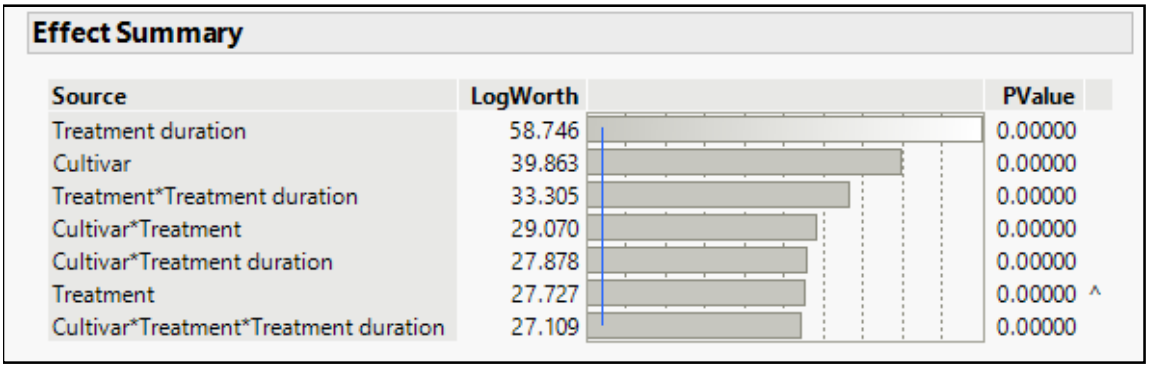
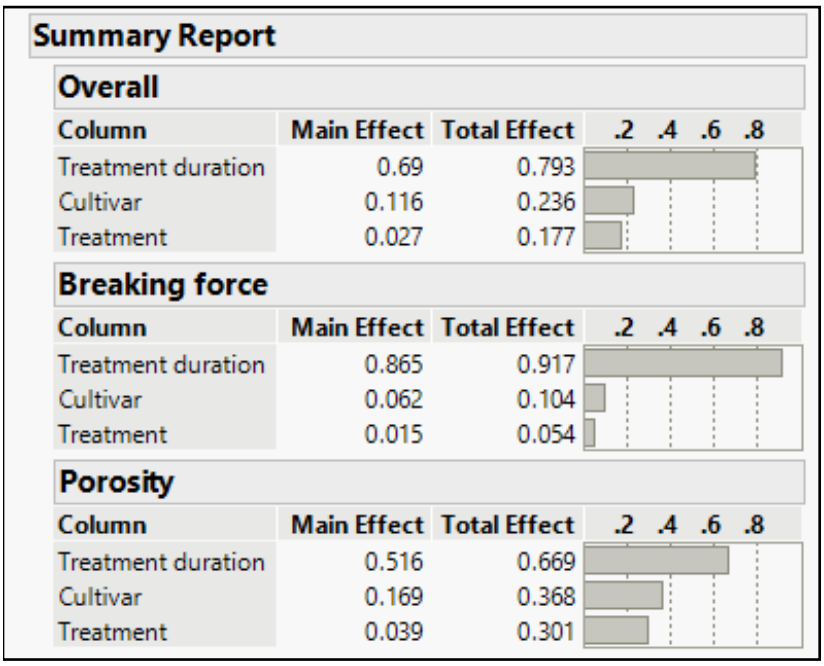


Figure 3. Effect summary of breaking force and porosity as affected by cultivar, treatment, treatment duration, and their interactions at a significance level of $P<0.05$.



Summary Report

Overall

Column	Main Effect	Total Effect
Treatment duration	0.69	0.793
Cultivar	0.116	0.236
Treatment	0.027	0.177

Breaking force

Column	Main Effect	Total Effect
Treatment duration	0.865	0.917
Cultivar	0.062	0.104
Treatment	0.015	0.054

Porosity

Column	Main Effect	Total Effect
Treatment duration	0.516	0.669
Cultivar	0.169	0.368
Treatment	0.039	0.301

Figure 4. Summary report showing the level of influence of the individual factors on porosity and breaking force.

IV. CHAPTER 2: Effect of Protein Denaturation and Lipid Removal on Rice Physicochemical Properties

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ABSTRACT

The physicochemical properties of rice are influenced by their chemical components and their interactions. It has been demonstrated that the interactions among rice chemical components are enhanced by protein denaturation (PD) via heat treatment and by lipid removal (LR) via solvent extraction. The objective of this study was to investigate the impacts of PD, LR, and their combined treatments on the gelatinization and pasting properties, and swelling power, water solubility of four brown rice flours. Rice protein was denatured under a vacuum at 100°C, and rice lipid was removed via hexane for varying times. The combined treatments were done in two ways, PD followed by LR (PD-LR), and LR followed by PD (LR-PD). The results showed that PD increased gelatinization temperatures, while LR decreased gelatinization temperatures. Both PD and LR reduced pasting and swelling properties; however, PD resulted in a greater decrease than LR. Polar lipids are proposed to serve as bridges to link denatured proteins and starch granules in the combined treatments, resulting in greater decreases in the pasting and swelling properties than the individual treatments. The results obtained in this study demonstrate the importance of starch-lipid-protein interactions on the physicochemical properties of brown rice flour.

Keywords: Rice; Protein denaturation; Lipid removal; Pasting properties; Gelatinization properties

INTRODUCTION

Rice flour is growing in popularity as a food ingredient due to its hypoallergenicity and bland taste. Rice flour properties are predominantly governed by starch with amylopectin responsible for starch swelling and pasting properties whereas amylose is responsible for inhibiting starch swelling (Tester & Morrison 1990). However, proteins and lipids also impact rice flour functionality.

Rice protein content, which ranges from 4.3 to 18.2%, positively correlates with hardness, but negatively correlates with the adhesiveness of cooked rice (Champagne, Wood, Juliano, & Bechtel, 2004; Derycke et al., 2005; Lyon, Champagne, Vinyard, & Windham, 2000). Cereal proteins are classified into four fractions according to their different solubilities (Osborne, 1924): water-soluble albumin, salt-soluble globulin, alcohol-soluble prolamins and alkali-soluble glutelin. Rice quality is more affected by glutelin and prolamin than by albumin and globulin because of their higher contents. Glutelin is the predominant protein fraction in rice, accounting for 60–80% of the total protein content and may restrict starch swelling through its interaction with starch (Kim, Lee, Yoon, Lim, & Kim, 2013). Baxter, Blanchard, & Zhao (2014) reported a greater glutelin content to be associated with an increase in rice flour gel hardness. In contrast, prolamin, which constitutes 20–30% of rice protein, causes significant decreases in gel hardness, but significant increases in the breakdown viscosity of starch gel (Baxter, Blanchard, & Zhao, 2004). In addition to their distinct impacts on rice physicochemical properties, the individual protein fractions also respond differently to various treatments (Chrastil, 1990; Ju, Hettiarachchy, & Rath, 2001; Shi, Wu, & Quan, 2017). Ju et al. (2001) reported that the solubility of rice glutelin decreased more substantially than the other protein fractions with increasing temperature due to its greater

sensitivity to heat. An increase in storage temperature can also lead to the oxidation of rice protein fractions (Chrastil, 1990).

Although lipids constitute only 1.6–3.1% of the total weight of brown rice, they significantly impact the physicochemical properties of rice (Champagne et al., 2004; Juliano & Perez, 1983). Lipids are not uniformly distributed throughout the rice kernel but are highly concentrated in the outer layers and progressively decrease toward the center of the kernel (Godber & Juliano, 2004; Zhou, Robards, Helliwell, & Blanchard, 2002). Rice lipids are usually classified as non-starch lipids, which are primarily triacylglycerols in the bran, and starch lipids, which are mostly phospholipids and predominantly associated with starch granules in the endosperm (Juliano, 1983). Rice cultivars with higher total lipid contents have greater gelatinization temperatures and require longer cooking times than those with lower total lipid contents (Juliano & Perez, 1983). The removal of lipids from milled rice flour resulted in significant decreases in the pasting temperature and the final and setback viscosities (Liang, 2001), because lipids complexed with amylose and hence restricted the reassociation of starch molecules during cooling.

The impacts of proteins and lipids on rice properties have been investigated primarily with respect to their individual effects. As a composite material, the mechanical, physicochemical, and functional properties of rice are affected not only by the individual chemical components but also by their interactions (Kim & Ami, 1993; Zweifel, Handschin, Escher, & Conde-Petit, 2003). Our previous work (Oppong Siaw, Wang, McClung, & Mauromoustakos, 2021) demonstrated the impacts of proteins and lipids on rice kernel porosity and hardness by protein denaturation and lipid removal via heat treatment and solvent extraction, respectively. The results showed that porosity was primarily influenced by the quantity of proteins and lipids, whereas kernel hardness was more influenced by the adhesion strength of the protein-starch matrix, which was proposed to

be strengthened by higher glutelin content and lower residual lipid content. Therefore, it was hypothesized that the increased interactions among chemical components in rice would also influence their physicochemical and functional properties and subsequently their applications as ingredients in food. The present study investigated the impacts of the combined protein denaturation and lipid removal treatment compared with the individual treatments on the gelatinization and pasting properties, swelling power, and water solubility of the treated brown rice flours.

MATERIALS AND METHODS

Materials

Rough rice of four long-grain rice cultivars with moisture contents of ~12% and varying protein and lipid contents were obtained and stored at 4 °C. Cocodrie from 2009 and Sierra from 2014 were produced by the USDA-ARS, Dale Bumpers National Rice Research Center, Stuttgart, AR. CL153 and CLXL745 from the 2018 crop year were produced by the University of Arkansas Rice Research and Extension Center at Harrisburg, AR. Rough rice was dehulled with a Satake rice machine (Model THU, Satake Engineering Co., Ltd., Tokyo, Japan), and broken brown rice kernels were separated from brown head rice using a shaker table (Grainman Machinery Mfg. Corp., Miami, FL). Brown head rice was used for this study.

Protein denaturation and/or lipid removal treatments

The procedures for protein denaturation, lipid removal and their combined treatments were detailed in our previous work (Oppong Siaw et al., 2021). Protein denaturation of brown head rice kernels was achieved via oven heat treatment at 100 °C for 0, 30, 60 and 90 min. Rice lipids were extracted at room temperature for 0, 30, 90 and 180 min using hexane. The combined treatments

were done in two ways: protein denaturation for 60 min first, followed by lipid removal for 30, 90 and 180 min (PD-LR), and lipid removal for 30, 90 and 180 min first, followed by protein denaturation for 60 min (LR-PD). Brown rice of treated and untreated rice was ground into flour using a Cyclone Sample Mill (Udy Corp. Fort Collins, CO).

Thermal properties

The thermal properties of untreated and treated brown rice flour were determined using a differential scanning calorimeter (Diamond DSC, PerkinElmer, Shelton, CT). Approximately 4.0 mg (db) of ground flour and 8 μL of deionized water were placed into a stainless-steel pan. The pan was scanned from 25 °C to 120 °C at 10 °C/min and the onset, peak, and end gelatinization temperatures and enthalpy (J/g) were recorded.

Pasting properties

The pasting properties of untreated and treated brown rice flour were determined using a Rapid ViscoAnalyser (RVA, Newport Scientific Pty. Ltd, Warriewood NSW, Australia). Rice slurry was prepared by mixing 3.0 g of brown rice flour (12% moisture basis) with 25 g deionized water. The slurry was first held at 50 °C for 1.5 min, heated from 50 °C to 95 °C at 5 °C/min, held at 95 °C for 5 min, cooled from 95 °C to 50 °C at 5 °C/min, and then finally held at 50 °C for 5 min. The peak, trough and final viscosities were recorded. Breakdown viscosity was calculated via the difference between peak and trough viscosity, and setback viscosity was calculated via the difference between final and peak viscosity.

Swelling power (SP) and water solubility index (WSI)

Swelling power (SP) and the water solubility index (WSI) were measured following the method of Holm, Björck, Asp, Sjöberg, & Lundquist (1985) with slight modifications. Brown rice flour (0.5 g, db) was suspended in 30 mL deionized water in a 50-mL centrifuge tube, and vortexed for 1 min. The suspension was heated in a water bath (Boekel/Grant ORS-200, Boekel Scientific, Feasterville, PA) at 95 °C for 30 min with gentle stirring. The heated sample was then cooled rapidly to room temperature in an ice water bath and finally, was centrifuged (Clinical 200, VWR International, Germany) at 2817×g for 20 min. The supernatant was carefully poured into a pre-weighed aluminum pan and dried at 105 °C for 12 h, and the remaining gel was collected and weighed. SP and WSI were calculated using the following formula:

$$SP\ (g/g) = \frac{Weight\ of\ gel\ (g)}{(Weight\ of\ flour\ - Weight\ of\ dried\ supernatant)\ g}$$

$$WSI\ (\%) = \frac{Weight\ of\ dried\ supernatant\ (g)}{Weight\ of\ flour\ (g)} \times 100$$

Statistical analysis

A completely randomized design (CRD) with a full 4 × 4 × 4 factorial treatment design, with two replications, was used to establish the effect of cultivar (Cocodrie, Sierra, CL153 and CLXL745), treatment (PD, LR, PD-LR, and LR-PD) and treatment duration (30, 60, 90, 180) on the swelling power, water solubility, and the gelatinization and pasting properties of treated and untreated brown rice flours. Data was analyzed using JMP Pro software (version 15.2, SAS Software Institute, Cary, NC). Tukey's Honestly Significant Difference (HSD) test was used for testing main and interaction factor effects at the 5% significance level to separate treatment

combination means. Pearson's correlation coefficient was used to determine the significance of linear relationship among the responses.

RESULTS AND DISCUSSION

Gelatinization properties

The gelatinization properties of rice are associated with the sensory quality of cooked rice and the energy and time required for cooking (Waters, Henry, Reinke, & Fitzgerald, 2006). The gelatinization temperatures and enthalpies of brown rice before and after the protein denaturation treatment and the lipid removal treatment are presented in Table 1. Both gelatinization temperatures and enthalpy increased gradually but significantly with increasing heat treatment duration for all cultivars. After 90 min of the heat treatment, the onset and peak temperatures increased by approximately 3 °C, the end temperature increased by 5–6 °C, and the enthalpy increased by approximately 2 J/g across all cultivars. The observed increases in these properties are attributed to the increased protein-protein and protein-starch interactions due to the protein denaturation and binding of denatured proteins to starch granules in the rice endosperm, respectively, which impedes water migration and starch swelling, thus leading to greater gelatinization temperatures and enthalpy (Paulik, Jekle, & Becker, 2019). Juliano (1984) proposed that denatured proteins became agglomerates via disulfide bond formation, which hindered heat transfer and increased gelatinization temperatures. Recently, Paulik et al. (2019) reported an increase in gelatinization temperature when wheat flour was heat treated for 13 min at increasing temperatures of 20, 40, 65, 80, 95 and 110 °C, but this increase in gelatinization temperatures was not observed when wheat starch was subjected to the same treatments. López-Barón, Gu, Vasanthan, and Hoover (2017) reported that the addition of native proteins had no effect on gelatinization temperatures, but the addition of denatured or hydrolyzed proteins resulted in a

significant increase in wheat starch gelatinization temperatures. The confocal laser scanning micrographs (CLSM) revealed that very few non-starch components were buried inside the starch granules with the addition of native proteins, but protein-starch interaction was noted when denatured or hydrolyzed proteins were added. López-Barón et al. (2017) further proposed that protein may interact with starch by forming a layer of coating on the surface of starch and by being embedded within the interior of starch granules. The increased gelatinization enthalpy is proposed to arise from the reorganization of starch molecules into a more crystalline and thermally stable structure (Zweifel, Conde-Petit, & Escher, 2000) and an increase in protein-starch interaction during the heating treatment. Zweifel et al. (2000) reported that a high drying temperature (100 °C) resulted in less permeable and more rigid starch granules, and consequently more thermostable granules. CLXL745 displayed the greatest increase in gelatinization enthalpy for all heat durations, which is attributed to its large amylopectin content (Oppong Siaw et al., 2021), since amylopectin is primarily responsible for starch crystalline structure.

In contrast to the protein denaturation treatment, a smaller yet significant decrease in gelatinization temperatures (0.57–1.61 °C) was observed following an increase in lipid removal time (Table 1). The changes in gelatinization temperature were not as large as those from the heat treatment, likely due to the smaller amount of lipid content relative to the protein content in rice kernels, and only non-starch lipids were extracted during lipid removal with hexane. Zhou, Robards, Helliwell, & Blanchard (2007) reported that the removal of non-starch lipids played a minor role in influencing starch gelatinization properties. The removal of starch lipids, however, has a more pronounced influence on the gelatinization properties. Zhang et al. (2019) reported a decrease of 2.07–6.69 °C in gelatinization temperature after removing starch lipids in milled rice. The greatest decrease in the onset gelatinization temperature of the four rice cultivars occurred in

CL153, which was attributed to its greatest decrease (0.86%) in residual lipids after 180 min of lipid removal (Oppong Siaw et al., 2021). The removal of lipids improved water access to rice flour, and consequently reduced gelatinization temperatures (Morrison, 1995). Similar to the protein denaturation treatment, the gelatinization enthalpy increased significantly across all cultivars following lipid removal. A negative correlation ($r = -0.50$, p-value = 0.0040) was found between residual lipid content and gelatinization enthalpy, which agrees with Zhang et al. (2019) who observed lower gelatinization enthalpy in high-lipid rice cultivars.

The results for the combined effects of protein denaturation for 60 min and lipid removal for varying durations on the gelatinization properties of treated rice are presented in Table 2. There was an increase in gelatinization temperatures and enthalpy with increasing lipid removal time for the combined treatments, although the individual treatments exerted opposite effects on the gelatinization temperatures. The protein denaturation (PD) treatment, regardless of the combining sequence with lipid removal, exerted a greater impact on gelatinization temperature than the lipid removal (LR) treatment, therefore both PD-LR and LR-PD resulted in increased gelatinization temperatures. Because hexane primarily removed nonpolar lipids, the proportion of polar lipids increased with increasing lipid removal time by hexane, and thus enhanced the starch and denatured protein interaction through the mediation of the polar lipids. Greenblatt, Bettge, & Morris (1995) proposed that polar lipids were capable of mediating proteins and starch interactions by acting as “bridges” between the starch granule surface and proteins. This protein-polar lipid-starch interaction further impeded water migration and starch swelling, thus leading to greater increases in gelatinization temperatures. The protein denaturation first, followed by the lipid removal (PD-LR) treatment had significantly higher gelatinization temperatures and generally higher enthalpy than the lipid removal first, followed by protein denaturation (LR-PD) treatment

irrespective of lipid removal time. For the PD-LR treatments, the structural change from protein denaturation is proposed to enclose the lipids and subsequently limit the amount of lipids extracted during the subsequent lipid removal treatment (Oppong Siaw et al., 2021). The enclosed nonpolar lipids increased the hydrophobicity of treated starch granules, and thus limited water penetration and subsequent starch swelling. When LR was conducted prior to PD, more lipids were removed, and therefore the hydrophobicity of the resulted starch granules was lower, which explains the lower gelatinization temperatures but greater gelatinization enthalpy by the LR-PD treatment compared with the PD-LR treatments.

Pasting properties

There was a gradual yet significant decrease in all pasting viscosities in all rice cultivars after the heat treatment, and the extent of the decreases was more pronounced after 60 min of heat treatment (Table 3, Supplementary Fig. 1). The formation of protein-protein and protein-starch interactions from the heat treatment is proposed to be primarily responsible for the decrease in all pasting viscosities because of restricted starch swelling from these interactions. The restriction of starch swelling, in turn, better preserved starch granule integrity, and protected the starch from mechanical shearing, leading to a decrease in breakdown viscosity (Hamaker & Griffin, 1993). The decrease in breakdown viscosity after 90 min of the heat treatment followed the pattern of CLXL745 > CL153 > Sierra > Cocodrie, which was attributed to the greater decrease in protein solubility observed in CLXL745 and CL153 than in Sierra and Cocodrie in the previous study (Oppong Siaw et al., 2021).

The impacts of lipid removal on the pasting properties (Table 3, Supplementary Fig. 2) were similar to those of protein denaturation but to a lesser extent, likely due to the small amount

of lipids (0.52–0.86%) that were extracted from rice kernels (Oppong Siaw et al., 2021). Although the removal of nonpolar lipids improved water access to starch and slightly decreased gelatinization temperatures, the viscosity development was predominantly governed by the protein-starch interaction in rice flour. The decrease in nonpolar lipids led to better interaction between starch and proteins, which further limited starch swelling. Therefore, an increase in lipid removal time resulted in a decrease in pasting viscosities. The present results were consistent with those of Yang & Chang (1999), who reported gradual decrease in pasting viscosities when lipids were removed from the outer parts of milled rice kernels.

The pasting viscosities of the combined treatments were significantly lower than those of the individual treatments, for the same lipid removal time, irrespective of the order of the combined treatment (Table 4, Supplementary Figs. 3–4), suggesting the occurrence of synergistic interactions from the starch-polar lipid-protein interactions, as described in Section 3.1. The PD-LR treatment displayed greater setback viscosities but significantly lower peak, trough and breakdown viscosities than those of the LR-PD treatment for most conditions. The exposure of the hydrophobic groups from protein denaturation in both PD-LR and LR-PD treatments increased the hydrophobicity in the rice kernel. However, because less lipids were removed in the PD-LR treatment as compared to the LR-PD treatment, the hydrophobicity in the PD-LR treated kernels was greater than that in the LR-PD treated kernels. Therefore, the pasting viscosities, except setback viscosity, of the LR-PD treatment were greater than those of the PD-LR treatment.

Swelling power (SP) and water solubility properties (WSI)

The results of swelling power and the water solubility index of brown rice flour at 95 °C from the protein denaturation or the lipid removal treatment are presented in Fig. 1. Swelling power

(SP) represents the water holding capacity of starch and is primarily governed by the amylopectin fraction and inversely proportional to the rigidity of the starch granule (Lii, Tsai, & Tseng, 1996). All cultivars exhibited a decrease in swelling power with increasing denaturation time (Fig. 1A), which is similar to the change in peak viscosity as peak viscosity reflects the extent of swelling. Native CL153 and CLXL745 exhibited greater SP due to their higher amylopectin and lower protein and lipid contents (Oppong Siaw et al., 2021). Heat denaturation enhanced the surface hydrophobicity of protein, and thus decreased water availability, starch hydration, and water holding capacity (López-Barón et al., 2017). The greater decreases in SP of CL153 and CLXL745 correlate with their greater decreases in protein solubility due to their greater glutelin contents as reported in our previous study (Oppong Siaw et al., 2021). Glutelin, a type II protein body, is more susceptible to conformational change, which enhances its ability to bind to starch and thus greater restricts the degree of starch swelling compared with type I protein bodies like prolamins, which better retain their conformation during heat treatment (Iida, Amano, & Nishio, 1993; Ogawa et al., 1987). The water solubility index (WSI) of rice flour indicates the amount of leached soluble components and is primarily composed of amylose due to its linear structure (Ding, Ainsworth, Tucker, & Marson, 2005). Therefore, CLXL745, with the lowest amylose content of the four cultivars, had the lowest WSI (Oppong Siaw et al., 2021). The denaturation of proteins restricted starch swelling, and thus decreased the swelling power of starch granules, and subsequently the amount of leached soluble components (Hasjim, Li, & Dhital, 2012). Therefore, there was a gradual decrease in the WSI of rice flour as protein denaturation time increased. The results also support the negative correlation observed between SP ($r = -0.47$, p-value = 0.0073) and the WSI ($r = -0.56$, p-value = 0.0009) with increasing protein denaturation time.

The removal of lipids resulted in a decrease in both the SP and WSI of brown rice flour (Fig. 1B), and the decrease was not as significant as that in the protein denaturation treatment, which is similar to the trend of the pasting properties. Because the four rice cultivars had inherently different lipid contents, no correlation ($r = -0.13$, p-value = 0.46) was observed between the SP and the lipid removal time when considering all cultivars. However, a very strong negative correlation ($r = -0.96$ to -0.98, p-value = <0.0001) was noted between the SP and the lipid removal time within each cultivar. CL153, which had the greatest decrease in SP (0.63 g/g) and WSI (4.38%) among the four cultivars, had the greatest decrease in residual lipids (0.86%) after 180 min of lipid removal. Similarly, CLXL745, which had the smallest decrease in the SP (0.25 g/g) and WSI (1.19%) among the four cultivars, had the smallest decrease in residual lipids (0.52%).

Both the PD-LR and LR-PD treatments exhibited a similar trend in the SP and WSI as the individual treatments with a gradual decrease in the SP and WSI with increasing lipid removal time (Fig. 2). The decrease in both the SP and WSI of the combined treatments was greater than that of the individual treatments as occurred with their pasting properties, supporting the synergistic effect from the protein-polar lipid-starch interactions. The SP and WSI values of the PD-LR treatment were lower than those of the LR-PD treatment irrespective of the lipid removal time, which supports the trend observed in the pasting viscosities.

Statistical summary

Data analysis showed that cultivar (Cocodrie, Sierra, CL153 and CLXL745), treatment (PD, LR, PD-LR, and LR-PD) and treatment duration (30, 60, 90, 180) and all their two-way and three-way interactions significantly affected the swelling, water solubility, gelatinization and

pasting properties of the brown rice flours as shown in Fig. 3A. To assess the factor (cultivar, treatment, treatment duration) importance on all the responses (swelling, water solubility, gelatinization and pasting properties), we ran simulations using independently resampled inputs (Fig. 3B). Cultivar exerted the greatest influence on the variability of the responses, thus highlighting the importance of rice chemical composition on the rice physicochemical properties. Subsequently, the overall main effect of the treatments (PD, LR, PD-LR, and LR-PD) on each of the responses were compared using Tukey's HSD test (Table 5). LR had the least impact on the physicochemical properties, implying the minor role that lipids play in rice flour functionalities and end-use. There was a significant difference between LR and PD-LR/LR-PD, but no significance difference between PD and PD-LR/LR-PD, thus highlighting the major role that proteins play in rice physicochemical properties.

CONCLUSIONS

Protein denaturation and lipid removal exerted contrasting effects on the gelatinization temperatures of treated rice flour, and these effects were enhanced by treatment time. The high temperature applied during the protein denaturation treatment promoted protein-protein and protein-starch interactions, leading to increased gelatinization properties, and decreased pasting viscosities and swelling properties. Polar lipids acted as bridges to link starch granules and denatured proteins, and the removal of non-polar lipids increased the concentration of polar lipids, which promoted protein-starch interactions and led to decreased pasting and swelling properties. The combined treatments resulted in more significant increases in gelatinization temperatures and decreases in pasting, swelling power and water solubility properties than the individual treatments, which was proposed to result from the synergistic interactions from the starch-polar lipid-protein interactions. The physicochemical properties of the combined treatments on rice cultivars were

affected by the sequence of the individual treatments (PD and LR), illustrating the unique differences in how PD and LR affect rice properties. The results suggest that heat treatment could be employed to produce brown rice flour with improved heat and shear stabilities for specific applications.

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TABLES AND FIGURES

Table 1. Gelatinization temperatures and enthalpies of rice cultivars subjected to protein denaturation and lipid removal for varying durations[a].

Cultivar	Treatment	Treatment duration (min)	Temperature (°C)			Enthalpy (J/g)
			Onset	Peak	End	
Cocodrie	Protein denaturation	0	71.23±0.03^{d}	75.97±0.02^{d}	79.69±0.01^{d}	8.29±0.01^{d}
		30	72.46±0.04^{c}	76.82±0.03^{c}	81.14±0.03^{c}	9.04±0.01^{c}
		60	73.56±0.05^{b}	77.85±0.02^{b}	83.44±0.03^{b}	9.68±0.05^{b}
		90	74.46±0.04^{a}	78.67±0.03^{a}	85.27±0.01^{a}	9.99±0.01^{a}
Sierra		0	70.59±0.01^{d}	74.46±0.04^{d}	78.44±0.04^{d}	7.88±0.02^{d}
		30	71.73±0.02^{c}	75.96±0.02^{c}	79.97±0.03^{c}	8.41±0.01^{c}
		60	72.85±0.04^{b}	76.28±0.02^{b}	81.37±0.02^{b}	9.12±0.01^{b}
		90	73.97±0.04^{a}	77.87±0.04^{a}	83.82±0.02^{a}	9.94±0.04^{a}
CL153		0	72.85±0.01^{d}	76.62±0.04^{d}	80.68±0.02^{d}	8.44±0.04^{d}
		30	73.38±0.02^{c}	77.95±0.02^{c}	81.95±0.05^{c}	9.11±0.01^{c}
		60	74.48±0.01^{b}	78.93±0.02^{b}	83.75±0.01^{b}	9.62±0.02^{b}
		90	75.97±0.01^{a}	79.47±0.01^{a}	86.84±0.02^{a}	10.42±0.02^{a}
CLXL745		0	73.44±0.02^{d}	78.44±0.01^{d}	82.77±0.03^{d}	8.67±0.02^{d}
		30	74.66±0.03^{c}	79.64±0.02^{c}	84.43±0.01^{c}	9.96±0.01^{c}
		60	75.18±0.02^{b}	80.47±0.01^{b}	86.33±0.02^{b}	10.49±0.01^{b}
		90	76.74±0.02^{a}	81.92±0.02^{a}	88.73±0.02^{a}	11.37±0.05^{a}
Cocodrie	Lipid removal	0	71.23±0.03^{a}	75.97±0.02^{a}	79.69±0.01^{a}	8.29±0.01^{d}
		30	71.15±0.05^{a}	75.67±0.01^{b}	79.49±0.02^{b}	8.75±0.01^{c}
		90	70.95±0.02^{b}	75.26±0.04^{c}	79.15±0.04^{c}	9.27±0.02^{b}
		180	70.56±0.03^{c}	74.88±0.02^{d}	78.67±0.03^{d}	10.06±0.05^{a}
Sierra		0	70.59±0.01^{a}	74.46±0.04^{a}	78.44±0.04^{a}	7.88±0.02^{d}
		30	70.45±0.04^{b}	74.23±0.01^{b}	77.98±0.01^{b}	8.29±0.01^{c}
		90	70.14±0.03^{c}	74.17±0.02^{b}	77.63±0.02^{c}	8.64±0.02^{b}
		180	70.02±0.01^{d}	73.86±0.02^{c}	77.23±0.02^{d}	9.22±0.03^{a}
CL153		0	72.85±0.01^{a}	76.62±0.01^{a}	80.68±0.02^{a}	8.44±0.04^{d}
		30	72.44±0.05^{b}	76.29±0.02^{b}	80.36±0.04^{b}	8.85±0.03^{c}
		90	71.84±0.01^{c}	76.07±0.04^{c}	79.88±0.02^{c}	9.12±0.02^{b}
		180	71.27±0.02^{d}	75.45±0.04^{d}	79.58±0.02^{d}	9.83±0.05^{a}
CLXL745		0	73.44±0.02^{a}	78.44±0.01^{a}	82.77±0.03^{a}	8.67±0.02^{d}
		30	73.14±0.03^{b}	78.40±0.01^{a}	82.47±0.02^{b}	9.02±0.02^{c}
		90	72.87±0.02^{c}	78.06±0.06^{b}	81.81±0.02^{c}	9.79±0.03^{b}
		180	72.43±0.02^{d}	77.85±0.02^{c}	81.16±0.01^{d}	10.17±0.01^{a}

[a]Mean values of two replicates with the same letter in the same column within the same cultivar are not significantly different ($P<0.05$) based on Tukey's HSD test.

Table 2. Gelatinization temperatures and enthalpies of rice cultivars after the combined treatments of protein denaturation for 60 min and lipid removal for varying durations[a].

Cultivar	Treatment	Lipid removal time (min)	Temperature (°C)			Enthalpy (J/g)
			Onset	Peak	End	
Cocodrie	PD-LR	30	73.15±0.01[a]	77.11±0.01[a]	81.69±0.02[a]	9.41±0.01[b]
	LR-PD		72.71±0.04[b]	76.91±0.01[b]	81.22±0.03[b]	9.94±0.03[a]
	PD-LR	90	74.40±0.02[a]	78.05±0.02[a]	83.98±0.02[a]	10.26±0.01[b]
	LR-PD		73.60±0.04[b]	77.65±0.06[b]	83.54±0.04[b]	10.64±0.02[a]
	PD-LR	180	75.66±0.02[a]	78.97±0.01[a]	85.88±0.01[a]	10.52±0.01[b]
	LR-PD		74.36±0.03[b]	78.95±0.02a	85.43±0.01[b]	10.90±0.01[a]
Sierra	PD-LR	30	71.88±0.02[a]	75.85±0.01[a]	79.60±0.01[a]	8.94±0.03[b]
	LR-PD		71.50±0.01[b]	75.33±0.01[b]	79.14±0.02[b]	9.11±0.01[a]
	PD-LR	90	72.93±0.04[a]	76.60±0.02[a]	81.66±0.01[a]	9.53±0.02[b]
	LR-PD		72.66±0.01[b]	76.23±0.02[b]	81.21±0.01[b]	9.88±0.01[a]
	PD-LR	180	73.97±0.01[a]	77.93±0.02[a]	83.91±0.01[a]	10.44±0.02[b]
	LR-PD		73.49±0.01[b]	77.44±0.02[b]	83.26±0.01[b]	10.81±0.02[a]
CL153	PD-LR	30	73.91±0.02[a]	77.74±0.06[a]	82.68±0.04[b]	9.40±0.03[a]
	LR-PD		73.58±0.02[b]	77.36±0.06[b]	83.87±0.04[a]	9.74±0.04[b]
	PD-LR	90	74.95±0.04[a]	78.66±0.07[a]	84.65±0.05[b]	10.17±0.03[a]
	LR-PD		74.66±0.03[b]	78.16±0.06[b]	85.06±0.00[a]	10.27±0.04[a]
	PD-LR	180	75.88±0.01[a]	79.76±0.05[a]	86.30±0.04[a]	10.93±0.02[b]
	LR-PD		75.45±0.04[b]	79.60±0.03[a]	86.46±0.26[a]	11.07±0.04[a]
CLXL745	PD-LR	30	75.27±0.02[a]	80.00±0.06[a]	84.84±0.03[a]	9.52±0.01[a]
	LR-PD		74.61±0.04[b]	78.94±0.03[b]	84.28±0.06[b]	9.37±0.03[b]
	PD-LR	90	75.95±0.04[a]	80.70±0.03[a]	86.95±0.03[a]	10.08±0.04[b]
	LR-PD		75.20±0.11[b]	79.99±0.04[b]	86.72±0.01[b]	10.56±0.03[a]
	PD-LR	180	77.14±0.08[a]	81.85±0.04[a]	88.62±0.06[a]	10.94±0.04[b]
	LR-PD		76.32±0.06[b]	80.62±0.02[b]	87.60±0.05[b]	11.24±0.04[a]

[a]Mean values of two replicates with the same letter in the same column within the same cultivar for the same time duration are not significantly different ($P<0.05$) based on Tukey's HSD test.

Table 3. Pasting viscosities of rice cultivars subjected to protein denaturation and lipid removal for varying durations[a].

Cultivar	Treatment	Treatment duration (min)	Viscosity (cP)				
			Peak	Trough	Breakdown	Final	Setback
Cocodrie	Protein denaturation	0	1595±1[a]	1156±11[a]	439±16[a]	3619±78[a]	2024±40[a]
		30	1556±19[a]	1130±23[b]	426±7[a]	2772±22[b]	1216±8[b]
		60	1272±26[b]	1020±9[c]	252±10[c]	2469±16[c]	1197±2[b]
		90	1216±13[b]	1015±10[c]	201±3[d]	2287±1[d]	1071±12[c]
Sierra		0	2413±10[a]	1863±39[a]	550±12[a]	5349±38[a]	2936±11[a]
		30	2370±2[a]	1854±23[a]	516±12[a]	4136±32[b]	1766±3[b]
		60	1922±12[b]	1644±12[b]	278±0[b]	3499±7[c]	1577±19[c]
		90	1829±63[b]	1588±49[b]	241±7[b]	3376±11[c]	1547±45[c]
CL153		0	2226±6[a]	1364±38[a]	862±4[a]	4354±49[a]	2128±42[a]
		30	2185±23[a]	1354±20[b]	831±16[ab]	4179±28[b]	1994±4[b]
		60	2019±17[b]	1308±1[b]	711±31[c]	3444±33[c]	1425±13[c]
		90	1780±3[c]	1307±9[b]	473±6[d]	3049±19[d]	1269±30[d]
CLXL745		0	2495±30[a]	1439±6[a]	1056±18[a]	4766±37[a]	2271±5[a]
		30	2444±21[a]	1417±8[a]	1027±9[b]	4472±8[b]	2028±13[b]
		60	2240±2[b]	1377±11[b]	863±11[c]	3794±36[c]	1554±8[c]
		90	1957±3[c]	1365±7[b]	592±3[d]	3036±6[d]	1079±11[d]
Cocodrie	Lipid removal	0	1595±1[a]	1156±11[a]	439±16[a]	3619±78[a]	2024±40[a]
		30	1490±16[b]	1064±16[b]	426±8[ab]	3008±16[b]	1518±16[b]
		90	1381±1[c]	1049±8[b]	332±7[ab]	2598±3[c]	1217±8[c]
		180	1340±27[c]	1020±24[b]	320±28[b]	2457±25[c]	1117±28[c]
Sierra		0	2413±10[a]	1863±39[a]	550±12[a]	5349±38[a]	2936±11[a]
		30	2359±47[ab]	1846±30[a]	513±9[a]	5236±22[a]	2877±16[b]
		90	2323±16[ab]	1829±4[a]	494±16[b]	5036±16[a]	2713±16[c]
		180	2270±37[b]	1780±10[a]	490±16[b]	4961±24[b]	2691±9[d]
CL153		0	2226±6[a]	1364±38[a]	862±4[a]	4354±49[a]	2128±42[a]
		30	2017±42[b]	1230±13[b]	787±22[a]	4073±78[b]	2056±6[ab]
		90	1954±28[bc]	1196±1[b]	758±21[b]	3938±11[bc]	1984±14[b]
		180	1852±53[c]	1145±37[b]	707±16[b]	3752±2[c]	1900±17[c]
CLXL745		0	2495±30[a]	1439±6[a]	1056±18[a]	4766±37[a]	2271±5[a]
		30	2355±2[b]	1321±21[b]	1034±39[a]	3865±84[b]	1510±45[b]
		90	2140±19[c]	1226±19[bc]	914±19[b]	3600±35[c]	1460±15[bc]
		180	2082±49[c]	1208±47[c]	874±1[b]	3523±35[c]	1441±19[c]

[a]Mean values of two replicates with the same letter in the same column within the same cultivar are not significantly different ($P<0.05$) based on Tukey's HSD test.

Table 4. Pasting viscosities of rice cultivars after the combined treatment of protein denaturation for 60 min and lipid removal for varying durations[a].

Cultivar	Treatment	Lipid removal time (min)	Viscosity (cP)				
			Peak	Trough	Breakdown	Final	Setback
Cocodrie	PD-LR	30	1386±6[b]	1046±2[b]	340±2[b]	2417±18[a]	1031±10[a]
	LR-PD		1457±34[a]	1032±11[a]	425±1[a]	2410±28[a]	953±14[b]
	PD-LR	90	1142±14[b]	889±6[b]	243±5[b]	2134±21[b]	992±4[a]
	LR-PD		1405±13[a]	996±8[a]	409±10[a]	2333±16[a]	928±10[a]
	PD-LR	180	1027±11[b]	889±8[b]	138±5[b]	1883±13[b]	856±11[a]
	LR-PD		1336±24[a]	1008±9[a]	328±4[a]	2187±64[a]	851±19[a]
Sierra	PD-LR	30	1223±8[b]	809±8[b]	414±6[a]	2575±9[b]	1352±4[a]
	LR-PD		1470±18[a]	1028±4[a]	442±17[a]	2673±32[a]	1203±4[b]
	PD-LR	90	1211±1[b]	820±1[b]	391±7[a]	2538±6[a]	1327±13[a]
	LR-PD		1435±4[a]	1001±1[a]	434±14[a]	2578±4[a]	1143±10[b]
	PD-LR	180	1194±20[b]	826±21[b]	368±6[b]	2472±18[b]	1278±4[a]
	LR-PD		1380±17[a]	965±5[a]	415±4[a]	2514±15[a]	1134±1[b]
CL153	PD-LR	30	1513±23[b]	1134±8[b]	379±10[b]	2826±8[b]	1313±6[a]
	LR-PD		1787±2[a]	1263±2[a]	524±1[a]	3054±6[a]	1267±4[b]
	PD-LR	90	1428±3[b]	1134±5[b]	294±7[b]	2734±6[a]	1306±8[a]
	LR-PD		1733±4[a]	1242±6[a]	491±6[a]	2780±6[a]	1047±6[b]
	PD-LR	180	1424±2[a]	1257±6[a]	167±6[b]	2691±7[a]	1267±15[a]
	LR-PD		1374±12[b]	1149±0[b]	225±8[a]	2400±6[b]	1026±4[b]
CLXL745	PD-LR	30	2018±42[b]	1350±4[a]	668±24[b]	3210±90[a]	1192±34[a]
	LR-PD		2111±46[a]	1260±19[a]	851±30[a]	3107±48[a]	996±9[b]
	PD-LR	90	1880±62[a]	1335±47[a]	545±15[a]	2951±70[a]	1071±36[a]
	LR-PD		1891±17[a]	1333±3[a]	558±4[a]	2847±19[b]	956±2[b]
	PD-LR	180	1740±33[b]	1312±16[a]	428±16[b]	2772±52[a]	1032±2[a]
	LR-PD		1853±7[a]	1303±6[a]	550±16[a]	2666±5[b]	813±15[b]

[a]Mean values of two replicates with the same letter in the same column within the same cultivar for the same time duration are not significantly different ($P<0.05$) based on Tukey's HSD test.

Table 5. Main effect of treatment on gelatinization, pasting and swelling properties.

Treatment	Gelatinization Properties				Pasting Properties						
	Temperature (°C)				Viscosity (cP)						
	Onset	Peak	End	Enthalpy (J/g)	Peak	Trough	Breakdown	Final	Setback	Swelling power (g/g)	Water solubility index (%)
PD	73.59[a]	77.96[a]	83.08[a]	9.40[ab]	1970[a]	1387[a]	582[ab]	3662[a]	1693[ab]	11.32[b]	11.58[ab]
LR	71.58[b]	75.98[b]	79.81[b]	9.02[b]	2018[a]	1358[a]	660[a]	4008[a]	1990[a]	11.79[a]	12.73[a]
PD-LR	73.95[a]	78.04[a]	83.27[a]	9.59[a]	1619[b]	1163[b]	455[c]	3081[b]	1460[bc]	11.19[b]	11.04[b]
LR-PD	73.51[a]	77.67[a]	83.04[a]	9.80[a]	1747[b]	1212[b]	535[bc]	3102[b]	1355[c]	11.35[b]	11.38[b]

[a]Mean values with the same letter in the same column are not significantly different (P<0.05) based on Tukey's HSD test.

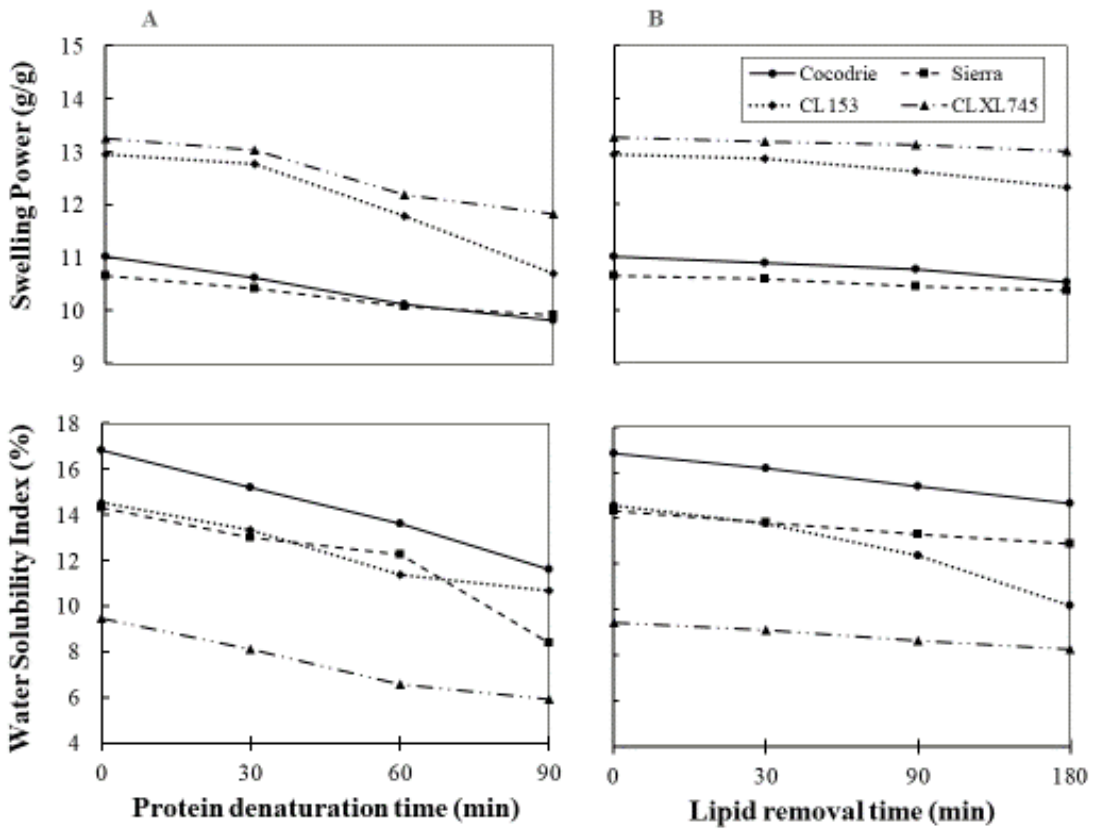


Figure 1. Swelling power and water solubility index of rice cultivars after A) protein denaturation and B) lipid removal.

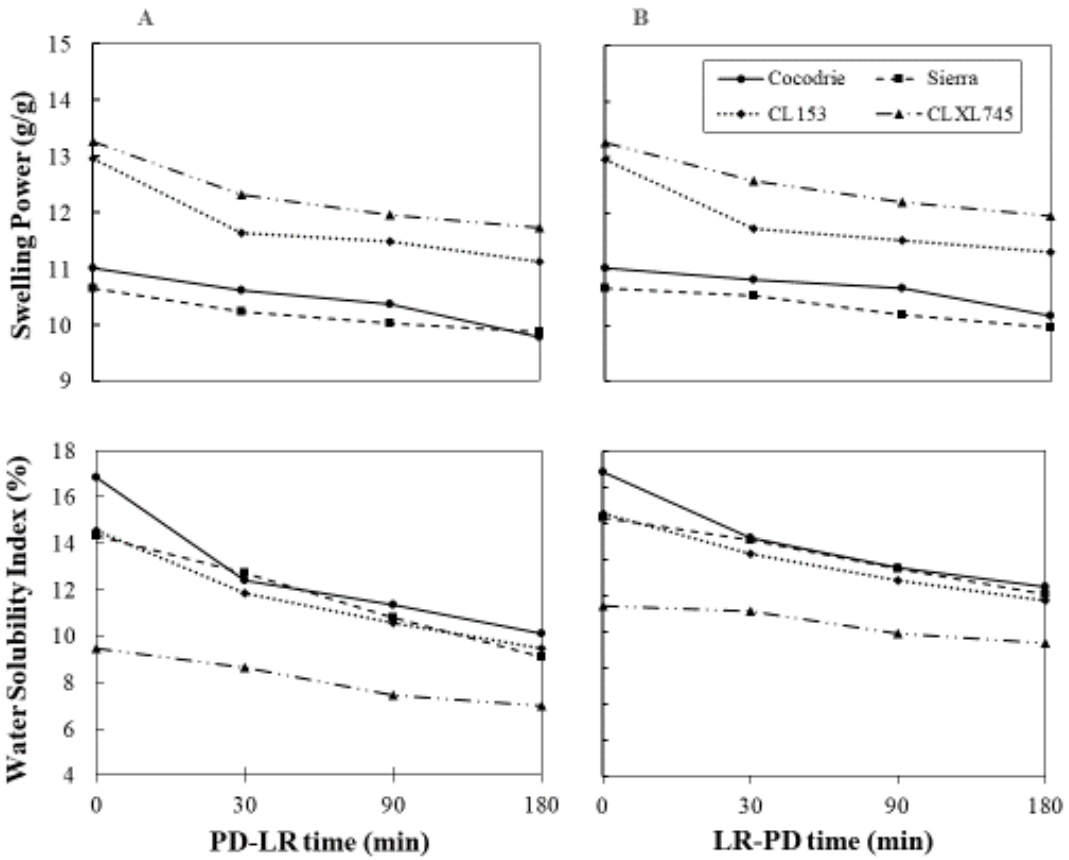


Figure 2. Swelling power and water solubility index of rice cultivars after A) protein denaturation first, followed by lipid removal (PD-LR) and B) lipid removal first followed by protein denaturation (LR-PD).

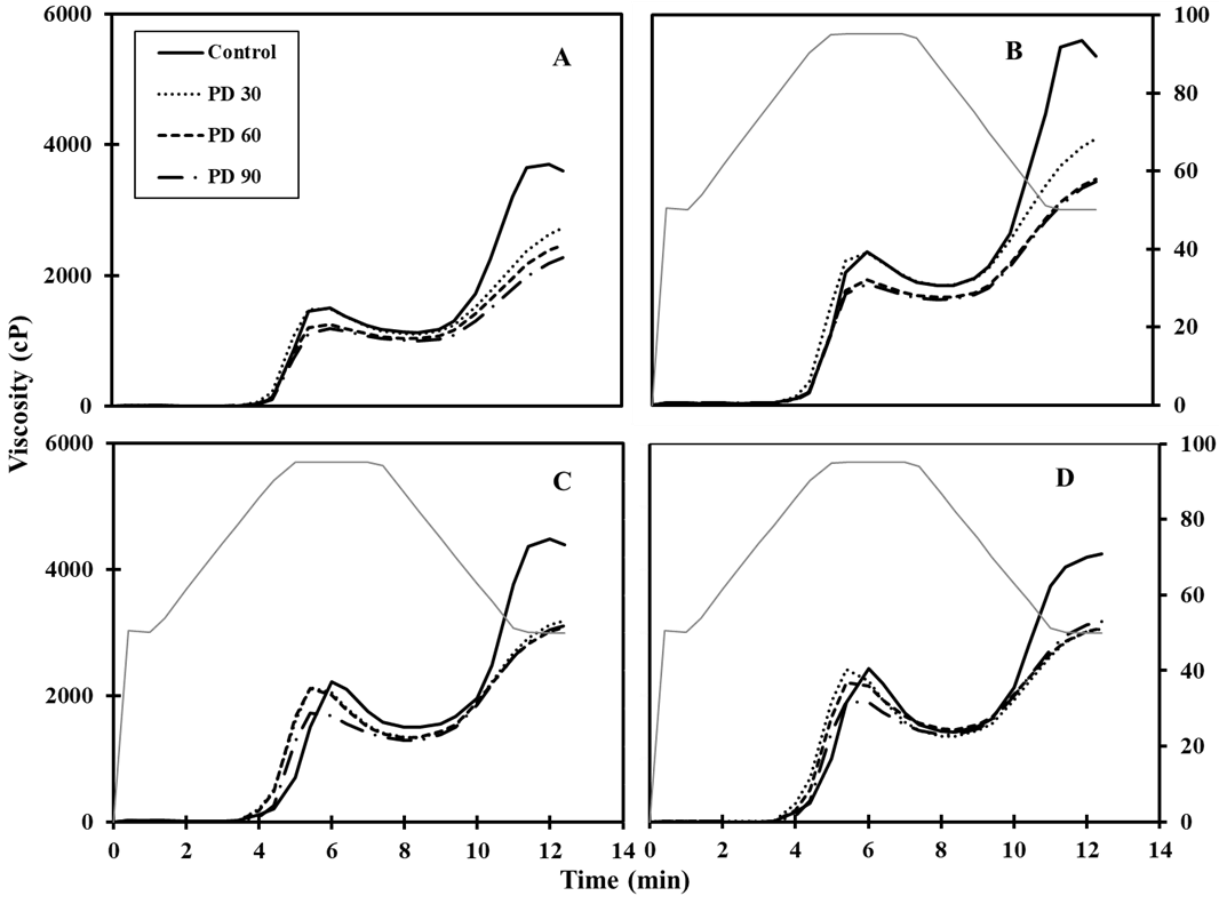
A.

Parameter	LogWorth	P-value
Cultivar	115.334	0.00000
Treatment duration	107.100	0.00000
Treatment	106.649	0.00000
Treatment*Treatment duration	96.226	0.00000
Cultivar*Treatment	69.329	0.00000
Cultivar*Treatment duration	67.113	0.00000
Cultivar*Treatment*Treatment duration	58.206	0.00000

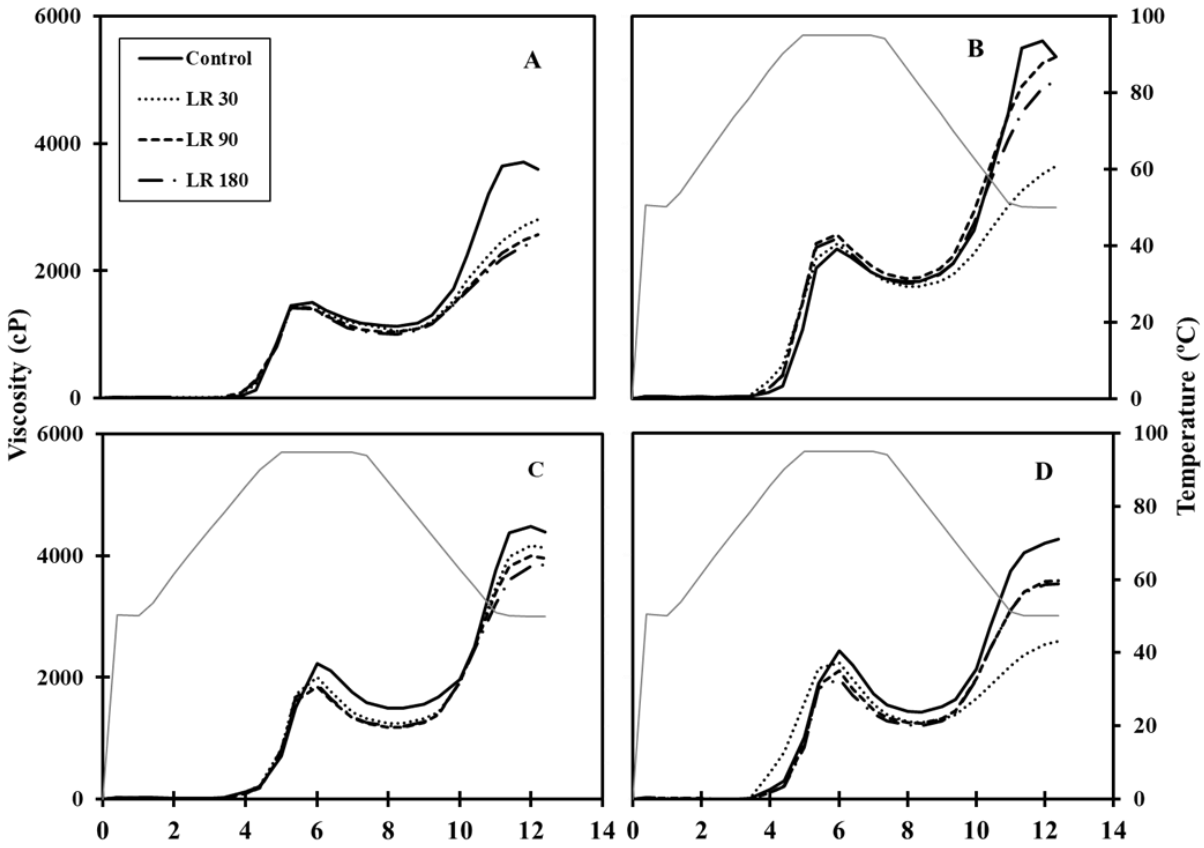
B.

Parameter	Main Effect	Total Effect
Cultivar	0.386	0.487
Treatment duration	0.302	0.415
Treatment	0.142	0.287

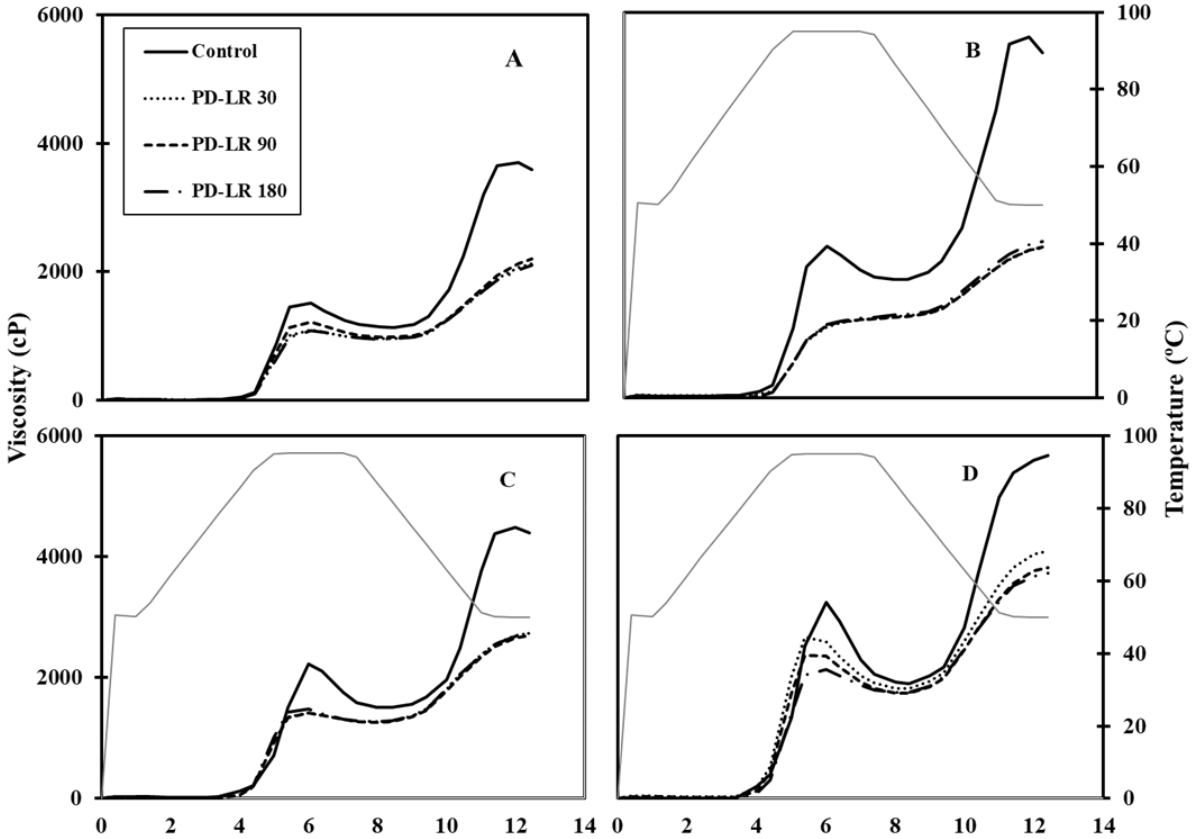
Figure 3. Overall effect summary of A) response variables as affected by treatment design factors and their interactions. B) level of influence of the individual factors by themselves (main effect) and the total effect that includes all interactions involving that effect on the physicochemical properties.



Supplementary figure 1. Pasting profiles of four rice cultivars (A=Cocodrie, B=Sierra, C=CL153, D=CLXL745) after protein denaturation.
PD, Protein denaturation; treatment time (min).

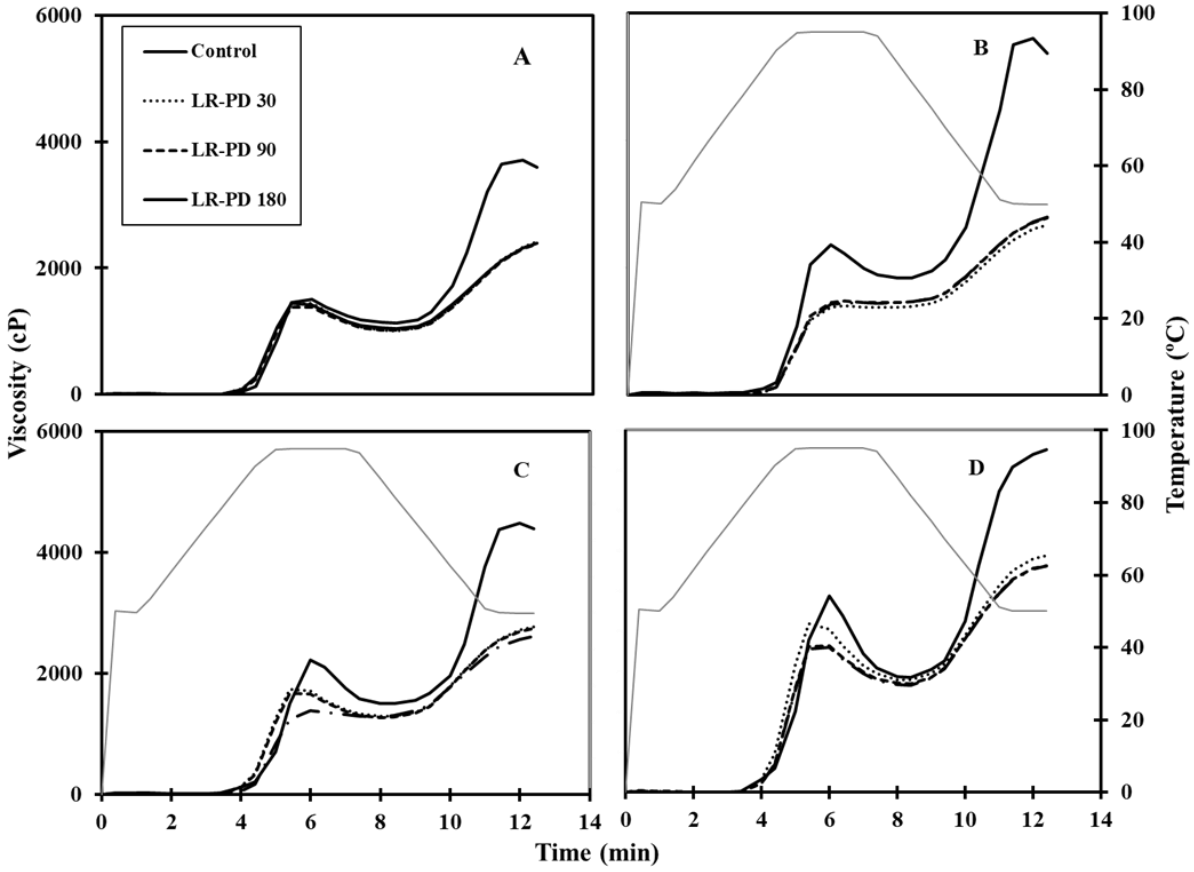


Supplementary figure 2. Pasting profiles of four rice cultivars (A=Cocodrie, B=Sierra, C=CL153, D=CLXl745) after lipid removal.
LR, Lipid removal; number, treatment time (min).



Supplementary figure 3. Pasting profiles of four rice cultivars (A=Cocodrie, B=Sierra, C=CL153, D=CLXl745) after combined treatment.

PD-LR, Protein denaturation first for 60 min followed by lipid removal; number, lipid removal time.



Supplementary figure 4. Pasting profiles of four rice cultivars (A=Cocodrie, B=Sierra, C=CL153, D=CLXl745) after combined treatment.
LR-PD, Lipid removal first followed by protein denaturation for 60 min; number, lipid removal time.

V. CHAPTER 3: Bran Layer Thickness and Chemical Composition on Rice Milling Properties

Michelle Oppong Siaw, Anna M. McClung, Andy Mauromoustakos, Ya-Jane Wang

ABSTRACT

Rice milling involves applying forces to remove bran from brown rice to produce milled rice. The physical and chemical properties of a rice kernel may affect both the amount of bran removed during milling and the amount of head rice yield (HRY) produced which is a determinant of crop value. This study investigated bran thickness and bran chemical composition as factors that influence bran removal and HRY as a result of milling. Brown rice was milled in 5 or 10 sec increments until a surface lipid content (SLC) of ~0.4% was reached, and the bran fractions were collected, quantified, and analyzed. The results showed brown rice kernels with initial higher SLCs required longer milling times to achieve 0.4% SLC, but the longer milling times did not translate into lower HRYs or higher bran yields. Bran chemical composition had a more significant impact on the milling characteristics than bran thickness. Arabinoxylans had the greatest impact on the HRY, followed by lipids and proteins. The significant interaction between arabinoxylans and proteins on HRY and bran yield, indicates the probability of crosslinking between proteins and arabinoxylans. This study demonstrates the importance of rice bran chemical components and their interactions on rice milling quality.

Keywords: Head rice yield; Bran yield; Bran thickness; Surface lipid content; Arabinoxylans

INTRODUCTION

Surface lipid content (SLC) is the mass percentage of lipids that remains on the surface of rice kernels after milling, and it affects the quality, stability, and end-use functionality of rice (Chen, Marks, & Siebenmorgen, 1997). Brown rice kernels are susceptible to rancidification during storage due to the high concentration of lipids in the bran in contrast to when the outer bran layers are removed from the rice kernel during milling producing shelf-stable milled rice. The price of rice is determined by milling quality, which is primarily characterized by total milled rice yield (MRY) and head rice yield (HRY); thus, it is important that processors produce rice with high milling quality. The MRY represents the mass fraction of milled rice, which includes both head rice and broken kernels from the original rough rice mass. The HRY represents the mass of milled rice kernels that retain three-fourths or more of the original kernel length (USDA, 1990) as compared to the original rough rice mass. The milling process is a mechanical process that applies a series of abrasive and frictional forces to remove bran layers from the brown rice kernels (Lu & Siebenmorgen, 1995). The rate of bran removal determines the milling duration required to attain a given degree of milling (DOM). The DOM is also a measure of the extent to which bran is removed from brown rice during milling, and affects both MRY and HRY (Andrews, Siebenmorgen, & Mauromostakos, 1992; Chen & Siebenmorgen, 1997). Bhashyam & Srinivas (1984) reported that a 2% increase in rice bran removal time resulted in a 4% increase in kernel breakage.

The physical and chemical properties of a rice kernel affect its milling properties. Chen & Siebenmorgen (1997) reported that thinner kernels (<1.67 mm) were milled at a greater bran removal rate as indicated by lower SLC than thicker kernels. Grigg & Siebenmorgen (2013) studied the impact of kernel thickness on the milling yields of two purelines and two hybrid cultivars of long-grain rice and reported that the MRYs and HRYs of thick kernels (>2 mm) were

greater than those of thin kernels (<2 mm). However, even with a similar kernel thickness, the rate of bran removal varies among different rice cultivars. Siebenmorgen, Matsler, & Earp (2006) found that the SLC levels of hybrids were lower than those of purelines of long-grain rice across several milling durations, suggesting that different rice cultivars may have unique physical and/or chemical properties that influence their milling characteristics.

The impacts of bran thickness and chemical composition on milling characteristics have been investigated in other cereals such as wheat. Greffeuille, Abecassis, L'Helgouac'h, & Lullien-Pellerin (2005) studied the differences in the aleurone layer removal between hard and soft common wheats during grain milling. They reported that the degree of adhesion between the aleurone layer and the starchy endosperm affected the starch content of bran, flour yield and flour purity. They further explained that mechanical resistance to removal of the aleurone tissue in hard wheat resulted in a higher flour yield and a lower flour purity due to the contamination of the flour with bran. Greffeuille, Abecassis, Lapierre, & Lullien-Pellerin (2006) later reported that the wheat bran mechanical properties are related to the structure and chemical composition of the wheat cell walls. Similar to wheat bran, rice bran is composed of 12-17% protein, 13-23% lipid, 34-54% carbohydrates, and 8-18% ash (Saunders, 1985). The carbohydrates in rice bran include 13.8% starch, 9.5-16.9% hemicellulose, 5.9-9.0% cellulose, and 5.5-6.9% free sugars. (Lu & Luh, 1991). Arabinoxylans (AX) is the predominant rice bran hemicellulose and constitutes 5.63-6.82% of the rice bran (Hashimoto, Shogren, Bolte, & Pomeranz, 1987). These constituents are not homogeneously distributed throughout the bran, and thus, their distribution may affect rice milling performance. We hypothesized that the thickness and the chemical composition of rice bran layers are major factors controlling bran removal and HRY of rice kernels. The objective of this study

was to investigate the relationship between the SLC, bran layer thickness and composition of nine U.S. long-grain rice cultivars in relation to their milling properties of bran yield and HRY.

MATERIALS AND METHODS

Materials

Rough rice of nine long-grain rice cultivars, including five purelines (Cybonnet, Deltabelle, Rondo, Roy J and Sierra) and four hybrids (FPRT7521, XPRT753, XL745 and XL756), with moisture contents of ~12% were collected and evaluated. The cultivars, Roy J from 2015, Deltabelle from 2017, Cybonnet and Rondo from 2018, and Sierra from 2019 were produced in Stuttgart, AR and provided by the USDA Dale Bumpers National Rice Research Center. The hybrids from the 2020 crop year were provided by the University of Arkansas Rice Research and Extension Center in Harrisburg, AR. Rough rice was cleaned using a dockage tester (Carter-Day Company, Minneapolis, MN) and dehulled with a Satake rice dehusker (Model THU, Satake Engineering Co. Ltd. Tokyo, Japan) to obtain brown rice. Brown broken rice was separated from brown head rice using a double-tray sizing device (Model 61, Grain Machinery Mfg. Corp. Miami, FL). Brown head rice was then graded into three thickness fractions, including <1.78 mm, 1.78-1.88 mm, and 1.88-1.98 mm, using a precision sizer (ABF2, Carter-Day Company, Minneapolis, MN). The thickness range of 1.78-1.88 mm was found to be the predominant fraction of brown rice kernels among the nine cultivars, and therefore was selected for this study to reduce variation and maintain kernels of uniform size.

Milling characteristics

Brown rice samples of each cultivar were milled using a laboratory mill (McGill No. 2, RAPSCO, Brookshire, TX.) at intervals of 5 or 10 sec until a surface lipid content of ~0.4% was

reached. The bran fraction after each milling duration was collected and weighed. Head rice kernels were separated from brokens. Head rice yield (HRY) and bran yield were calculated by the following equations.

$$Head\ rice\ yield\ (\%) = \frac{Weight\ of\ head\ rice}{Weight\ of\ brown\ rice\ (as\ is)} \times 100$$

$$Bran\ yield\ (\%) = \frac{Weight\ of\ bran}{Weight\ of\ brown\ rice\ (as\ is)} \times 100$$

Surface lipid content

The surface lipid content (as-is basis) of brown rice kernels after different milling durations was determined using a near infrared spectrophotometer (NIR, DA7200; Perten Instrument, Huddinge, Sweden), which was indicative of the degree of milling (DOM) (Chen et al., 1997). Approximately 50 g of each sample was poured into a 75-mm diameter cup and scanned (reflectance) over an NIR wavelength range 950-1650 nm at 5-nm wave-length increments. Three scans were conducted on each milling duration.

Rice kernel dimensions

Kernel dimensions (length, width, and thickness) of head rice samples from each milling fraction were measured in duplicates using an image analysis system (SeedCount 5000, Next Instruments, Condell Park, NSW, Australia). Approximately 1000 kernels of head rice were weighed and placed in a 32 mm-thick tray (152 mm × 100 mm × 20 mm), with no two kernels allowed to be in contact. The SeedCount instrument was used to scan each kernel in the tray to determine the average length, width, and thickness of the kernel.

Bran thickness

The cross-sectional morphology of brown rice kernels after different milling durations was observed via scanning electron microscopy (SEM) (PHILIPS XL30, FEI-Phillips, Hillsboro, OR) at an accelerating voltage of 10 kV. The rice kernels were manually cracked crosswise and mounted on an aluminum stub with double-stick tape, and then placed inside the SEM. Representative micrographs of each sample were taken at 1000× magnification. Bran thickness was measured from the micrographs on a micrometer scale. Three measurements on the same kernel were recorded for each of eight brown rice kernels for each cultivar.

Chemical composition

Bran fractions and milled rice flours, after varying milling durations, were analyzed for their chemical components according to Approved Methods of the American Association of Cereal Chemists (AACC, 2000). Moisture content was determined according to AOAC Method 930.15; crude protein utilized the micro Kjeldahl method according to Approved Method 46-12.01 with a conversion factor of 5.95. Crude lipids followed the Approved Method 30-20 using hexane as the solvent; and ash content followed the Approved Method 08–0 by dry ashing. The Arabinoxylan (AX) content was determined following the method of Douglas (1981). Approximately 4.5 mg of bran and flour were weighed into glass tubes and 2 ml of distilled water was added, followed by 10 mL of the freshly prepared extracting solution (110 mL glacial acetic acid, 2 mL hydrochloric acid, 5 mL 20% w/v phloroglucinol in ethanol, and 1 mL of 1.75% w/v glucose in water). The tubes were sealed and placed in a 100 °C boiling water bath (OLS200L, Grant Instruments Ltd, Cambridgeshire, Germany) for 25 min while shaking, then cooled rapidly under flowing cold water. The absorbance was quickly read at 552 nm and 510 nm using a spectrophotometer (Genesys 20, Thermo Fisher Scientific, USA). The percentage of AX in the bran and flour was

calculated by subtracting the values at 510 nm from 552 nm and comparing the results with a calibration curve.

Statistical analysis

All milling treatments were replicated twice, and all physical and chemical analyses were conducted in duplicates, and analyzed with JMP Pro software (version 16.2, SAS Software Institute, Cary, NC) using Tukey's Honestly Significant Difference (HSD) test. The level of significance was set at 5% for mean comparison. Analysis of variance (ANOVA) created from a stepwise regression control model was used to establish the effect of physical and chemical properties of rice kernels and their interactions on bran yield and head rice yield.

RESULTS AND DISCUSSION

Milling characteristics of rice cultivars

The U.S. rice industry associates 0.4% surface lipid content (SLC) with optimal head rice yield and better storability of rice (Basutkar, Siebenmorgen, Wang, & Patindol, 2015). Therefore, in this study, rice kernels were milled until they reached a SLC of 0.4%. The milling curves of SLC in relation to milling time of the nine rice cultivars are illustrated in Figure 1. There was a decrease in SLC with increasing milling duration for all cultivars, but the decreases varied among cultivars. In general, purelines, compared to hybrids, had higher initial SLC values ranging from 1.81-2.07% and required longer milling times of 60-80 sec to reach 0.4% SLC, with the exception of Roy J, which had the lowest initial SLC at 1.62% and required only 40 sec to reach 0.4% SLC. In contrast, hybrids had lower initial SLCs of 1.71-1.77% and required 40-50 sec to achieve 0.4% SLC. This is in agreement with Siebenmorgen et al. (2006) who reported that hybrids, XL7 and

XL8, with lower initial SLCs reached their required final SLC in a shorter milling time than purelines Cocodrie, Cypress and Wells, which had higher initial SLCs.

Longer milling times are usually associated with greater bran removal, lower head rice yields (HRY), and consequently lower economic value. However, the results of the nine cultivars milled to attain 0.4% SLC challenge this concept (Figure 2). For instance, Deltabelle and Roy J had the highest HRY at 86.15% and 84.80%, respectively, but were milled for 60 and 40 sec, respectively, to reach 0.4% SLC. In contrast, XL745 and XL756 were milled for a similar duration of 50 sec but had HRYs of 83.60% and 68.87%, respectively. These results illustrate that there was no correlation between HRY and milling duration ($r = -0.26$) at 0.4% SLC for both purelines and hybrids suggesting that there are other factors affecting HRY production in response to milling.

Rice bran makes up 6-11% of the brown rice weight (Juliano & Tuaño, 2019), and is removed during milling to produce milled rice. During the milling process, rice kernels were forced against each other via a steel-ribbed cylinder rotating inside a metal screen. The frictional forces created between the rice kernels and the metal screen and between the individual rice kernels removed the bran layer from the grain and caused a reduction in kernel dimensions. As milling progressed, the amount of bran removed from the rice kernels increased, but the amount removed varied greatly among cultivars with the same milling duration and target SLC of 0.4% (Figure 3). FPRT7521 reached 0.4% SLC after milling for 40 sec with a bran yield of 9.61%, whereas Rondo had a bran yield of 8.00% after milling for 80 sec. Thus, it could be deduced that the length of milling time required to reach 0.4% SLC does not equate to the amount of bran removed, which is supported by their non-significant correlation ($r = 0.28$). There was, however, a significant negative correlation ($r = -0.62$, $p < 0.01$) between bran yield and HRY. These results suggest that

the bran layer is more easily removed from weak kernels with a low HRY than from strong kernels with a high HRY, which may be attributed to their differences in chemical composition and/or distribution of their chemical components.

Grain physical properties associated with rice milling characteristics

The dimensions of the nine brown rice kernels varied significantly among the nine cultivars by length, width, and thickness ranging from 7.32-7.75 mm, 2.42-2.70 mm, and 2.03-2.13 mm, respectively, although they are all marketed as long-grain rice cultivars (Table 1). Cybonnet, Roy J, Sierra and XL756 had greater kernel lengths (>7.50 mm) than the other cultivars, and thus are considered as extra-long grain rice cultivars (Khush, Paule, & de la Cruz, 1979). Cybonnet, Roy J, Sierra and XL756 cultivars were slender in shape with a length-to-width ratio of >3, whereas the rest of the cultivars in this study had a length-to-width ratio of 2.1-3.0 which differs from the standards for US long grains. The width dimensions of cultivars Deltabelle, Rondo, FPRT7521 and XPRT753 was >2.60 mm, while those of Cybonnet, Roy J, Sierra, XL745 and XL756 were all <2.50 mm, thus illustrating that both purelines and hybrid long grains varied in kernel dimensions.

There was a gradual but significant decrease in kernel dimensions with milling time as a result of bran removal, with length showing the greatest change, decreasing 0.33-0.54 mm, followed by width decreasing 0.12-0.28 mm, and thickness, which showed the smallest decrease of 0.05-0.17 mm. The greatest decrease in length for all cultivars occurred in the first 5 sec of milling, agreeing with Ren et al. (2021) who reported that during the first 0-10 sec of milling the seedcoat is fragmented and easily peeled. Cybonnet, Sierra, Roy J and XL756 had the greatest decreases in length after milling as extra-long cultivars have more tapered ends, and thus, more

likely to produce greater reductions in kernel length after milling compared to cultivars with thicker edges. Rondo and XPRT753 recorded the least amount of change in length; however, these cultivars exhibited the greatest decreases in width and thickness.

Bran thickness, as measured by the transverse cross-sectional morphology of brown rice kernels with scanning electron microscopy (SEM), varied significantly among rice cultivars, with XL745, a hybrid, having the greatest bran thickness of 38.21 μm and Roy J, a pureline, having the least at 23.90 μm (Table 1). Khin et al. (2013) studied the differences in the aleurone layer traits of 321 rice varieties and reported the aleurone layer thickness ranges from 10-29.1 μm with a mean of 19.0 μm. The aleurone layer makes up a large portion of the rice bran thickness, and thus, variation in bran thickness is attributed to the thickness of the aleurone layer (Ogawa, Glenn, Orts, & Wood, 2003; Sapirstein, 2016). The representative SEM images of the transverse cross-section of the brown rice kernels from each cultivar are presented in Figure 4. The transverse cross-section images show the pericarp, aleurone layer and endosperm from the exterior to the interior of the rice kernel. There were variations in the appearance of the rice bran among the cultivars. For example, there is a clearer distinction between the bran layers and the endosperm in some cultivars (Cybonnet, Roy J, Sierra, XL745 and XPRT753) compared to others (Deltabelle, Rondo, FPRT7521 and XL756). Bran layers are made up of an outer pericarp, a middle testa layer and an interior aleurone layer. There was clear evidence of the pericarp and aleurone layer in all cultivars. The testa, however, was not as visible in all cultivars likely because the testa is the thinnest layer among the layers of the bran (Sapirstein, 2016). There were visible void spaces in the aleurone layers in cultivars like Cybonnet, XL756 and XPRT753 but no visible void spaces in other cultivars such as Sierra and Roy J. Thus, there was no consistent pattern of structural differences between the bran layers of purelines and hybrids in terms of bran thickness and the presence of

void space. The present results do not show the importance of bran thickness in controlling the ease of bran removal. The bran thickness was not significantly associated with milling time (r = 0.41), bran yield (r = 0.46), or HRY (r = 0.02) at 0.4% SLC.

Chemical composition of rice flours

The chemical composition of rice is impacted by several factors associated with the rice grain, including environmental and genotype variability, distribution of chemical constituents throughout the grain, and the thickness of the bran layer (Rosniyana, Hashifah, & Norin, 2007). The chemical composition of brown rice flour varied significantly among the nine cultivars (Table 2). The protein, lipid, ash, and arabinoxylan (AX) contents in the brown rice flour, prior to milling, ranged from 7.82-9.86%, 2.20-2.92%, 1.09-1.43%, and 0.26-0.58%, respectively. Purelines, with the exception of Roy J, had higher protein content than hybrids with Deltabelle having the greatest protein content and XPRT753 containing the least. Lipids are primarily concentrated in the bran layer of brown rice, with a smaller amount present in the endosperm (Champagne, Wood, Juliano, & Bechtel, 2004). Perdon, Siebenmorgen, Mauromoustakos, Griffin, & Johnson (2001) reported that because rice lipids are heavily concentrated in the bran, there is a concurrent decrease in total and surface lipid content during milling. In this study, Cybonnet and Sierra had the highest brown rice total lipid content (TLC) at 2.91% and 2.92%, respectively, whereas Roy J had the least at 2.20% (Table 2). After milling to SLC of 0.4%, Cybonnet and Deltabelle had the highest, TLC while Sierra was the lowest. These results demonstrate that varieties differ in the content and distribution of lipids throughout the kernel. The total ash content refers to any inorganic material, such as minerals, that remains after high heating removes water and organic material such as lipids and proteins. The brown rice ash analysis of the nine cultivars, demonstrated that Deltabelle had

the highest mineral content and Roy J had the least. Varieties also differed in ash content after milling to SLC of 4% (Table 2). Arabinoxylans (AX) form part of the non-starch polysaccharides in rice and have a lower concentration in the brown rice compared to the bran (Table 2 and 3). The AX content of the brown rice differed among rice varieties, with Deltabelle and Roy J having the highest whereas Sierra had the lowest (Table 2).

There was a significant decrease in protein, lipid, ash, and AX contents with increased milling time. However, the percentage decrease of protein content with respect to milling time differed among the varieties. For instance, the greatest decrease in the protein content of Cybonnet and FPRT7521 occurred within the first 5 sec of milling. Rondo, XPRT753, XL756, XL745 exhibited the greatest decrease in protein content after 10 sec of milling, and that of the remaining cultivars occurred after 20 sec of milling (Table 2). This difference among the varieties did not appear to be related to bran thickness (Table 1) and suggests a dissimilarity in distribution of proteins in the rice kernels. The lipid content, similar to the protein content, decreased significantly with increasing milling time. The total lipid content of all cultivars diminished more than 50% before reaching one-third of the total milling time, demonstrating the high concentration of lipids in the outer layers of the bran. The present results support the findings by Wang et al. (2021) who reported over a 50% decrease in lipid content of rice at one-half of the total milling time. Likewise, there was less than 30% of the total ash content remaining in the rice kernels at 0.4% SLC, agreeing with the results of Lamberts et al. (2007) who reported that more than 60% of rice mineral content is present in the bran layers. Similar to other components, increases in milling durations resulted in significant decreases in the AX content. There was less than 40% of the total AX content remaining in the rice kernels at 0.4% SLC, which confirms the presence of higher AX concentrations in the bran than in the endosperm. Hashimoto et al. (1987) reported that the AX

content of a commercial milled rice was 1.82%, compared to its bran content of 6.82%. Deltabelle had the greatest decrease in the percent of AX with increasing milling time, likely due to its higher AX content in the bran, compared to other cultivars (Table 2).

Chemical composition of bran associated rice milling characteristics

The chemical components of the rice bran collected after each milling duration until reaching 0.4% SLC were quantified and are presented in Table 3. Rice bran composition varies considerably due to variety, distribution of chemical components in the bran, and milling time (Rosniyana et al., 2007; Kalpanadevi, Singh, & Subramanian, 2018). There was an increase in bran protein and ash content, but a decrease in bran AX content with increasing milling time. Lipids exhibited a different trend from the other components, where it increased with milling time, and then began to decrease with continuous increasing milling time for most cultivars. The proteins in rice bran are stored as protein bodies and are primarily present in the aleurone layers; thus, the protein content in the bran increased significantly among cultivars with increasing milling time. Deltabelle had the highest protein content, whereas Rondo and XPRT753 had the lowest protein content in the bran at 0.4% SLC. Among the nine cultivars there was wide variation in the percentage increase in protein content with milling time to produce 0.4% SLC which was independent of starting protein content (at 5 sec) and whether the variety was a pureline or hybrid (Table 3). The trend of an increase and then a decrease in bran lipid content with increasing milling time indicates lipids are more concentrated in the exterior part of the aleurone layer, whereas other components, like proteins, are more concentrated in the interior part of the aleurone layer. The ash content in the bran varied, ranging from 6.91% in FPRT7521 to 11.65% in Rondo at 5 sec of milling. However, after 40 sec of milling and reaching 0.4% SLC, FPRT7521 bran had the greatest

increase in ash content, reaching 11.32%. The results demonstrate that the distribution of minerals in the bran layer differs among rice cultivars.

The bran AX content at 5 sec of milling ranged from 4.45% in FPRT7521 to 8.23% in Deltabelle. AX is mostly concentrated in the bran of cereals, with the concentration decreasing from the pericarp to the aleurone layers. This trend is different from that of other chemical components like proteins, lipids, and ash. This is because, AX is a hemicellulose which is highly concentrated in the cell walls of cereals, i.e., outer pericarp, compared to the other chemical components like proteins and lipids which are highly concentrated in the inner pericarp and the aleurone (Sibakov, Lehtinen, & Poutanen, 2013). Swennen, Courtin, Lindemans, & Delcour (2006) reported that approximately 38% of wheat bran AX is found in the pericarp, with about 25% present in the epidermis layer, 25% in the aleurone layer, and the remaining 12% of the AX in the testa and the hyaline layer of the wheat bran. The AX content in the bran collected at 0.4% SLC ranged from 2.55% in Sierra to 5.31% in Deltabelle and Roy J, values which are lower than the 5.63-6.82% reported by Hashimoto et al. (1987) after they analyzed AX content in two commercial rice brans. Izydorczyk (2009) reported that the amount and structure of arabinoxylans in cereals may vary with the genus, species, and variety. For instance, the range of AX content is 6.1-22.1% in wheat bran (Gebruers et al., 2008), 12.1-14.8% in rye bran (Nyström et al., 2008), and 4.8-9.8% in barley bran (Andersson et al., 2008). AX contains ferulic acid, a phenolic compound that is covalently linked to the arabinose residues via an ester linkage, and adjacent AX chains can be crosslinked with each other via di-ferulic acid bridges (Peyron, Chaurand, Rouau, & Abecassis, 2002). The crosslinking of AX promotes tissue cohesion and maintains the structural integrity of the bran by increasing bran firmness (Buanafina, 2009; Chateigner-Boutin et al., 2016). Peyron et al. (2002) reported a significant positive correlation between wheat bran AX and the

bran mechanical strength. In the present study, bran AX at 0.4% SLC had a significant positive correlation ($r = 0.94$, $p < 0.001$) with HRY and a significant negative correlation ($r = -0.60$, $p < 0.01$) with bran yield, supporting the results by Sibakov et al. (2013) who reported an inverse relationship between bran yield and the AX content in wheat bran. The lower the amount of bran removed from a whole kernel suggests the stronger the kernel will be due to the strengthening contribution of AX and consequently, will result in a higher HRY.

Similar to the AX, there was a significant positive correlation ($r = 0.51$, $p < 0.05$) between bran protein content and HRY at 0.4% SLC. Proteins are proposed to function as bioadhesives to bind starch and lipids together, and thus, higher protein content improves the kernel strength. There was a negative correlation between the amount of bran removed and the bran lipid content at 0.4% SLC ($r = -0.55$, $p < 0.05$). Because the removal of the bran is primarily due to the frictional forces between the individual kernels and between the kernels and the rotating cylinder of the rice mill, higher lipid content in the bran may reduce this friction, thus leading to less of the bran being removed. Unlike other chemical components, bran ash content did not have any significant impact on the amount of bran removed nor on the HRY, agreeing with Fares, Troccoli, & Di Fonzo (1996) who reported no association between wheat bran yield and the ash content in debranned wheat samples.

Statistical summary

The effects of bran thickness and its constituents (proteins, and lipids and arabinoxylans) and their interactions on the individual milling characteristics of HRY and bran yield were analyzed using a stepwise regression control model, and the results are summarized in **Table 4**. Ash was not included in this statistical analysis because it did not have any correlation with bran

yield or HRY. Arabinoxylans (AX) was the most significant factor affecting HRY, followed by lipids, whereas bran thickness and proteins had no significant impact. There was also a significant interaction between bran AX and proteins. Piber & Koehler (2005) reported the presence of covalent linkages between proteins and AX in wheat, which was formed between tyrosine in proteins and the ferulic acid of AX. Therefore, the significant interaction between AX and proteins in this study is proposed to result from the occurrence of cross linkage between tyrosine and ferulic acid. Similar to HRY, bran yield was significantly affected by AX. There was also a significant interaction between bran AX and proteins on bran yield, which illustrates the importance of these two chemical components on milling quality. Although bran thickness had no significant effect on the HRY and bran yield, a significant interaction was observed between bran thickness and AX on bran yield and HRY, likely because AX is primarily present in the bran and thus more AX is found in increased bran thickness.

CONCLUSIONS

The results demonstrate no difference in the milling characteristics between purelines and hybrids. Rice kernels with initial higher SLCs required longer milling times to achieve the target 0.4% SLC, but the longer milling times did not correspond to lower HRYs. However, a higher bran yield was correlated with a lower HRY. Increasing milling time resulted in a proportional decrease in proteins, lipids, ash, and AX content in the rice kernel, but the rate of decrease varied among cultivars. In contrast, there was a general proportional increase in the proteins, lipids, and ash content in the rice bran following increasing milling time, but the rate of increase varied from cultivar to cultivar. Bran thickness did not have significant impact on the milling time, bran yield, nor HRY, but the chemical components in the bran played a significant role on the milling characteristics. Among the chemical components studied, AX had the greatest impact on the

milling characteristics of HRY and bran yield. AX had a significant positive impact on HRY, but a significant negative impact on bran yield, which is attributed to its ability to increase bran firmness and maintain kernel structural integrity. There was a significant interaction between AX and protein on HRY and bran yield, which suggests the occurrence of crosslinking between proteins and arabinoxylan. Bran lipid content had a significant effect on the head rice yield, indicating that the presence of lipids may influence the head rice yield during rice milling, which may contribute to the rice milling quality. These results signify that the rice milling characteristics are influenced by the amount of bran removed during milling, which is determined by the chemical composition of the bran and the interactions of these components, rather than physical properties.

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FIGURES AND TABLES

Table 1. Bran thickness and kernel dimensions of nine rice cultivars subjected to varying durations of milling to attain a SLC of 0.4%

Properties	Milling time (sec)	Cybonnet	Deltabelle	Rondo	Roy J	Sierra	FPRT7521	XL745	XL756	XPRT753
Bran thickness (μm)	0	34.44±1.60^{c}	38.22±0.56^{a}	28.45±0.87^{f}	23.90±1.03^{h}	37.32±1.72^{b}	33.16±1.40^{d}	38.21±1.68^{a}	31.73±1.07^{e}	24.92±1.11^{g}
Length (mm)	0	7.75±0.01^{a}	7.35±0.01^{a}	7.27±0.02^{a}	7.62±0.02^{a}	7.64±0.01^{a}	7.34±0.01^{a}	7.32±0.01^{a}	7.52±0.01^{a}	7.49±0.01^{a}
	5	7.56±0.01^{b}	7.21±0.00^{b}	7.11±0.00^{b}	7.49±0.03^{b}	7.56±0.00^{b}	7.24±0.04^{b}	7.16±0.01^{b}	7.40±0.01^{b}	7.39±0.01^{b}
	10	7.53±0.01^{b}	7.18±0.01^{b}	7.07±0.00^{c}	7.35±0.03^{c}	7.53±0.00^{bc}	7.21±0.01^{b}	7.12±0.00^{c}	7.25±0.01^{c}	7.35±0.00^{b}
	20	7.37±0.00^{c}	7.10±0.00^{c}	7.04±0.01^{d}	7.33±0.00^{cd}	7.48±0.00^{cd}	7.06±0.00^{c}	7.06±0.00^{d}	7.20±0.02^{cd}	7.26±0.01^{c}
	30	7.34±0.01^{cd}	7.10±0.02^{c}	7.00±0.00^{e}	7.26±0.02^{de}	7.45±0.01^{de}	7.02±0.00^{c}	7.04±0.01^{de}	7.18±0.01^{de}	7.24±0.03^{c}
	40	7.33±0.01^{de}	7.08±0.02^{cd}	7.00±0.00^{e}	7.23±0.01^{e}	7.41±0.01^{e}	6.95±0.00^{d}	7.02±0.00^{e}	7.16±0.01^{de}	7.18±0.01^{d}
	50	7.31±0.01^{e}	7.04±0.00^{d}	6.99±0.01^{ef}		7.32±0.00^{f}		6.99±0.00^{f}	7.14±0.01^{e}	7.14±0.00^{d}
	60	7.26±0.00^{f}	6.99±0.00^{e}	6.97±0.03^{fg}		7.28±0.03^{fg}				
	70	7.21±0.01^{g}		6.95±0.00^{gh}		7.23±0.03^{g}				
	80			6.93±0.05^{h}		7.14±0.02^{h}				
Width (mm)	0	2.44±0.01^{a}	2.67±0.00^{a}	2.66±0.00^{a}	2.42±0.00^{a}	2.47±0.01^{a}	2.70±0.00^{a}	2.47±0.01^{a}	2.49±0.00^{a}	2.67±0.00^{a}
	5	2.43±0.01^{ab}	2.64±0.00^{b}	2.63±0.02^{a}	2.39±0.01^{ab}	2.45±0.00^{a}	2.68±0.01^{a}	2.43±0.00^{b}	2.48±0.00^{ab}	2.65±0.00^{a}
	10	2.38±0.00^{bc}	2.62±0.00^{b}	2.59±0.01^{b}	2.32±0.03^{b}	2.45±0.00^{a}	2.58±0.01^{b}	2.40±0.00^{bc}	2.47±0.00^{ab}	2.49±0.02^{b}
	20	2.35±0.01^{c}	2.58±0.01^{c}	2.54±0.03^{c}	2.21±0.02^{c}	2.42±0.02^{b}	2.52±0.01^{c}	2.37±0.00^{c}	2.44±0.00^{bc}	2.45±0.00^{bc}
	30	2.30±0.03^{d}	2.53±0.00^{d}	2.49±0.00^{d}	2.18±0.02^{c}	2.42±0.01^{b}	2.47±0.00^{d}	2.34±0.02^{d}	2.42±0.02^{c}	2.43±0.02^{cd}
	40	2.30±0.01^{d}	2.52±0.00^{d}	2.47±0.03^{de}	2.17±0.00^{c}	2.38±0.01^{c}	2.45±0.00^{d}	2.30±0.00^{e}	2.41±0.02^{cd}	2.41±0.00^{cd}
	50	2.26±0.01^{de}	2.50±0.01^{e}	2.45±0.01^{ef}		2.36±0.00^{cd}		2.29±0.00^{e}	2.37±0.00^{d}	2.39±0.01^{d}
	60	2.24±0.01^{e}	2.47±0.00^{f}	2.43±0.01^{fg}		2.35±0.00^{cd}				
	70	2.21±0.00^{e}		2.43±0.03^{fg}		2.34±0.00^{d}				
	80			2.40±0.08^{g}		2.34±0.00^{d}				
Thickness (mm)	0	2.04±0.00^{a}	2.06±0.01^{a}	2.03±0.00^{a}	2.10±0.00^{a}	2.05±0.00^{a}	2.08±0.00^{a}	2.04±0.00^{a}	2.04±0.00^{a}	2.13±0.01^{a}
	5	2.04±0.01^{a}	2.04±0.00^{a}	2.02±0.00^{a}	2.09±0.00^{ab}	2.04±0.00^{ab}	2.06±0.01^{a}	2.04±0.00^{a}	2.03±0.00^{ab}	2.10±0.01^{ab}
	10	2.02±0.00^{b}	2.02±0.01^{b}	2.01±0.00^{ab}	2.07±0.00^{ab}	2.03±0.00^{b}	2.03±0.01^{b}	2.02±0.00^{b}	2.02±0.00^{ab}	2.07±.01^{bc}
	20	2.01±0.00^{bc}	1.99±0.00^{c}	1.99±0.02^{b}	2.06±0.01^{b}	2.01±0.00^{c}	2.00±0.01^{c}	2.01±0.00^{c}	2.01±0.01^{ab}	2.04±0.01^{cd}
	30	2.00±0.00^{cd}	1.97±0.01^{d}	1.96±0.00^{c}	2.02±0.01^{c}	2.00±0.00^{c}	1.97±0.01^{d}	2.00±0.00^{d}	2.00±0.01^{bc}	2.01±0.00^{de}
	40	2.00±0.00^{cd}	1.95±0.01^{e}	1.95±0.02^{cd}	2.00±0.02^{c}	1.98±0.00^{d}	1.95±0.01^{d}	1.98±0.00^{e}	1.98±0.01^{cd}	1.98±0.01^{ef}
	50	1.99±0.00^{de}	1.92±0.00^{f}	1.93±0.01^{de}		1.97±0.01^{e}		1.97±0.00^{f}	1.95±0.01^{d}	1.96±0.00^{f}
	60	1.99±0.00^{de}	1.90±0.00^{g}	1.89±0.01^{ef}		1.95±0.00^{f}				
	70	1.99±0.01^{e}		1.89±0.01^{f}		1.95±0.00^{f}				
	80			1.86±0.06^{g}		1.92±0.01^{g}				

Mean values of two replications with the same letter in the same column within the same cultivar for length, width and thickness, and for the row representing bran thickness among all cultivars, are not significantly different (P<0.05) based on Tukey's HSD test

Table 2. Flour chemical composition of nine rice cultivars subjected to varying durations of milling to attain a SLC of 0.4%

Parameter (%, db)	Milling time (sec)	Cybonnet	Deltabelle	Rondo	Roy J	Sierra	FPRT7521	XL745	XL756	XPRT753
Proteins	0	8.97±0.00^{a}	9.86±0.00^{a}	8.68±0.02^{a}	7.86±0.03^{a}	9.10±0.00^{a}	8.62±0.00^{a}	8.88±0.00^{a}	8.73±0.00^{a}	7.82±0.00^{a}
	5	8.54±0.03^{b}	9.73±0.00^{b}	8.63±0.01^{a}	7.76±0.04^{b}	8.96±0.00^{b}	8.29±0.00^{b}	8.79±0.02^{a}	8.63±0.00^{b}	7.69±0.00^{b}
	10	8.47±0.04^{b}	9.56±0.00^{c}	8.35±0.02^{b}	7.47±0.00^{c}	8.82±0.02^{c}	8.11±0.11^{b}	8.50±0.00^{b}	8.37±0.00^{c}	7.30±0.06^{c}
	20	8.23±0.00^{c}	9.28±0.03^{d}	8.19±0.01^{c}	7.14±0.00^{d}	8.52±0.00^{d}	7.79±0.04^{c}	8.41±0.02^{c}	8.26±0.00^{d}	7.20±0.00^{cd}
	30	7.92±0.00^{d}	9.27±0.00^{e}	8.11±0.00^{d}	7.01±0.00^{e}	8.46±0.00^{e}	7.52±0.00^{d}	8.13±0.00^{d}	8.19±0.00^{e}	7.14±0.00^{d}
	40	7.81±0.00^{e}	9.16±0.01^{f}	8.03±0.00^{e}	6.16±0.00^{f}	8.38±0.01^{f}	7.42±0.00^{d}	8.10±0.00^{d}	8.15±0.00^{f}	6.92±0.00^{e}
	50	7.66±0.02^{f}	9.15±0.00^{g}	7.96±0.01^{f}		8.20±0.00^{g}		8.05±0.00^{e}	8.10±0.00^{g}	6.87±0.00^{e}
	60	7.62±0.02^{fg}	9.08±0.02^{h}	7.88±0.01^{g}		8.17±0.00^{g}				
	70	7.57±0.02^{g}		7.78±0.02^{h}		8.13±0.00^{h}				
	80			7.73±0.03^{i}		8.03±0.00^{i}				
Lipids	0	2.91±0.01^{a}	2.59±0.20^{a}	2.47±0.02^{a}	2.20±0.05^{a}	2.92±0.01^{a}	2.60±0.03^{a}	2.62±0.03^{a}	2.55±0.05^{a}	2.44±0.07^{a}
	5	2.37±0.09^{b}	2.32±0.15^{b}	2.36±0.14^{b}	1.87±0.01^{b}	2.36±0.03^{b}	2.08±0.01^{b}	2.04±0.07^{b}	2.01±0.03^{b}	2.11±0.01^{b}
	10	1.68±0.01^{c}	1.89±0.08^{c}	1.65±0.10^{c}	1.25±0.00^{c}	1.66±0.02^{c}	1.34±0.01^{c}	1.41±0.04^{c}	1.19±0.01^{c}	0.93±0.00^{c}
	20	1.11±0.03^{d}	1.02±0.18^{d}	1.24±0.06^{d}	0.55±0.01^{d}	0.92±0.03^{d}	0.67±0.01^{d}	0.82±0.03^{d}	0.60±0.01^{d}	0.73±0.03^{d}
	30	0.71±0.02^{e}	0.71±0.25^{e}	0.90±0.10^{e}	0.50±0.00^{d}	0.88±0.02^{d}	0.45±0.01^{e}	0.69±0.02^{d}	0.53±0.01^{d}	0.44±0.02^{e}
	40	0.61±0.02^{ef}	0.66±0.02^{e}	0.74±0.04^{f}	0.36±0.01^{e}	0.55±0.01^{e}	0.32±0.01^{f}	0.44±0.02^{e}	0.43±0.01^{e}	0.34±0.01^{ef}
	50	0.56±0.00^{fg}	0.49±017^{f}	0.61±0.05^{g}		0.48±0.01^{f}		0.33±0.01^{e}	0.26±0.00^{f}	0.28±0.01^{f}
	60	0.52±0.10^{fg}	0.47±0.23^{f}	0.52±0.06^{gh}		0.46±0.10^{f}				
	70	0.46±0.00^{g}		0.43±0.12^{hi}		0.42±0.00^{f}				
	80			0.34±0.09^{i}		0.24±0.01^{g}				
Ash	0	1.38±0.01^{a}	1.43±0.03^{a}	1.39±0.02^{a}	1.09±0.01^{a}	1.34±0.00^{a}	1.20±0.04^{a}	1.38±0.03^{a}	1.33±0.00^{a}	1.27±0.05^{a}
	5	1.20±0.03^{b}	1.37±0.04^{a}	1.31±0.04^{a}	0.92±0.00^{b}	1.18±0.04^{b}	1.00±0.02^{b}	1.12±0.01^{b}	1.08±0.01^{b}	1.14±0.04^{b}
	10	0.89±0.00^{c}	1.09±0.00^{b}	1.01±0.00^{b}	0.64±0.02^{c}	0.84±0.03^{c}	0.73±0.02^{c}	0.84±0.03^{c}	0.76±0.01^{c}	0.63±0.01^{c}
	20	0.68±0.00^{d}	0.68±0.03^{c}	0.82±0.07^{c}	0.44±0.00^{d}	0.62±0.01^{d}	0.44±0.00^{d}	0.57±0.00^{d}	0.52±0.00^{d}	0.43±0.01^{d}
	30	0.63±0.01^{d}	0.50±0.01^{d}	0.65±0.05^{d}	0.36±0.01^{e}	0.57±0.01^{d}	0.39±0.01^{de}	0.45±0.02^{e}	0.44±0.00^{e}	0.38±0.00^{d}
	40	0.51±0.02^{e}	0.46±0.02^{de}	0.58±0.08^{d}	0.30±0.00^{f}	0.42±0.01^{e}	0.32±0.01^{e}	0.37±0.01^{f}	0.35±0.01^{f}	0.35±0.00^{de}
	50	0.44±0.02^{f}	0.41±0.05^{e}	0.46±0.03^{e}		0.41±0.00^{e}		0.34±0.00^{f}	0.33±0.00^{f}	0.28±0.01^{e}
	60	0.38±0.05^{f}	0.32±0.04^{f}	0.42±0.11^{ef}		0.41±0.04^{e}				
	70	0.32±0.00^{g}		0.38±0.01^{fg}		0.40±0.01^{e}				
	80			0.33±0.06^{g}		0.31±0.01^{f}				
Arabinoxylans	0	0.41±0.01^{a}	0.58±0.03^{a}	0.39±0.01^{a}	0.55±0.02^{a}	0.26±0.01^{a}	0.38±0.01^{a}	0.43±0.02^{a}	0.31±0.01^{a}	0.35±0.02^{a}
	5	0.35±0.02^{b}	0.51±0.01^{b}	0.35±0.02^{a}	0.44±0.02^{b}	0.24±0.00^{ab}	0.36±0.01^{b}	0.38±0.02^{b}	0.30±0.02^{a}	0.33±0.01^{a}
	10	0.31±0.01^{c}	0.49±0.02^{b}	0.29±0.02^{b}	0.39±0.01^{c}	0.23±0.01^{bc}	0.26±0.02^{c}	0.31±0.02^{c}	0.26±0.01^{b}	0.29±0.02^{b}
	20	0.27±0.00^{d}	0.40±0.01^{c}	0.25±0.02^{c}	0.29±0.02^{d}	0.21±0.01^{cd}	0.20±0.01^{d}	0.27±0.00^{cd}	0.21±0.00^{c}	0.21±0.01^{c}
	30	0.23±0.01^{e}	0.29±0.02^{d}	0.21±0.01^{d}	0.25±0.00^{de}	0.20±0.01^{d}	0.15±0.01^{e}	0.24±0.02^{d}	0.18±0.01^{d}	0.19±0.02^{cd}
	40	0.20±0.01^{f}	0.25±0.01^{de}	0.18±0.01^{d}	0.22±0.02^{e}	0.19±0.01^{d}	0.12±0.00^{f}	0.18±0.01^{e}	0.14±0.01^{e}	0.15±0.00^{d}
	50	0.17±0.01^{g}	0.19±0.00^{ef}	0.13±0.00^{e}		0.16±0.01^{e}		0.14±0.01^{e}	0.07±0.00^{f}	0.08±0.00^{e}
	60	0.15±0.01^{g}	0.17±0.01^{f}	0.11±0.01^{ef}		0.14±0.00^{ef}				
	70	0.14±0.00^{g}		0.09±0.00^{ef}		0.12±0.00^{fg}				
	80			0.09±0.01^{f}		0.10±0.01^{g}				

Mean values of two replications with the same letter in the same column under the same parameter are not significantly different (P<0.05) based on Tukey's HSD test

Table 3. Bran chemical composition of nine rice cultivars subjected to varying durations of milling to attain a SLC of 0.4%

Parameter (%, db)	Milling time (sec)	Cybonnet	Deltabelle	Rondo	Roy J	Sierra	FPRT7521	XL745	XL756	XPRT753
Proteins	5	15.82±0.01^{h}	17.19±0.02^{f}	14.82±0.02^{g}	15.10±0.00^{e}	15.75±0.00^{i}	15.56±0.00^{e}	15.58±0.00^{f}	16.11±0.00^{f}	15.00±0.00^{f}
	10	16.13±0.00^{g}	17.27±0.00^{e}	14.96±0.01^{fg}	15.91±0.01^{d}	16.12±0.00^{h}	16.07±0.03^{d}	15.94±0.00^{e}	16.23±0.00^{e}	15.05±0.00^{e}
	20	16.34±0.01^{f}	17.32±0.02^{e}	15.07±0.02^{ef}	16.30±0.00^{c}	16.34±0.02^{g}	16.28±0.01^{c}	16.02±0.00^{d}	16.31±0.00^{d}	15.13±0.00^{d}
	30	16.79±0.02^{e}	17.51±0.01^{d}	15.16±0.01^{de}	16.47±0.00^{b}	16.42±0.00^{f}	16.51±0.01^{b}	16.39±0.00^{c}	16.50±0.01^{c}	15.55±0.00^{c}
	40	16.99±0.00^{d}	17.60±0.01^{c}	15.28±0.01^{d}	16.82±0.00^{a}	16.71±0.00^{e}	16.99±0.00^{a}	16.96±0.00^{b}	16.57±0.00^{b}	15.59±0.00^{b}
	50	17.33±0.00^{c}	18.09±0.02^{b}	15.46±0.04^{c}		16.81±0.00^{d}		17.90±0.00^{a}	16.75±0.00^{a}	15.69±0.00^{a}
	60	17.51±0.00^{b}	18.50±0.04^{a}	15.54±0.04^{bc}		16.91±0.00^{c}				
	70	17.68±0.00^{a}		15.62±0.05^{ab}		17.00±0.00^{b}				
	80			15.70±0.08^{a}		17.05±0.00^{a}				
Lipids	5	25.24±0.11^{de}	24.53±0.16^{b}	24.66±0.02^{d}	25.59±0.22^{c}	23.47±0.13^{e}	24.60±0.12^{b}	23.02±0.01^{d}	23.87±0.06^{d}	24.49±0.09^{d}
	10	27.46±0.07^{a}	26.38±0.06^{a}	26.53±0.30^{c}	26.48±0.04^{b}	25.63±0.33^{cd}	26.57±0.14^{a}	24.91±0.20^{c}	26.19±0.50^{c}	26.66±0.09^{c}
	20	27.82±0.04^{a}	26.23±0.26^{a}	27.28±0.24^{bc}	26.98±0.08^{a}	26.74±0.14^{a}	26.70±0.25^{a}	28.52±0.11^{a}	27.86±0.06^{a}	27.21±0.18^{ab}
	30	26.80±0.28^{b}	26.07±0.04^{a}	27.40±0.06^{ab}	27.25±0.00^{a}	26.41±0.14^{ab}	26.51±0.31^{a}	28.13±0.04^{a}	27.38±0.18^{ab}	27.50±0.08^{a}
	40	26.41±0.16^{bc}	26.11±0.01^{a}	27.59±0.08^{ab}	24.23±0.01^{d}	26.12±0.05^{bc}	26.04±0.27^{a}	27.67±0.02^{b}	27.44±0.05^{ab}	26.60±0.10^{c}
	50	25.92±0.13^{c}	26.12±0.03^{a}	28.16±0.00^{a}		25.86±0.05^{bcd}		24.50±0.13^{c}	26.94±0.11^{bc}	26.86±0.14^{bc}
	60	25.35±0.02^{d}	26.48±0.05^{a}	27.91±0.12^{ab}		25.76±0.05^{cd}				
	70	24.77±0.17^{e}		27.66±0.24^{ab}		25.65±0.05^{cd}				
	80			27.40±0.35^{ab}		25.52±0.06^{d}				
Ash	5	10.27±0.04^{f}	11.16±0.40^{c}	11.65±0.08^{e}	9.55±0.31^{b}	10.97±0.02^{g}	6.91±0.02^{e}	11.37±0.15^{c}	11.06±0.16^{d}	11.07±0.04^{d}
	10	10.54±0.02^{e}	12.22±0.22^{b}	12.53±0.04^{d}	10.45±0.16^{a}	11.38±0.04^{f}	9.19±0.03^{d}	12.13±0.01^{b}	11.29±0.01^{cd}	11.82±0.04^{c}
	20	10.68±0.04^{d}	12.40±0.13^{ab}	12.97±0.12^{c}	10.69±0.12^{a}	11.44±0.02^{f}	10.05±0.03^{c}	12.22±0.04^{b}	11.38±0.15^{bcd}	11.95±0.06^{c}
	30	10.88±0.02^{c}	12.68±0.09^{ab}	13.21±0.10^{c}	10.81±0.01^{a}	11.69±0.02^{e}	10.54±0.05^{b}	12.31±0.08^{b}	11.69±0.16^{abc}	12.34±0.04^{b}
	40	10.92±0.02^{c}	12.70±0.14^{ab}	13.54±0.04^{b}	10.93±0.02^{a}	11.79±0.04^{d}	11.32±0.04^{a}	12.48±0.21^{ab}	11.88±0.10^{ab}	12.79±0.04^{a}
	50	11.18±0.03^{b}	12.92±0.08^{ab}	13.68±0.06^{ab}		11.85±0.01^{cd}		12.82±0.08^{a}	12.00±0.13^{a}	12.91±0.03^{a}
	60	11.25±0.01^{ab}	12.98±0.05^{a}	13.74±0.03^{ab}		11.90±0.02^{bc}				
	70	11.31±0.02^{a}		13.80±0.00^{a}		11.95±0.01^{ab}				
	80			13.84±0.12^{a}		12.05±0.02^{a}				
Arabinoxylans	5	6.45±0.19^{a}	8.23±0.03^{a}	4.88±0.13^{a}	7.64±0.25^{a}	4.84±0.05^{a}	4.45±0.01^{a}	5.71±0.18^{a}	5.01±0.21^{a}	4.90±0.07^{a}
	10	5.62±0.10^{b}	7.56±0.22^{b}	4.61±0.14^{ab}	6.64±0.35^{b}	4.65±0.06^{a}	4.37±0.03^{a}	5.17±0.20^{ab}	4.82±0.22^{a}	4.32±0.08^{b}
	20	5.07±0.14^{c}	6.97±0.00^{c}	4.36±0.02^{bc}	5.90±0.14^{bc}	4.36±0.02^{b}	4.17±0.02^{a}	4.89±0.03^{bc}	4.01±0.18^{b}	4.06±0.15^{b}
	30	4.51±0.07^{d}	6.67±0.24^{cd}	4.26±0.00^{bcd}	5.59±0.04^{c}	4.29±0.03^{b}	3.78±0.05^{b}	4.55±0.14^{cd}	3.87±0.13^{b}	3.79±0.18^{bc}
	40	4.15±0.14^{de}	6.15±0.05^{de}	4.15±0.19^{cde}	5.31±0.02^{c}	3.74±0.02^{c}	3.43±0.18^{b}	4.33±0.14^{cd}	3.21±0.13^{c}	3.50±0.13^{cd}
	50	4.00±0.01^{e}	5.78±0.14^{ef}	3.91±0.04^{def}		3.34±0.08^{d}		4.07±0.14^{d}	3.07±0.05^{c}	3.20±0.18^{d}
	60	3.97±0.00^{e}	5.31±0.01^{f}	3.80±0.02^{ef}		3.18±0.08^{de}				
	70	3.94±0.02^{e}		3.74±0.02^{f}		3.02±0.09^{e}				
	80			3.68±0.01^{f}		2.55±0.06^{f}				

Mean values of two replications with the same letter in the same column under the same parameter are not significantly different (P<0.05) based on Tukey's HSD test

Table 4. Analysis of variance (ANOVA) table for factors affecting head rice yield and bran yield.

Parameter	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Head rice yield					
Bran thickness	1	1	0.15903	0.1651	0.6930
Proteins	1	1	4.44044	4.6112	0.0573
Lipids	1	1	10.49270	10.8962	0.0080*
Arabinoxylans	1	1	126.77624	131.6513	<.0001*
Arabinoxylans*Bran thickness	1	1	7.38779	7.6719	0.0198*
Arabinoxylans*Proteins	1	1	14.10195	14.6442	0.0033*
Bran thickness*Proteins	1	1	13.31979	13.8320	0.0040*
Bran yield					
Bran thickness	1	1	0.03070347	1.8732	0.2083
Proteins	1	1	0.02506608	1.5293	0.2513
Lipids	1	1	0.01218067	0.7432	0.4137
Arabinoxylans	1	1	0.15410758	9.4022	0.0154*
Arabinoxylans*Bran thickness	1	1	0.28137696	17.1670	0.0032*
Arabinoxylans*Proteins	1	1	0.12873701	7.8544	0.0231*
Arabinoxylans*Lipids	1	1	0.06142769	3.7478	0.0889
Bran thickness*Proteins	1	1	0.07813358	4.7670	0.0605
Bran thickness*Lipids	1	1	0.00351632	0.2145	0.6556

* denotes statistical significance at $p<0.05$

DF = Degrees of freedom

Nparm = Number of parameters

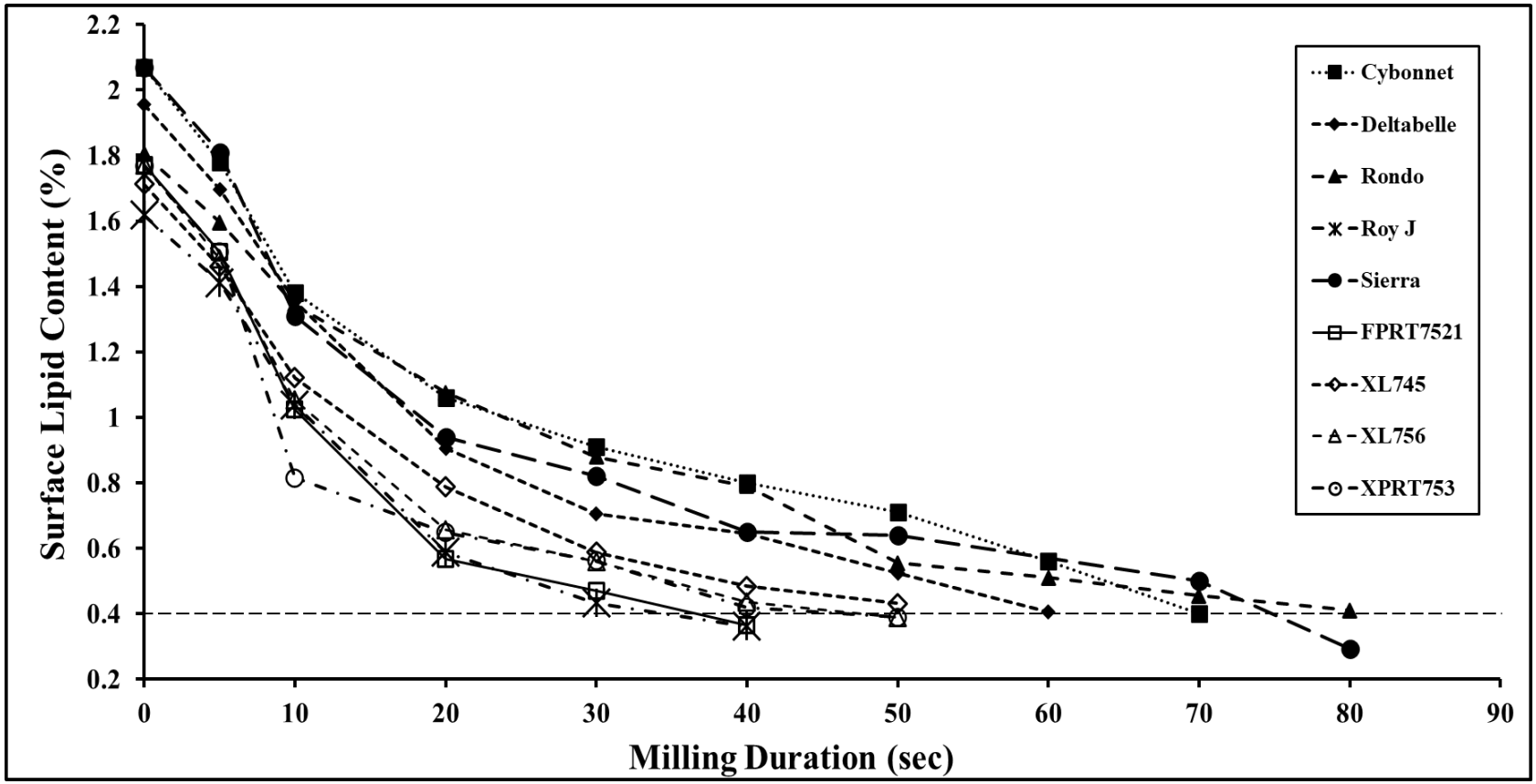

Figure 1. Surface lipid content of nine rice cultivars following incremental milling durations

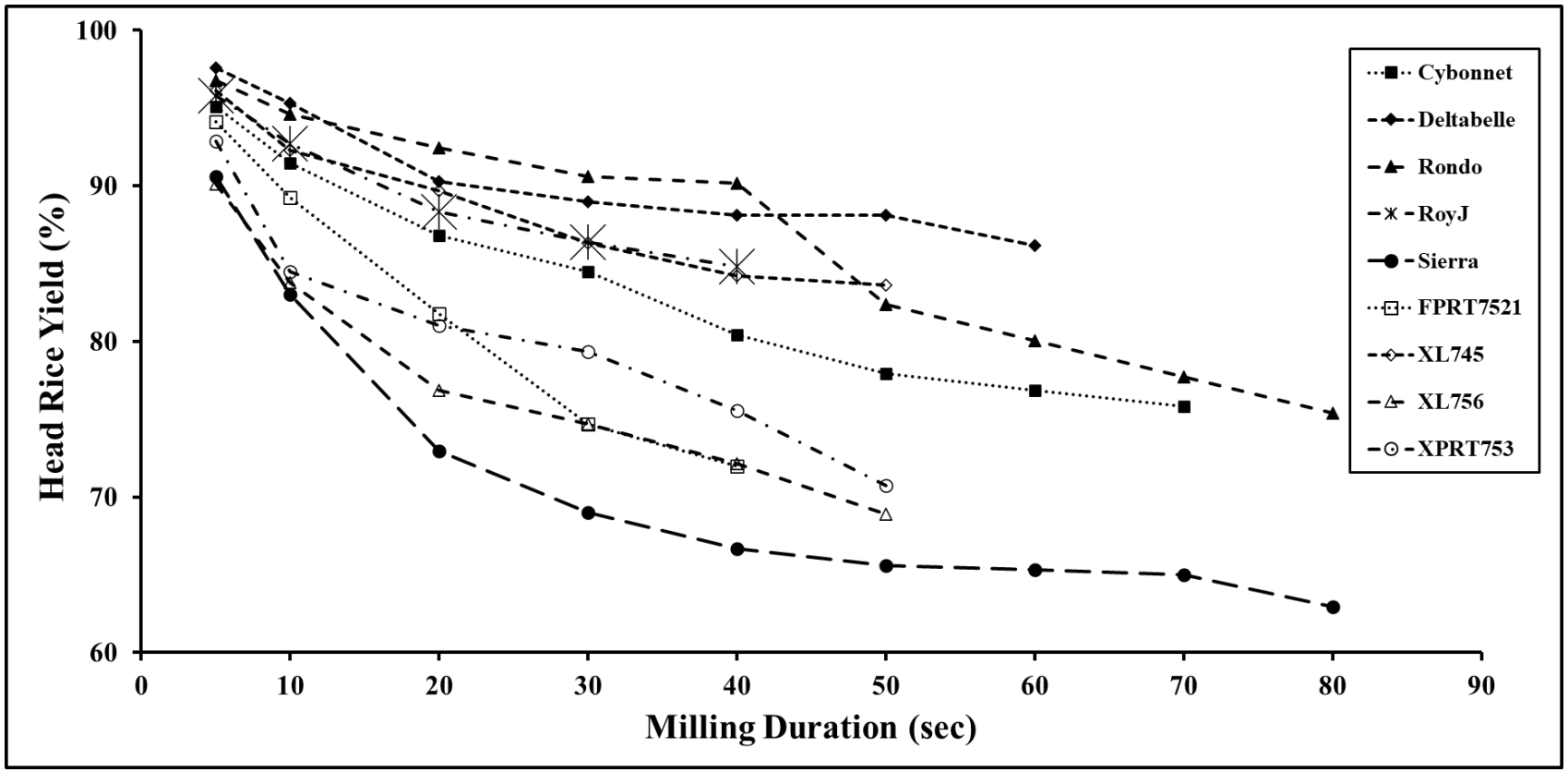

Figure 2. Head rice yield of nine rice cultivars following incremental milling durations

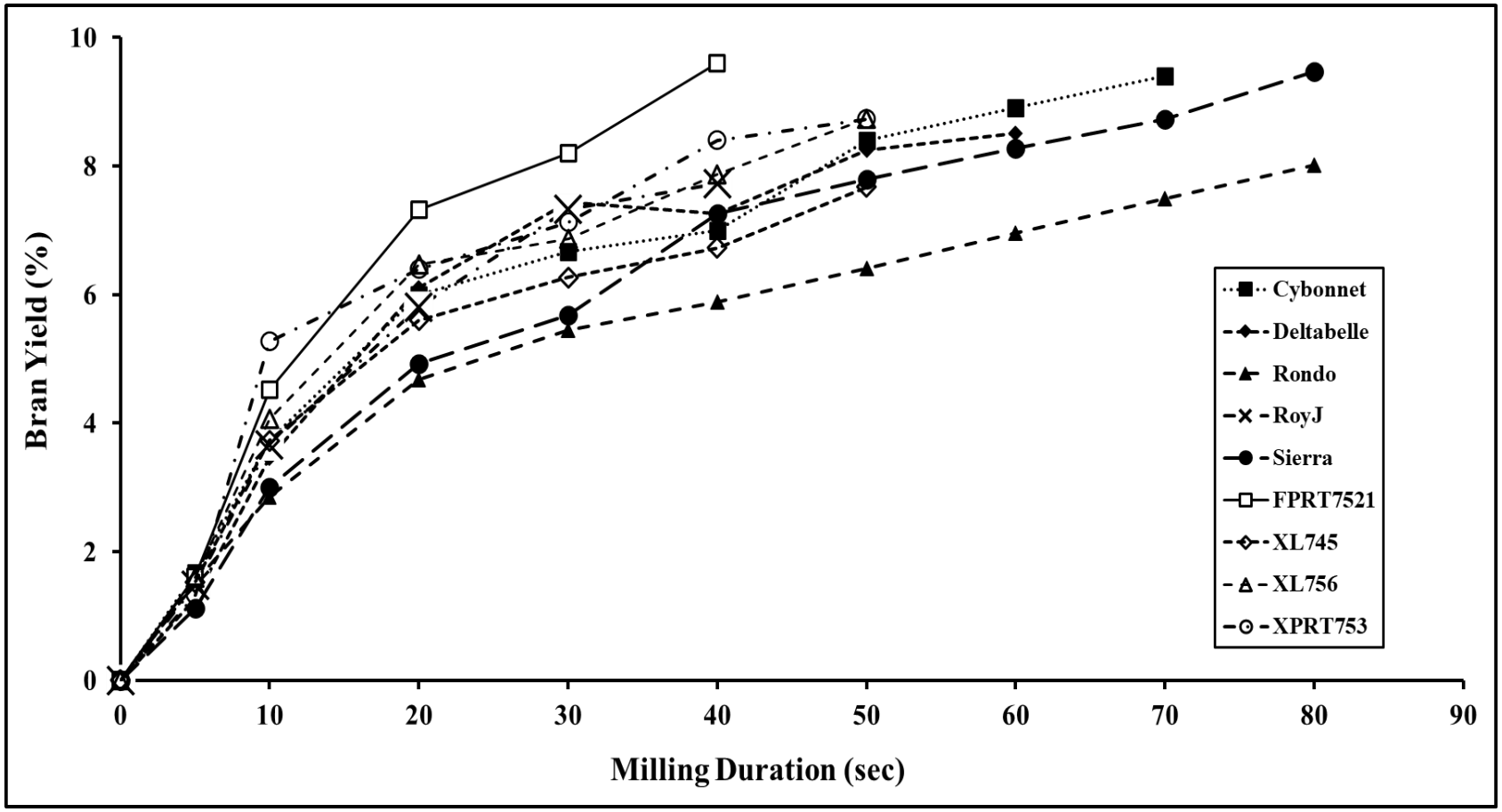

Figure 3. Bran yield of nine rice cultivars following incremental milling durations

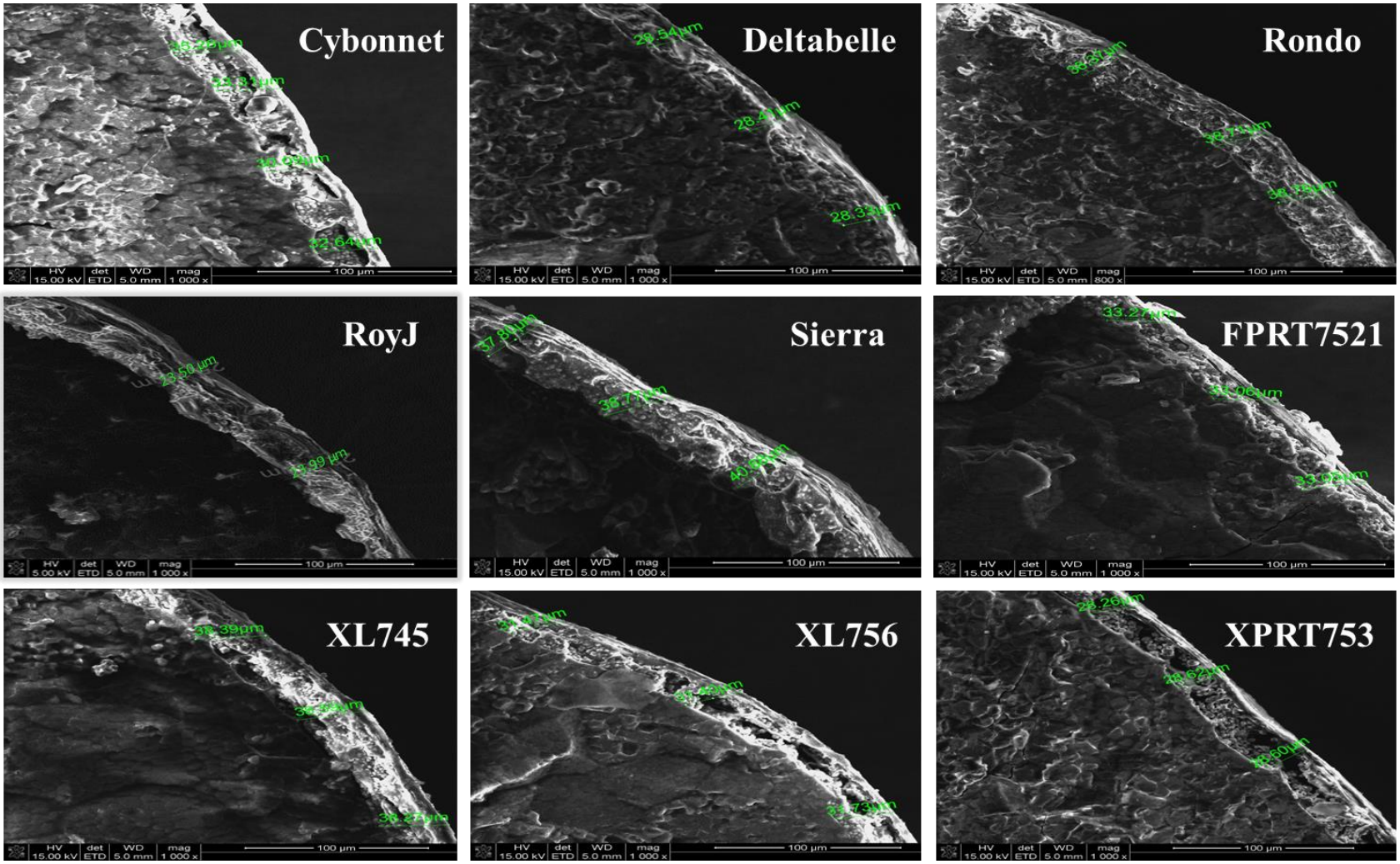

Figure 4. Scanning electron microscopy of bran thickness of brown rice kernels

VI. OVERALL CONCLUSIONS

This research demonstrated the importance of rice chemical components and their interactions on rice hardness, physicochemical properties, and milling properties. Protein denaturation (PD) by heat treatment resulted in enhanced interactions between proteins and starch, which improved continuity of the protein-starch matrix, reduced porosity, and consequently increased head rice yield (HRY). Non-polar lipids weakened the hydrophilic protein-starch matrix, and thus lipid removal (LR) decreased porosity and improved the mechanical strength of rice kernels. PD and LR had similar impacts on pasting viscosities but exerted contrasting effects on the gelatinization temperatures. The removal of non-polar lipids increased the concentration of polar lipids, which is proposed to serve as bridges linking denatured proteins and starch granules. The synergistic interactions of starch, polar lipids, and proteins caused significant increases in gelatinization temperatures and decreases in pasting viscosities, swelling power and water solubility. Rice milling properties were greatly influenced by rice bran chemical components, but not influenced by the bran thickness. Among bran chemical components, arabinoxylans (AX) had the greatest impact on HRY and bran yield. There were significant interactions between arabinoxylans and proteins on HRY and bran yield, indicating the probability of crosslinking between proteins and arabinoxylans. Developing cultivars with higher bran AX content and low lipid content can improve rice milling properties and subsequently the economic value of rice cultivars.