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EFFECT OF AMYLOSE AND PROTEIN OXIDATION ON THE THERMAL, RHEOLOGICAL, STRUCTURAL, AND DIGESTIVE PROPERTIES OF WAXY AND COMMON RICE FLOURS AND STARCHES

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EFFECT OF AMYLOSE AND PROTEIN OXIDATION ON THE THERMAL, RHEOLOGICAL, STRUCTURAL, AND DIGESTIVE PROPERTIES OF WAXY AND COMMON RICE FLOURS AND STARCHES

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Agriculture, Food, and Environment at the University of Kentucky

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2013

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The effects of oxidation by sodium hypochlorite (0, 0.8, 2, and 5%, NaOCl), the presence of endogenous proteins, and amylose content on waxy and common rice flours (WF, CF) and starches (WS, CS) were investigated in terms of in vitro starch digestibility, morphology and surface properties, and thermal and rheological characteristics.

The concentration of NaOCl had an effect on all the samples including WF, CF, WS, and CS. The carbonyl and carboxyl group contents increased up to 25 and 10 folds (P < 0.05) of oxidized starches (WS, CS), respectively. Only mild oxidation (P < 0.05) occurred in flours (WF, WS). In addition, endogenous proteins were oxidized according to amino acid analysis and SDS–PAGE results. Glu+Gln, Gly, His, Arg, Tyr, and Lys were more sensitive to NaOCl oxidation. Disulfide bonds, hydrophobic force, and hydrogen bonds were involved in protein polymerization after NaOCl oxidative modification. In granular state, the in vitro starch digestibility of WF, WS, and CS decreased by 5% NaOCl oxidation. After gelatinization, only 2 and 5% oxidized WS had lower digestibility.

Scanning electron microscopy and confocal laser scanning microscopy further demonstrated that protein existed on the surface of starch granules and had aggregation by oxidation. X-ray diffraction patterns showed the crystallinity of 5% oxidized flours and starches was reduced compared with all their non-oxidized samples.

Thermal and rheological properties were analyzed by differential scanning calorimetry and rheometer, respectively. Starch gelatinization peak temperature of flours (WF, RF) was increased by 3 °C, but starches (WS, CS) had a significantly decrease by 8 °C. Viscoelastic patterns were dramatically changed by oxidation. Oxidized WF and CF had increased in both viscosity and elasticity by oxidation, whereas both WS and CS had significantly lower viscoelasticity after oxidative modification.
KEYWORDS: Rice proteins and starches, oxidative modification, \textit{in vitro} digestibility, thermal and rheological properties, starch granule structural properties

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Student's signature

10/21/2013
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Rice is a staple consumed worldwide. Milled rice can be divided into three grades, head rice, second heads, and brewers' rice (Mitchell, 2009; Verma, 2010). Head rice is the group that consists of at least three-fourth of the whole kernel. Second heads are at least one-half of the whole kernel but less than three-fourth. Brewers' rice is only about one-quarter of the length of the full kernel (USA Rice Federation, 2013). Second heads and brewers' rice (also referred to as "broken rice") are much less valuable compared with the high quality head rice. Broken rice kernels are sold at discounted prices at only 7 cents/lb compared to head rice that costs 24 cents/lb. Yet, there is little difference in terms of functionality between broken rice and head rice. Therefore, broken rice, a by-product of rice milling, is increasingly milled into rice flour to be used as a functional and nutritional additive in baby foods, breakfast cereals, and snacks or for the manufacture of rice starch to be used in pharmaceutical and cosmetic products (Bao and Bergman, 2004). The main difference between rice flour and rice starch is that the former contains approximately 7–8% protein (primarily glutelin) whereas the latter has less than 1%.

The high demand for rice flour and starch products is driven by the concern of the consumer with allergic responses to gluten (which is almost absent in rice) and their unique functional properties, including white color, bland flavor, and superior freeze-thaw stability of pastas (Mitchell, 2009; Shirani and Ganesharane, 2009; Marti et al., 2011). In addition, rice flour and starch have excellent paste properties due to the amylose to amylopectin ratio.
Despite the usefulness of rice flour and starch as versatile functional food additives, in recent years, the high digestibility of starch presents various nutritional and health controversies, particularly with regards to obesity and diabetes. Obesity has nearly doubled since 1980, and 347 million people have diabetes worldwide today. This is largely due to unhealthy diets with high amounts of carbohydrates (World health organization, 2013). Many factors can influence the digestion rate of starch, including the starch source, starch-protein interaction, particle size, phenolic compounds, and the presence of fiber and antinutrients such as lectins, phytates, and enzyme inhibitors (Colonna et al., 1992; Mahasukhonthachat et al., 2010; Kandil, 2012).

In recent years, a tremendous amount effort has been put in developing slowly digested starch or resistant starch food products. These include high amlyose maize created by autoclaving and cooling cycles, and starches modified chemically by acetylation, hydroxypropylation, octenyl succinylation, or oxidation (Englyst, 1982; Pomeranz, 1992; Nugent, 2005; Carlos-Amaya et al., 2011; Thompson et al., 2011).

Oxidative modification by means of alkaline hypochlorite can introduce carbonyl and carboxyl groups in starch granules so as to improve functionality and creat a variety of other uses in the food industry (Li and Vasanthan, 2003; Sandhu et al., 2008). The low viscosity and neutral taste of oxidized starch are reported to be applicable in many products, such as salad dressing, mayonnaise, coating and sealing agents, emulsifiers, dough conditioners, and binding agents (Mazur et al., 1989; Konoo et al., 1996; Lawal, 2004). It has been reported that mild periodic oxidation reduced enzyme hydrolysis reactivity on the amorphous zones of starch granules (Gallant et al., 1992). The susceptibility of starch to enzyme hydrolysis is influenced more by the substituted groups.
in starch molecules than by the degree of disintegration of starch granules (Juansang et al., 2012). Hypochlorite oxidation treatment was reported to reduce corn starch digestibility (Chung et al., 2008). Later, Simsek et al. (2012) reported that oxidizing black and pinto bean starches with ozone increased levels of resistant starch.

Protein-starch association has been proven to contribute to the formation of resistant starch (Saura-Calixto et al., 1992). Proteins found on the surface of starch granules may act as physical barriers to starch digestion (Svihus et al., 2005). It has been demonstrated that high protein starchy foods, such as legumes, play an important role during the dietary management of diabetes (Simpson et al., 1981). There was a lower blood glucose response and reduced digestion rate of legumes compared to conventional cereal foods which generally contain half the amount of protein as legumes (Jenkins et al., 1980). Most starch granules in pasta remain embedded in the protein network (Jenkins et al., 1987). Microscopic observations show that the gelatinized starch granules of white bread are embedded in a relatively thin protein network (Fardet, 2006). Holm et al. (1985) reported that the availability of starch to α-amylase in raw and boiled wheat decreased substantially when pepsin was omitted. This implies that entrapments of starch granules or molecules with a protein matrix resulted in the decrease the digestion of starch by α-amylase.

Protein oxidation is one of the major causes for human aging and disease in the biological world; however, protein oxidation can be either helpful or detrimental in the food industry. Under severe oxidative stress, undesirable oxidative changes can occur that result in off-flavors, degradation, color changes, and poor water holding capacity (Xiong, 2000; Liu et al., 2010). Whereas, mild oxidative stress enhanced fresh meat
hydration and myofibril swelling capacity (Delles et al., 2011; Liu et al., 2011). Cui et al. (2012) demonstrated that surface activity improved while hydrodynamic behavior diminished with the oxidation of whey protein. Mild oxidation induced protein unfolding and facilitated myosin cross-linking by microbial transglutaminase (Li et al., 2012). Moreover, decreased digestibility of myosin was observed due to protein crosslinking induced by oxidation (Liu and Xiong, 2000). It was proposed that protein crosslinking might be the greatest factor that influences sorghum protein digestibility (Duodu et al., 2003).

Studies have shown that decreased protein digestibility is positively correlated to resistant starch formation (Lanfer Marquez and Lajolo, 1990). The external surface of the starch granule is the first barrier to hydration, enzyme attack, and chemical modifying agents. Rice protein in the endosperm tightly associates with the surface of starch granules; as a result, it is difficult to produce protein-free rice starch. Also, the reactive free aldehyde groups of oxidized starch are capable of reacting with free amino-or imino groups to form rigid structures (Tharanathan, 2005). This indicates that oxidation and the presence of proteins, including both endosperm and SGAPs (starch granule associated proteins), could have significant impacts on the starch properties and, more importantly, starch digestibility of rice.

Therefore, we hypothesize that starch and protein modification by oxidation could affect the physicochemical properties of rice flour and starch; rice endosperm and SGAPs protein cross linking and aggregation could occur outside of starch granule; protein aggregates might generate physical barriers with lower accessibility for enzyme penetration; and oxidatively generated carbonyl and carboxyl groups inside the starch
granule could induce conformational changes in the starch molecule that interrupt enzyme attack.

To test these hypotheses, the following objectives were proposed for my dissertation study:

1) to analyze the influence of oxidation on rice starch and protein structure;
2) to evaluate the in vitro digestibility of modified rice flours and starch;
3) to illustrate the molecular structure changes of rice flour and starch after oxidative treatment;
4) to elucidate the thermal stability and rheological properties of oxidatively modified rice flours and starch.
CHAPTER 2
LITERATURE REVIEW

Rice (*Oryza sativa* from Asia or *Oryza glaberrima* from Africa) is one of the oldest and most widely consumed staple cereals in the world. Dietary rice can be traced to over 5,000 years ago (Zhou et al., 2002). Only wheat exceeds rice in terms of production and food consumption. Almost half of the world's population is dependent on rice. Although 90% of rice is produced and consumed in Asia, the production and consumption of rice in the United States has increased more than 40% from 1980 to 1995 and an additional 25% from 1995 to 2005 (Michell, 2009). More than 117,000 varieties of rice, including wild rice, the ancestors of traditional and heirloom varieties of rice, and modern varieties, have been collected by the International Rice Gene Bank (International rice research institute, 2013).

2.1 Chemical Composition, Nutritional Value, Functionality, and Application of Rice

2.1.1 Chemical composition

Rice grain is comprised of the hull (16-28%, dry basis, db) and the caryopsis as shown in Figure 2.1 (Blakeney, 1984; Juliano, 1985). Brown rice, the caryopsis, is produced by removing the hull during milling. The composition of rice caryopsis is pericarp (1-2%), aleurone plus seed coat and nucellus (4-6%), embryo (2-3%), and starchy endosperm (89-94%). White rice is produced with further milling to remove the
pericarp, seed coat, aleurone layer, and embryo, resulting in partial loss of protein, lipid, fiber, minerals, vitamins, free amino acids, and free fatty acids.

This dissertation focuses mainly on white rice which is the most popular rice grain consumed in the world. Rice grain composition is dependent upon the specific variety and the growth environment. Starch, protein, and lipid, the three most abundant components (Table 2.1) of milled rice, will be individually introduced in this section.

Figure 2.1. A detailed structure of the rice grain (Adapted from Blakeney, 1984).
Table 2.1. Proximate content (%) of fractions in rough, brown, and milled rice at 14% moisture.*

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Rough</th>
<th>Brown</th>
<th>Milled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>53.4</td>
<td>66.4</td>
<td>77.6</td>
</tr>
<tr>
<td>Protein</td>
<td>5.8-7.7</td>
<td>7.1-8.3</td>
<td>6.3-7.1</td>
</tr>
<tr>
<td>Crude fat</td>
<td>1.5-2.3</td>
<td>1.6-2.8</td>
<td>0.3-0.5</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>7.2-10.4</td>
<td>0.6-1.0</td>
<td>0.2-0.5</td>
</tr>
<tr>
<td>Crude ash</td>
<td>2.9-5.2</td>
<td>1.0-1.5</td>
<td>0.3-0.8</td>
</tr>
<tr>
<td>Free sugars</td>
<td>0.5-1.2</td>
<td>0.7-1.3</td>
<td>0.22-0.45</td>
</tr>
</tbody>
</table>


2.1.1.1 Starch

Starch is the most abundant natural source of polysaccharide. In the rice grain, the central endosperm region is composed of a considerable amount of polygonal starch granules surrounded by protein (Bechtel and Pomeranz, 1978).

As with all other grains, rice starch granules are formed with numerous starch molecules that can be fractioned into linear amylose and highly branched amylopectin (Figure 2.2). The main variation in rice starch composition is caused by the relative proportion of these two molecules. Amylose content also varies between rice varieties, from a low of 0-2% (waxy rice) to a high of up to 25% (common rice). The term waxy is used to describe the vitreous or waxy surface when a kernel is cut. Waxy rice starches contain very little amylose due to natural mutations in the gene responsible for encoding granule bound starch synthase, essential for the synthesis of amylose (Rahman et al., 2000). Waxy rice is also called glutinous or sticky rice due to its cooking properties and sweet rice because of its use in Japanese Mochi cakes.
Rice starch granules, 3-8 µm in size with irregular polygonal shapes, are the smallest of the grains produced by plants (Jane, 2009). Rice starch granules show birefringence under polarized light. Like most other cereal starches, A-type X-ray diffraction patterns can be observed (Zobel, 1964). The starch granule structure is organized into concentric alternating crystalline and amorphous layers (Vandeputte et al., 2003; Svihus et al., 2005). These structures are termed growth rings. Because of this stable semicrystalline structure, starch granules are not soluble in water at room temperature. Amylose is a linear molecule that consists of α (1→4) linked D-glucopyranosyl units, whereas amylopectin has short α (1→4) linked D-glucosyl chains with 5-6% randomly distributed α (1→6) linked D-glucopyranosyl units (Figure 2.2).
(Vandeputte et al., 2003; Pérez et al., 2009). The amorphous layers mainly consist of amylose. Amylose forms single or double helices within the native granule structure (Buléon et al., 1998). Single helices can give rise to a central cavity that can be filled with compounds such as fatty acids, iodine, and alcohols. Due to this helical structure, amylose can be produced by 1-butanol in the form of precipitates (Bao and Bergman, 2004). Double helices formed by amylopectin branches induce the semi-crystalline nature of starch (Vandeputte et al., 2003). Rice starch chains display a natural twist with a helical conformation in which six anhydroglucose units present each turn. The helix is a composite of hydroxyl groups of glucosyl residues located on the outer surface and hydrophobic internal cavity (Zhou et al., 2002). The crystalline lamellae are 90-10 nm thick and consist of the double helical clusters of amylopectin side chains (Oostergetel and Bruggen, 1989; Jenkins et al., 1993). Three types of branch chains, A-, B-, and C-chains, are found in amylopectin (Figure 2.3). A-chains are those linked with B- or C-chains by their reducing ends through α (1→6) linkages, and B-chains are linked to another B- or C-chain and also branched by A-chains. Only one reducing end is present in each amlyopectin due to its single C-chain (Pérez et al., 2009).

Figure 2.3. Schematic diagram of amylose (a) and amylopectin (b). (Pérez, Baldwin, et al., 2009)
2.1.1.2 Protein

Protein is the second most abundant constituent in rice. The protein content in milled rice ranges from 6.3 to 7.1% (Table 2.1, Juliano and Bechtel, 1985; Kennedy and Burlingame, 2003). Most of the proteins found in seeds are storage proteins, also called endosperm proteins. Structural and metabolic proteins and starch granule associated proteins (SGAPs) are also present in the rice kernel. The external surface of the starch granule is the first barrier to hydration, enzyme attack, and chemical modifying agents. Rice protein in the endosperm tightly associates on the surface of starch granules, and, as a result, it is difficult to produce protein free rice starch. This indicates the presence of proteins, including both endosperm and SGAPs, could have significant effects on the starch properties. Also, proteins found on the surface of starch granules may act as physical barriers to starch digestion (Svihus et al., 2005).
Rice endosperm proteins are classified into four types: water soluble albumins (9-11%), salt soluble globulins (7-15%), alcohol soluble prolams (2-4%), and acid or alkali soluble glutelins (80-90%). The high percentage of glutelins and low percentage of prolams in rice imparts the rice protein with very unique characteristics among the cereal proteins (Payne and Rhodes, 1982). Albumins and globulins, rich in the aleurone layer but decreasing toward the center of kernel, are usually removed during milling. Whereas, glutelins have the inverse distribution in rice endosperm in the forms of protein bodies (Houston et al., 1968). These spherically shaped protein bodies bind strongly to the compound starch granules with disulfide bonds and hydrophobic bonds (Lim et al., 1999).

Among these four protein fractions, albumin contains the highest lysine content, followed by glutelin, globulin, and prolamin. Due to the low content of prolamin in rice endosperm proteins, rice has higher lysine content than other cereals. Globulin is the highest in the sulfur amino acids including cysteine and methionine. Prolamin contains the most glutamic acid, leucine, tyrosine, and phenylalanine (Juliano and Boulter, 1985). Glutamine, asparagine, arginine, glycine, and alanine are the most abundant amino acids in rice glutenin. Glutelin, or oryzain, is the major storage protein in rice with two major subunits from 19 to 25 and 34.5 to 39 kDa. According to SDS-PAGE patterns from rice embryo, pericarp, and aleurone layers, glutelin mainly exists in the endosperm of the rice grain (Villareal and Juliano, 1978). It is insoluble in water due to the highly ordered structure by hydrophobic, hydrogen, and disulfide bonds. It is mainly soluble in acidic (pH below 3.0) or alkaline (pH above 10.0) solutions. The amide groups in glutamine and
asparagine side chains promote aggregations of glutelin (Wen and Luthe, 1985). This may explain the lack of functional properties of rice glutelin.

SGAPs are defined as proteins that are distinctly different from storage proteins and naturally located on the surface of or as integral components within the starch granules (Baldwin, 2001). The presence of protein in or on starch might influence the physicochemical properties of starch such as swelling, solubility, and gelatinization temperature. The surface protein on the granule could also be a barrier to granule hydration, enzyme attack, and chemical reaction (Chan et al., 2012).

2.1.1.3 Lipid

Rice contains very low amount of lipids. The majority of lipids are located in the bran and embryo of the rice grain. The lipids in the endosperm are associated with protein bodies, and also bounded with starch granules (Juliano, 1985). Generally, isolation of starch from rice flour mainly involves protein removal (Bao and Bergman, 2004). In non-waxy, common rice starch, there is 0.3-0.4% bound lipids, whereas, waxy rice contains even less (0.03%) (Morrison and Azudin, 1987). Among the starch bound lipids, roughly 32% are free fatty acid and 68% are lysophosphatidyl choline (Morrison et al., 1987).

2.1.1.4 Others

There are trace amount of phosphorus in rice, including phosphate-monoesters and phospholipids. In common rice, 0.048% (db) of phospholipids and 0.013% (db) of phosphate-monoesters were reported, whereas in waxy rice, only 0.003% (db) phosphate-monoesters were detected (Lim et al., 1975; Jane, et al., 1996; Bao and Bergman, 2004).
2.1.2 Nutritional value

Because of their high nutritive quality and good functionality, rice products are very competitive ingredient in commercial applications (Bean and Nishita, 1985; Ju et al., 2001). More importantly, the unique protein profile (lack of gliadin) of rice makes rice a highly desirable hypoallergenic cereal.

2.1.3 Functionality and application

Rice starch is widely applied as an ingredient in various food and industrial products based on its advantages over other starches. These properties includes bland flavor, white color, small granule size, better freeze-thaw stability, more acid resistance, and hypoallergenicity (Mitchell, 2009; Marti, 2011).

2.1.3.1 Gelatinization and rheological properties

Before heating in water, starch granules are insoluble and only absorb a limited amount of water. Gelatinization is an irreversible process taking place in the presence of water and heat with the disruption of the starch granule molecular order, granule swelling, crystallite melting, loss of birefringence, and starch solubilization. It is critical to optimize the heat input, temperature, and cooking time to completely gelatinize starch during processing to minimize cost and develop functionality such as viscosity and elasticity.

In the first step of gelatinization, the viscosity of starch slurry will increase rapidly due to the granule swelling with increasing temperature. The peak viscosity is achieved when granules swollen have been balanced with the granules broken by stirring. With
further heating and stirring, more granules rupture and fragment causing a decrease in viscosity. During cooling, some of the starch molecules will partially re-associate to form a gel which is called retrogradation.

Pasting temperatures of the starch granule differ with starch origins due to the varying accessibility of the granule to hydration. The accessibility of hydration is dependent upon the ratio of amylose to amylopectin, protein residue, and lipid concentration (Taggart, 2004). Starch granule properties and the degree of amylose leaching during gelatinization are the major factors responsible for starch rheological behavior. Many researchers have attempted to compare the viscosity between rice flour and starch, waxy rice and common rice. Bao et al., (1999), and Vandeputte et al., (2003) reported that amylose content is the main factor for pasting viscosity, however, other results showed two similar amylose content rice flours expressed very different pasting properties. When comparing rice flour and its starch, distinct viscosity was observed mainly due to the influence of proteins (Wang et al., 2002; Fitzgerald et al., 2003).

**2.1.3.2 Retrogradation and gelation**

During cooling below the starch crystallites melting temperature, retrogradation or gel formation will occur quickly due to dispersed amylose re-associate to form ordered structures (Marti et al., 2011). This would cause increased viscosity, gel firming, and texture staling in starch food systems which will profoundly affect the final product quality, consumer acceptance, shelf life, and nutritional properties (Billiaderis 1991). Although starch retrogradation is considered a negative factor in baking, some studies showed that it had a positive correlation with extruded products quality (Karim et al., 2000). Meanwhile, retrogradation is a critical processing step for starchy noodles, in
order to decrease stickiness, prevent dissolution during boiling and increase chewiness (Tan et al., 2009). Also, many studies have indicated that retrogradation is a reliable way to produce resistant starch (See 2.2.1).

2.2 Resistant Starches

For nutritional purposes, Englyst, Kingman, et al. (1992) classified starches into three categories, rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Resistant starch (RS) is defined as the sum of starch and products of starch degradation not absorbed in the small intestine and can survive prolonged incubation with $\alpha$-amylase and other amylolytic enzymes (Englyst, et al., 1982; Goñi, García-Diz et al., 1996; Nugent, 2005). It has been recognized that RS has potential functions similar to dietary fiber in the large intestine (Cummines and Englyst, 1991).

2.2.1 Type and Production

Four types of resistant starches, RS1, RS2, RS3, and RS4, have been classified (Table 2.2) based on their origins and physical characteristics. RS1 is a physically inaccessible starch, for example pulses. Thick cell walls make it is difficult to break down pulses during cooking and digestion in the stomach. Rough ground cereal grains belong to the RS1 category. RS2 is native resistant starch granules with B-type X-ray patterns including unripe banana starch, raw potato starch, and high amylose starch species such as Eurylon®, Novelose® 240, Hylon® VII, and Hi-Maize™. Higher gelatinization temperatures (above 120 °C) are required for this type. RS3 are retrograded starches. Retrogradation is the collective process of starch gelatinization then cooling before
reorganization into linear chain starch. Amylose is more susceptible to retrogradation due to its linear structure. Currently, several commercial RS3 derived from high-amylose are available, such as Novelose® 330 and Neo-amylose. RS4 are chemically modified starches such as starch esters, ethers, and cross-linked. Summaries of the commercial RS are presented in Table 2.3.

Table 2.2. Classification of resistant starches.*

<table>
<thead>
<tr>
<th>Type</th>
<th>Main characteristic</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS1</td>
<td>Physically inaccessible to digestive enzymes</td>
<td>Whole or partially milled grains, seeds, lentils</td>
</tr>
<tr>
<td>RS2</td>
<td>Granular starch with B-type crystallinity</td>
<td>Raw potatoes, green bananas, some legumes, high amylose starch</td>
</tr>
<tr>
<td>RS3</td>
<td>Retrograded starch</td>
<td>Cooked and cooled starchy food, such as stale bread, Novelose 330, Neo-amylose</td>
</tr>
<tr>
<td>RS4</td>
<td>Chemically modified starch</td>
<td>Starch esters, ethers, and cross linked starch</td>
</tr>
</tbody>
</table>

* Englyst et al., 1992; Thompson, 2000; Nugent, 2005.

2.2.2 Health benefits

In general, digestible starches can be hydrolyzed by α-amylases, glycoamylase, and sucrase-isomaltase in the small intestine to yield free glucose for energy. However, RS plays a role similar to dietary fiber without releasing calories. The energy value of RS is approximately 2 kcal/g which is significantly lower than digestible starch at 4.2 of kcal/g (Liversey, 1994). This would make RS an attractive ingredient to many food manufacturers, especially for breads that are traditionally high on the glycaemic index (GI). GI is a physiological concept to classify carbohydrate foods. It is indicated by the
incremental area under the blood glucose curve after consumption of 50 g carbohydrate, divided by the curve area of control food, usually white bread or glucose (Ludwig and Eckel, 2002). Researchers continue to debate whether RS has potential effects on glucose response, insulin response, satiety, and weight control. Positive effects were often observed shortly after intake of a high RS meal, within the first 2 to 8 h (Higgins, 2004). RS did not increase satiety in the studies of Holm and Bjorck, (1992), Mèance, et al., (1999), whereas, studies conducted by Raben et al., (1994), Skrabanja et al., (2001) proved RS imparted satiety.

Further, many investigations on both animal and human fecal content showed that RS could ferment in the large intestine resulting in the production of carbon dioxide, methane, hydrogen, lactic acid, and, more importantly, short chain fatty acids (SCFA) including butyrate, propionate, and acetate (Phillips et al. 1995; Ferguson et al., 2000; Henningsson et al., 2003; Muir et al., 2004). Therefore, intake of RS may help improve colonic health as well as dietary fiber. Different types of RS have been reported to produce different SCFA under certain circumstances. RS2 from raw potato starch increased butyrate production in humans and rats, whereas RS3 increased the concentration of acetate in pigs but not humans (Cummings et al., 1996; Martin et al., 2000). SCFA production, particularly butyrate, is associated with improved colonic function and could be measured as marker of colorectal cancer (Nugent, 2005). Animal studies (rats) have proved that RS appears to have a protective effect on colonic functions (Conlon and Bird, 2003; Toden et al., 2003).
### Table 2.3. Health benefits, functionalities, and applications of commercial resistant starches.

<table>
<thead>
<tr>
<th>Product</th>
<th>Type</th>
<th>Source</th>
<th>Producer</th>
<th>Health benefits</th>
<th>Functional benefits</th>
<th>Applications</th>
</tr>
</thead>
</table>
Table 2.3 Health benefits, functionalities, and applications of commercial resistant starches. (Continued) *

<table>
<thead>
<tr>
<th>Product</th>
<th>Type</th>
<th>Source</th>
<th>Producer</th>
<th>Health benefits</th>
<th>Functional benefits</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>maltodextrins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neo-amylose</td>
<td>RS3</td>
<td>Sucrose</td>
<td>Südzucker (German)</td>
<td>Prebiotic.</td>
<td></td>
<td>Pharmaceuticals. Food. Cosmetics.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>from corn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Van Munster et al., 1994; Champ, 2004, and the following links

http://eu.foodinnovation.com/docs/HYLONVII.pdf
http://www.hi-maize.com/Himaize/USA/Home
http://listofcompanies.co.in/opta-food-ingredients-inc/
2.3 Modifications of Starch

Starch is widely used in the food, paper, and textile industries. However, native starches have inadequate tolerance during manufacturing. For instance, swollen starch granules are fragile to shearing during processing, this would cause the loss of viscosity and textural stability. The undesirable properties of native starch give rise to limitations during application. Therefore, many types of modification including chemical, physical, and enzymatic (Figure 2.3), have been applied to starches to enhance their desirable attributes and minimize their defects. These modification treatments enable the evolution of new processing technologies and market trends. It is also very common to utilize more than two modification treatments on the same product to maximize overall performance.

Figure 2.5 Chemical and enzymatic modifications of starch (Adapted from Taggart, 2004)
2.3.1 Physical modifications

Pregelatinization, radiation, and mechanical processing are three physical treatments that are frequently used for starch modification. Pregelatinization, named precooked starch, usually involves cooking and drying by one of many ways including drum drying, extrusion, and spray drying. Instant starch food products are the main application for this process. Radiation, for example microwave, has various effects on corn, potato, and cassava starches. They include increased gelatinization temperature, improved stability, reduced peak viscosity, and increased amylolytic enzyme susceptibility (Marquette et al., 1982; Muzimbaranda and Tomasik, 1994). Ultrasound, ball milling, and pressure treatments have also been used to achieve higher gel strength and cold water swelling starches (Meuser et al., 1978; Morrison et al., 1994; Mason, 2009).

2.3.2 Chemical modifications

Chemical modifications of starch are generally through the introduction of functional groups, which involve crosslinking, esterification, etherification, and oxidation of the available hydroxyl groups on the glucopyranosyl units. Commercially modified starches are produced by the addition of certain concentration reagents into starch slurries under controlled alkaline conditions (pH 7-9 for esterification, pH 11-12 for etherification) and temperature (< 60 °C). Hydrochloric or sulfuric acid is used to neutralize the reaction slurry, and afterward, water washing then drying (< 40 °C) will yield the modified starch powder (Chiu and Solarek, 2009).
Crosslinking using bifunctional reagents to link hydroxyl groups in starch granules by covalent bonds slows down the rate of granule swelling and protects the starch granule from rupture during shearing (Felton and Schopmeyer, 1943; Wu and Seib, 1990; Taggart, 2004; Mason, 2009). The United States Food and Drug Administration (FDA) stipulates that no more than 0.1% phosphoryl chloride, 1% sodium trimetaphosphate, or 0.12% adipic acetic mixed anhydride by weight of starch can be used in food grade crosslinked modified starches (FDA, 1995). Epichlorohydrin that can produce diester linkage is banned in the US. Crosslinking modification can improve the acid, heat, shear, and freeze-thaw cycle stability of starches (Wu and Seib, 1990). Phosphorylated starch pastes are clear of high viscosity and resistant to retrogradation.

Stabilization is the addition of substituents to the hydroxyl groups of starch. There are four major substitutions, starch acetates, starch hydroxylpropyl ethers, starch monophosphate esters, and starch sodium octenylsuccinates, applied in food ingredients (FDA, 1995). These substituents sterically interrupt the starch polymers association thus inhibiting retrogradation and improving water holding capacity and cold temperature stability. Acetylated rice starch increased solubility, viscosity, and lowered the initial pasting temperature according to Jae et al. (1993) and Gonzalez and Perez (2002). Octenylsuccinylation can produce modified starch with emulsifying capability.

2.3.3 Enzymatic modifications

α-amylase, β-amylase, iso-amylase, and pullulanase are commonly applied in starch hydrolysis to give lower molecular weight fragments, higher DE (dextrose
equivalent) syrup, de-branch amylopectin. Glucose, maltose, oligosaccharide, polysaccharides can be produced depending on the extent of enzyme hydrolysis (ref).

2.4 Starch Oxidation

Two objectives, bleaching and enhancing physicochemical properties, are achieved by treating starch with oxidants. Recently, there has been an increasing interest to utilize oxidation to enhance starch functionalities such as low viscosity, binding, film forming, high stability, and clarity. Oxidized starch has already been applied in foods as coating and sealing agents in confectionary, as emulsifier and dough conditioner for bread, as gum arabic replacer, and as binding agent in batter applications (Kuakpetoon and Wang, 2001; 2006).

Sodium hypochlorite is a widespread commercial oxidant used for starch oxidation in the food industry compared to other less commonly used oxidants such as periodate, chromic acid, permanganate, and nitrogen dioxide (Kuakpetoon and Wang, 2001; Mason, 2009). First, hydroxyl groups on starch molecules are randomly oxidized to carbonyl groups and then to carboxyl groups (Mason, 2009). Consequently, the level of starch oxidation, which takes place mainly at the hydroxyl groups of C-2, C-3, and C-6 positions, can be indicated by the number of carboxyl and carbonyl groups on oxidized starch (Kuakpetoon and Wang, 2001). The dialdehyde or dicarboxylic acid of oxidized starch can cross-link with amino/imino groups of protein. It was reported that the interaction of small amounts of dialdehyde starch with amino groups of wheat gluten improved dough properties and the quality of bread (Tharanathan, 2005).
2.5 Protein Oxidation

During food storage and processing, different conditions (temperature, light) or treatments (heating, pressure, radiation, food additives) would cause protein quality loss due to protein oxidation. Physical and chemical changes in oxidized proteins include amino acid destruction, decreases in protein solubility due to protein polymerization, loss of enzyme activity, formation of amino acid derivatives including carbonyls, and increase (mild oxidation) or decrease (intensive oxidation) in protein digestibility (Xiong, 2000; Liu and Xiong, 2000). Free radicals are the leading causes for the protein oxidation during food processing and storage. Amino acid side chains, the peptide backbone are the targets for free radicals attack on the proteins. Amino acid with reactive side chains such as sulfhydryl, thioether, amino group, imidazole ring, indole ring are more susceptible to be oxidized (Roubal and Tappel, 1966). Protein aggregates can be induced by oxidizing agents through both non-covalent and covalent forces. Oxidation can expose the nonpolar amino acid residues that result in hydrophobic association of proteins. Hypochlorous acid has been reported to cause protein side-chain modification, backbone fragmentation, and cross-linking (Pattison, 2001).

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CHAPTER 3

THE ROLE OF PROTEINS IN THE CHEMICAL CHANGES AND IN VITRO DIGESTIBILITY OF OXIDATIVELY TREATED RICE FLOURS AND STARCHES VARYING IN AMYLOSE CONTENT

Waxy rice flour / starch          Common rice flour / starch

Oxidized with sodium hypochlorite
0, 0.8%, 2%, 5%

Chemical composition
  Moisture
  Protein
  Total starch
  Amylose

Starch oxidation
  Carbonyl group
  Carboxyl group

Protein modification
  Amino acid composition
  Protein electrophoresis patterns

Color

In vitro starch digestibility
3.1 Summary

Unhealthy diet with high calories is a recognized cause for obesity and diabetes. Recent efforts have focused on developing low-glycemic index diets, for example, resistant starches, through chemical modifications. In the present study, we investigated the impact of oxidative stress applied to endosperm proteins as well as SGAPs on the digestibility of waxy (WF) and common (CF) rice flours and their respective starches (WS, CS). Raw WF, CF, WS, and CS were treated with 0.8, 2, and 5% sodium hypochlorite (NaOCl) at pH 9.5 and 35°C for 50 min. Carbonyl and carboxyl group contents of oxidized starches rose up to 25 and 10–fold, respectively, with increasing the oxidant concentration, but only moderate increases were observed in flours ($P < 0.05$). Raw starches (WS, CS) and WF were less digestible ($P < 0.05$) than nonoxidized; however, for all heated (gelatinized) samples, the digestibility increased ($P < 0.05$) by the oxidant treatments. The decreased digestibility of WF may be explained by endogenous protein aggregation revealed in SDS–PAGE. The results indicated that oxidative modification is a potential technique to lower the digestibility of WF due to the susceptibility of starch granules to oxidants and the presence of endogenous proteins.
3.2 Introduction

The over-consumption of cereal and other starch-based foods has been recognized as an important cause for a variety of health issues, including diabetes and obesity. On the basis of nutritional consideration, starch can be classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992). RDS induces a rapid increase in blood glucose and insulin levels after ingestion. SDS prolongs the release of glucose, thus preventing hyperglycaemia-related diseases. RS reduces starch availability for digestion and produces short chain fatty acids in the large bowl through fermentation, which is beneficial for colon health and protects against colorectal cancer (Muir et al., 2004; Lehmann and Robin, 2007). Therefore, starch ingredients with high levels of SDS and RS can improve nutritive function of foods.

The digestibility of starch is dependent on the chemical and physical nature of the starch. Many factors, both intrinsic and extrinsic, can affect the digestibility of starch therefore the calorie intake. The physical form of the cereal (whole grain vs refined flour), starch granule size and shape, native α-amylase inhibitors, endogenous proteins, pigments (red pericarp in rice), degree of starch gelatinization, and amylose/amylopectin ratio have been reported to be important factors affecting starch digestibility (Thorne et al., 1983; Holm et al., 1985; Perera et al., 2000). Raw starch is generally less digestible than cooked starch (Dreher et al., 1984; Singh et al., 2010). Waxy rice starch is more susceptible to pancreatic α-amylase than common rice starch (Evers and Juliano, 1976).

Considerable efforts have been made in the past decades to develop technologies that may be used to reduce starch digestibility. Oxidative modification represents one potential and novel approach. Oxidized starch is produced by reacting starch with a
specific amount of oxidizing reagents under controlled pH and temperature conditions. Many oxidizing reagents are used to modify starch, for example, periodate, hypochlorite, and hydrogen peroxide (Veelaert et al., 1995; Li and Vasanthan, 2003; Tolvanen et al., 2009). Sodium hypochlorite is the most widely used oxidizing agent in the food industry (Floor et al., 1989; Forssell et al., 1995; Sánchez-Rivera et al., 2005). The oxidative reaction occurs primarily at the hydroxyl groups of the C-2, C-3 and C-6 positions on a D-glucopyranosyl unit to form carbonyls first and then to carboxyls (Kuakpetoon and Wang, 2001, 2008). The carbonyl and carboxyl derivatives are not recognized by α-amylase; therefore, such oxidative modification reduces the digestibility of starch (Wolf et al., 1999; Chung et al., 2008).

Previous studies have shown that starch, both in raw and cooked wheat, was encapsulated by a protein matrix which restricted the availability of starch to α-amylase (Batey and Alexander, 1982; Holm et al., 1985). Also, oxidation has been reported to enhance protein network formation (Liu et al., 2000; Xiong et al., 2010). Therefore, it can be hypothesized that exposures of starch and flours to oxidizing agents might reduce the digestibility of the consulting amylose and amylopectin. The objective of the present research was to investigate the molecular modification of both starch and protein in two types of rice flours following hypochlorite oxidation. The resulting alteration in starch digestibility was examined.
3.3 Materials and Methods

3.3.1 Materials

Waxy rice and common rice were procured from Walong Marketing Inc. (Buena Park, CA) and Pacific International Rice Mill, LLC (Woodland, CA), respectively. An aqueous solution of sodium hypochlorite (NaOCl) (containing 6% active chlorine) was purchased from RICCA Chemical Co. (Arlington, TX). Alpha-amylase (type VI-B from porcine pancreas, A-3176, 23 U/mg) and amyloglucosidase (from Aspergillus niger, A-7095, 300 U/mL) were purchased from Sigma Chemical Co. (St. Louis, MO). Glucose hexokinase liquid stable reagent (37.6 mM buffer, 2.1 mM ATP, 2.5 mM NAD, Hexokinase from recombinant yeast > 1500 U/L, Glucose-6-phosphate dehydrogenase > 2500 U/L, pH 7.7 at 20 °C) was purchased from Thermo Scientific (Middletown, VA).

3.3.2 Preparation of rice flours and starches

Waxy rice and common rice kernels were ground into flours for WF and CF samples using a laboratory mill (Nutrimill, L' Equip, St. George, UT). WS and CS were isolated according to the procedure of Baik et al. (1997) with some modifications. Rice kernels were soaked in deionized water (1:1, w/w) for 8 h then ground with two-fold 0.4% (w/v) NaOH solution using a Waring blender for 3 min. The suspension was passed through a 40 mesh sieve and allowed to select 4 °C for 24 h. The supernatant was removed and two-fold 0.4% (w/v) NaOH solution was added into the sediment. After stirring to thoroughly mix, the mixture was kept at 4 °C for 24 h. The sediments were collected and adjusted to neutral pH with 1 M HCl then washed twice with deionized
water by centrifugation (6552 g, 10 min) to remove the salts. Isolated starches were dried at 40 °C in a convention oven and ground with motor and pestle then passed through a 40 mesh sieve.

3.3.3 Oxidation

Oxidized rice flours and starches were prepared according to the method of Autio et al. (1996) with some modifications. Flour and starch slurries (40%, w/w) were prepared by adding deionized water to 100 g sample (dry basis, db) to a final weight of 250 g. The slurries were maintained at 35 °C and the pH was adjusted to 9.5 with 2 M NaOH. Sodium hypochlorite at 0.8, 2, and 5% (w/w, final concentration in starch slurry) were slowly added into the slurry over 30 min while maintaining the pH at 9.5 ± 0.1 with 1 M H$_2$SO$_4$. An additional 50 min reaction period was allowed while the pH was maintained at 9.5 using 1 M NaOH. After reaction, the slurries were adjusted to pH 7.0 with 1 M H$_2$SO$_4$ and dewatered by centrifugation at 6552 g for 10 min, then washed with two-fold volume of deionized water before drying in a convention oven at 40 °C for 48 h. The oxidized samples were ground with motor and pestle followed by passing through a 40 mesh sieve, then stored in a desiccator at room temperature.

3.3.4 Chemical profile analysis

3.3.4.1 Moisture content

All samples (ca. 2 g) weighed into small aluminum pans were dried in a 100 °C convention oven for 24 h and cooled in a desiccator. The moisture content was expressed as the weight difference before and after drying in percentage.
3.3.4.2 Protein content

Protein content all samples was analyzed using the vario Macro elemental analyzer (Elementar, Hanau, Germany). About 250 mg flours and 500 mg starches were weighed into reusable crucibles and then loaded elemental analyzer to conduct the nitrogen analysis. The nitrogen content was multiplied by 5.95 to convert into protein content in rice flour or starch (Juliano, 1994).

3.3.4.3 Total starch content

Starch content in native and oxidized rice flours and starches were measured according to Englyst et al. (1992). About 1 g sample was weighed and mixed with 25 mL acetate buffer (0.1 M, pH 5.2), then heated at 100 °C for 30 min. After cooling down to room temperature, 0.1 mL of heat stable α-amylase was added into gelatinized starch solution and boiled for 15 min. Potassium hydroxide (10 mL, 7 M) was added into chilled hydrolyzed sample, followed by shaking in an ice water bath for 15 min to thoroughly hydrolyze starch. Then, 1 mL of the reaction solution was mixed with 10 mL of 0.5 M acetic acid solution containing 0.2 mL 50 AGU/mL amylglucosidase. The mixture was incubated at 70 °C for 30 min. Total glucose from hydrolysis reaction was measured by glucose hexokinase liquid stable reagent by Konelab 30i (Thermo Electron Corp., Waltham, MA).

3.3.4.4 Amylose content

Amylose content was determined according to the procedure of Chrastil (1987). About 20 mg sample was mixed with 5 ml 85% methanol for 30 min at 60 °C then centrifuged at 1220 g for 10 min to remove lipid. The extraction was repeated twice. The pellet was solubilized with 0.5 M NaOH and then heated at 95 °C for 30 min with
shaking (110 stroke/min). The reaction solution (0.1 mL) was added to 5 mL of 0.5% trichloroacetic acid to precipitate protein. After centrifugation at 1220 g for 10 min, 0.05 mL of 0.01 N I₂-KI solution was added to the supernatant. After 30 min at room temperature, blue color was read the absorbance at 620 nm. A standard curve was prepared using by 0–100% pure potato amylase and the difference was made up with pure potato amylopectin.

3.3.5 Color measurement

A Minolta Chroma meter model CR-310 (Konica Minolta, Japan) was used for all color (\(L^*, a^*, b^*\)) determinations. \(L^*\) is a measure of the lightness from black to white recorded as 0–100, \(a^*\) value with the range from positive to negative values represents redness and greenness, respectively. In addition, \(b^*\) value with positive to negative values indicates yellowness and blueness, respectively. Whiteness (%) was calculated according to Lu et al. (2005).

\[
\text{Whiteness} = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}
\]

3.3.6 Starch carbonyl group analysis

The starch carbonyl content was analyzed according to Smith (1967). Four grams of flours or starches were suspended in 100 mL of deionized water in a 500 mL beaker and gelatinized at 100 °C for 20 min. The gelatinized sample was cooled to 40 °C and then adjusted to pH 3.2 with 0.1 N HCl. Hydroxylamine reagent (15 mL, 5%, w/v) containing 0.5 N NaOH was then added to react with the carbonyl groups in sample. The beaker was sealed with aluminum foil and placed in a 40 °C water bath for 4 h with

33
constant shaking (110 stroke/min). The excess hydroxylamine was determined by rapidly
titrating the reaction mixture to pH 3.2 with standardized 0.1 M HCl. The sample blank
was run with only hydroxylamine reagent. Carbonyl group content was calculated as:

\[
\text{Carbonyl content (\%)} = \frac{[(\text{Blank} - \text{Sample}) \times \text{HCl, M} \times 0.028 \times 100]}{\text{Sample weight in g (db)}}
\]

3.3.7 Starch carboxyl group analysis

The carboxyl group content was determined according to the procedure of
Chattopadhyay et al. (1997) with some modifications. Two grams of a sample were
suspended into 25 mL 0.1 N HCl and stirred for 30 min. After decanting the supernatant
out, the precipitate was washed with 400 mL of deionized water. The washed sample was
transferred into a 500 mL beaker, and the volume was brought to 300 mL with deionized
water. The starch slurry was completely gelatinized in a boiling water bath (100 °C) with
continuously shaking for 15 min. The hot dispersion was then brought to 450 mL with
deionized water and immediately titrated to pH 8.3 with standardized 0.01 N NaOH. The
sample blank was run with deionized water in the same manner. Carboxyl group content
was calculated as:

\[
\text{Carboxyl content (\%)} = \frac{[(\text{Sample} - \text{Blank}) \times \text{NaOH, N} \times 100]}{\text{Sample weight in g (db)}} \times 0.045
\]

3.3.8 Amino acid analysis

Native and oxidized rice flours and starches were analyzed by reverse-phase high-
performance liquid chromatography (RP-HPLC; Waters Corporation, Milford, MA) to
evaluate the amino acid composition according to the official procedure 982.30 (AOAC,
2005). About 0.2 g sample was hydrolyzed with 6 N HCl at 110 °C for 24 h. Norleucine was used as the internal standard.

3.3.9 SDS–PAGE

Proteins from both WF and CF were extracted with four different solvents: 0.1 M NaOH; 2% SDS; 2% SDS + 6 M urea; and 2% SDS + 6 M urea + 0.1% dithiothreitol (DTT) to determine different chemical bonds formed upon oxidative modification (Van der Borght, 2006). SDS–PAGE of extracted protein was run according to Laemmli (1970) with a 5–18% acrylamide gradient gel. All the samples before electrophoresis were treated with either 10% β-mercaptoethanol (β-ME) or without.

3.3.10 In vitro starch digestibility

Non-gelatinized and gelatinized flours and starches were subjected to in vitro starch digestibility based on the methods of Englyst et al. (1992) and Juansang et al. (2012) with some modifications. Porcine pancreatic α-amylase (0.435 g) was dispersed into 50 mL sodium acetate buffer (0.1 M, pH 5.2) containing 4 mM CaCl₂. After centrifugation at 1220 g for 5 min, a volume of 4.5 mL supernatant was mixed with 0.5 mL 15 U/mL amyloglucosidase. This enzyme solution was prepared freshly for each test.

For non-gelatinized samples, about 100 mg (db) sample and 10 glass beads (5 mm diameter) were loaded into a flask with 5 mL sodium acetate buffer (pH 5.2) and 5 mL enzyme solution. The mixture was incubated in a shaking water bath (110 stroke/min) at 37 °C to carry out the digestion process. To prepare gelatinized samples, 10% of the sample slurry was heated at 100 °C for 5 min. After cooling to room temperature, 100 mg
A gelatinized sample was subjected to the same digestive procedure as non-gelatinized samples.

After 20 min and 2 h digestion under the condition as described above, 1 mL reaction mixture was taken for the glucose content analysis. The mixture was centrifuged at 5478 g for 10 min. The supernatant was diluted to 10 times so that the glucose content would be in the glucose hexokinase liquid stable reagent test range. The liberated glucose in the supernatant was analyzed by glucose hexokinase liquid stable reagent by Konelab 30i (Thermo Electron Corp., Waltham, MA). The series of reactions involved in the assay were as follows:

\[
\text{Hexokinase} \quad \text{Glucose} + \text{ATP} \rightarrow \text{G-6-P} + \text{ADP} \\
\text{Glucose-6-phosphate dehydrogenase} \quad \text{G-6-P} + \text{NAD}^+ \rightarrow 6\text{-PG} + \text{NADH} + \text{H}^+
\]

The amount of NADH formed is proportional to the concentration of glucose in the sample and measured by the absorbance at 340 nm.

3.3.11 Statistical analysis

All the measurements were repeated in three trials with triplicate sample analysis except for the RP-HPLC which was repeated twice. Data were analyzed using the general linear model's procedure of Statistix software 9.0 (Analytical Software, Tallahassee, FL.). Analysis of variance (ANOVA) was performed to determine treatment effects. Significant differences \((P < 0.05)\) were determined using the least significant differences (LSD) procedure.
3.4 Results and Discussions

3.4.1 Chemical composition

Total moisture, protein, starch, and amylose contents of native and oxidized WF, WS, CF, and CS are summarized in Table 3.1. For native samples, all the samples had similar moisture contents (11.5%, 10.2%, 10.6%, and 11.4%, respectively). As expected, the protein content of flours (WF 7.30%, CF 8.20%) was much higher compared to starches (WS 1.56%, CS 1.31%) due to most of the endosperm proteins being removed from flours during starch preparation. The expected higher amounts total starch in both starch materials (WS 84.1%, CS 85.5%) compared to flours (WF 78.5%, CF 77.1%), were consistent with the protein content difference between flours and starches. Amylose was not detected in all waxy rice samples (WF, WS), whereas 4.72% and 10.14% amylose were found in CF and CS, respectively.

The sample protein content, especially in WF and CF, was reduced with the degree of oxidation induced by the concentration of the oxidant (NaOCl). This can be explained by the loss of the water-soluble proteins (albumins) due to the washing step after oxidation. In addition, oxidized both WF and CF samples had a higher starch content than native samples, whereas the samples from WS and CS had less. The amylose content decreased by the oxidation in CS but increased in CF. The increased amount in total starch of WF and CF and amylose content of CF compensated for the decrease of protein. Amylose in common corn starch has been reported to be more susceptible to oxidation and degraded at higher oxidation levels can explain the decreased amylose in CS (Wang and Wang, 2003; Kuakpetoon and Wang, 2006)
3.4.2 Color

Color is one of the most important sensory attributes in consumer acceptance of a food product. Table 3.2 shows the color parameters of WF, WS, CF, and CS with different oxidation degrees (0, 0.8, 2, and 5% NaOCl). $L^*$, $a^*$, and $b^*$ values and whiteness of native waxy and common rice flours and starches ranged from 95.4 to 100.9, –0.44 to –0.09, –0.09 to 4.59, 93.7 to 99.5%, respectively. The starch samples (WS, CS) were whiter ($P < 0.05$) in terms of higher lightness ($L^*$ value) and lower yellowness ($b^*$ value) when compared with flour samples (WF, CF). The endosperm proteins removed during starch preparation contributed to the lower yellowness ($b^*$ value) and higher whiteness in starches.

Oxidation significantly influenced all the color parameters including $L^*$, $a^*$, and $b^*$ of both flour (WF, CF) and starch (WS, CS) samples ($P < 0.05$). WF and CF exhibited significantly more changes, especially in $L^*$ value (decreased to 5), $b^*$ value (increased to 10), and whiteness (decreased to 10%), with increasing amount of NaOCl. These marked changes were in a sharp contrast with those occurring to starches (WS and CS), which exhibited only about 1% decrease of whiteness.

3.4.3 Starch modifications

The hydroxyl groups of starch molecules are first oxidized to carbonyl groups and then to carboxyl groups (Kuakpetoon and Wang, 2003). The concentrations of carbonyls and carboxyls, indicators of the degree of starch oxidation, in WF, WS, CF, and CS treated with different amounts of oxidant (0, 0.8, 2, and 5% NaOCl) are displayed in
Table 3.3. Both carbonyl and carboxyl contents of oxidized flours and starches rose sharply ($P < 0.05$) with increasing the NaOCl percentage except for the carboxyl content of WF that showed no significant change ($P > 0.05$).

The carbonyl content of oxidized flours increased up to 11 and 8 times, from 0.02 to 0.23% in WF, and 0.01 to 0.08% in CF, respectively. For starch samples, WS experienced about a 25-fold increase in carbonyls from 0.01 to 0.25%, compared with only about 2-fold increase in CS from 0.13 to 0.23% ($P < 0.05$) as the NaOCl treatment increased.

For carboxylic group content, there were more extensive changes in both WS (from 0.09 to 0.87%) and CS (from 0.15 to 0.87%) upon increasing the NaOCl concentration from 0 to 5%, comparing with WF (no difference) and CF (from 0.17 to 0.25%). It was reported that deproteinization, as a pretreatment before the starch ozonation, significantly increased the oxidation degree of starches prepared from corn, sago, and tapioca (Chan et al., 2012). This could explain why both WS and CS received higher extents of oxidative modification in terms of carbonyl and carboxyl group contents. Wang and Wang (2003) reported that common corn starch had higher carboxyl but similar carbonyl content than waxy corn starch after NaOCl oxidation. In contrast, both carbonyl and carboxyl contents significantly increased by the oxidation degree in waxy and common rice starches. It was observed oxidation was more intense with decreasing amylose content in corn starch (Kuakpetoon and Wang, 2008). This explained that low amylose starches (WF, WS) were more susceptible to oxidation.
3.4.4 Protein modifications

3.4.4.1 Amino acids

Essential amino acid composition is routinely determined as an indicator of protein efficiency ratio in food products. In addition, amino acid quantification can be used to understand the protein modification in terms of primary structure change. The amino acid composition of native and oxidized samples is presented in Table 3.4. Of the 20 amino acids, only 17 can be quantified by acid hydrolysis. Met, Cys, and Trp were excluded because of their sensitivity to strong acid conditions (AOAC, 2005).

The overall amino acid profiles of native WF and CF were quiet similar. After oxidation, the majority of amino acids exhibited significant losses ($P < 0.05$) in an oxidant dose-dependent manner for both WF and CF samples. Among the flour samples, Glu+Gln, Gly, His, Arg, Tyr, and Lys were more sensitive to oxidation, showing dramatic losses (56–74%) compared with others amino acids with 9–30% losses upon 5% NaOCl treatments. His, Tyr, and Lys are known to have a strong antioxidative capacity (Stadtman, 2003; Wang and de Mejia, 2005). Therefore, it was not surprising that they were easier to be oxidized than other amino acids. According to the absolute kinetic data of the amino acid reaction with hypochlorous acid at physiological pH (7.4), Cys and Met were most reactive, followed by Lys, His, Trp, and Tyr; the $\alpha$-amino groups of amino acids and peptides could also be modified (Pattison, 2001).

The isoelectric points (pI) of rice proteins (albumin, prolamin, globulin, acidic glutenlin) are reportedly from pH 4.1 to pH 4.8, except the basic glutenlin fraction that has pI of 8.5–9.3 (Shyur et al., 1988; Ju et al., 2001). Since the whole oxidation process was maintained at pH 9.5, the rigid rice protein structure would become loose and
hydrophobic amino acid side chain groups would become exposed. These amino acid side residues would competitively react with NaOCl with the hydroxyl groups on starch molecules. This can explain the lower starch carbonyl and carboxyl group contents in rice flours (WF, CF) compared with rice starches (WS, CS) as described above. In addition, as is mentioned in Chapter 2, rice albumin contains the highest amount of lysine. The decreased Lys content could be partially related to the albumin being removed by washing after oxidation treatment.

The amino acid content in WS and CS was very limited, and less than 0.1% was detected in native samples. After oxidation, the initial low amount decreased to an undetectable range. The results were consistent with protein analysis shown in Table 3.1. Most of the endosperm proteins was removed during starch isolation, and only the tightly bound protein and SGAPs were expected to remain with the starch granules.

3.4.4.2 SDS–PAGE

The polypeptide profile and molecular weight (MW) distribution of proteins extracted with different solvents from native and oxidized rice flours were characterized by 5–18% of acrylamide gradient gels shown in Figure 3.1 and 3.2. Native waxy and common rice protein had similar protein compositions which consisted of 10, 13, and 16 kDa prolamin, 22–23 kDa basic glutelin (B), 26 kDa globulin, 37–39 kDa acid glutelin (A), 57 kDa progluteline, and 90–110 kDa high molecular weight (HMW) polymers.

According to 0.1 M NaOH solvent SDS–PAGE results, the mild oxidation treatment by 0.8% NaOCl caused glutelin A and B cross-linked with each other as well as with other protein molecules via disulfide bonds based on the comparison between non-
reducing and reducing gels. It was reported that glutelin A and B contained 8 and 5 cysteine residues, respectively (Katsube-Tanaka et al., 2004). In the native state of rice protein, four of these cysteine residues form two disulfide bonds that one is an intermolecular bond between glutelin A and B and the other is an intramolecular disulfide bond in glutelin A (Van der Borght et al., 2006). This suggested that the available cysteine residues could participate in intermolecular cross-linking via SH/S–S interchange under mild oxidation condition to form high MW polymers. However, the reducing condition treatment did not fully recover the cross-linked glutelin A and B subunits, indicating that disulfide bonds were not only covalent linkages in the protein. Similar results were found in the higher oxidation treatments by 2 and 5% NaOCl, and the majority of the protein bands disappeared due to oxidation and were not fully recovered by the reducing agent (β-ME). It was implicated that there were other chemical bonds formed during oxidation treatments, for example, carbonyl-amine condensation, and dityrosine. Also, native samples (WF and CF) had HMW polymer bands presented on stacking gel and disappeared by oxidation. This indicated even larger polymers formed during oxidation that did not even enter the 3% stacking gel. The reducing condition treatment (with β-ME) was unable to disaggregate these polymers, indicating disulfide bonds were not the only one cross-linking formed during oxidation.

The 57 kDa progluteline in 0.8 and 2% NaOCl-oxidized WF and CF was recovered by the three individual treatments, i.e., 2% SDS; 2% SDS and 6 M urea; 2% SDS, 6 M urea and 0.1% DTT. Gluteline A and B subunits bands became more intense after SDS, urea and DTT dissolution suggesting that H-bonds and intermolecular disulfide bonds were buried in the hydrophobic core. Buried disulfide bonds were
reduced more slowly than others due to the more free energies of the transition states required for their reduction (Creighton et al., 1995). Prolamins (10–16 kDa) were recovered on all oxidized samples. Further, 2% SDS and 6 M urea partially recovered the HMW polymers in 0.8, 2% oxidized WF and in 0.8% CF indicating both hydrophobic and hydrogen bonds produced during oxidation.

In addition, the dialdehyde or dicarboxylic groups in starch molecules resulted from oxidation could have cross-linking with amino/imino groups of amino acid residues, implicating the unrecovered protein can be attributed to the protein and starch aggregates (Tharanthan, 2005).

3.4.5 In vitro digestibility of starch

The starch digestibility of non-gelatinized (uncooked) and gelatinized (cooked) flours and starches (WF, WS, CF, and CS) at 20 min and 2 h is presented in Figure 3.2 and Figure 3.3, respectively. Free glucose content (mg), the digestive product by α-amylase and amyloglucosidase, was used to indicate the starch digestibility.

In the granular state (non-gelatinized sample) (Figure 3.2), the digestibility (20 min and 2 h) of WF, WS, and CS decreased with 5% NaOCl, but the digestibility of CF was increased by all levels of oxidation (with 0.8, 2, and 5% oxidant) \((P < 0.05)\). The digestibility of WS decreased steadily with increasing the oxidant concentration while that of WF and CS remained unchanged until at 5% hypochlorite. WS displayed the most notable digestibility reduction (up to 38% in 20 min, and 34% in 2 h) of all samples, which was in concert with starch carbonyls formation (25–fold) as well as the production of more advanced oxidation products, i.e., the carboxyls (10–fold) (Table 3.3). It was
reported that depolymerization of starch could be induced by higher concentrations of oxidized treatment (Tharanathan, 2005). This can explain the digestibility of 5% WS increased compared with 0.8% and 2% WS.

Digestibility of oxidatively stressed samples was enhanced considerably by gelatinization (Figure 3.3). The accessibility to starch molecules by enzymes was improved when starch was more swollen due to cooking (Chung et al., 2008). Only WS had a lower digestibility after 2 h, and all other samples had a higher digestibility after the oxidation treatment ($P < 0.05$). This still could be attributed to higher oxidized modification occurred in WS.

### 3.5 Conclusions

Oxidation by hypochlorite is a potential technique to reduce the digestibility of WF, WS, and CS. For WS and CS, the decreased digestibility was attributed to the generation of carbonyl and carboxyl groups by the exposure to oxidants. For WF, the reduced digestibility was also due, at least in part, to the oxidative aggregation of endogenous proteins. Further studies are needed to compare the dynamics and stoichiometry of oxidative conversion of starch polyhydroxyl groups to aldehydes and carboxyls, and the impact of oxidative modification on the physicochemical properties of rice flours and starches.
Table 3.1. Chemical profiles (moisture, protein, total starch, and amylose content) of native and oxidized waxy and common rice flours and starches.

<table>
<thead>
<tr>
<th>Sample</th>
<th>NaOCl (%)</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Total starch (%)</th>
<th>Amylose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waxy rice flour (WF)</td>
<td>0</td>
<td>11.5</td>
<td>7.30</td>
<td>78.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>13.0</td>
<td>6.90</td>
<td>83.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.4</td>
<td>6.66</td>
<td>83.2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12.1</td>
<td>5.79</td>
<td>84.2</td>
<td>-</td>
</tr>
<tr>
<td>Waxy rice starch (WS)</td>
<td>0</td>
<td>10.2</td>
<td>1.56</td>
<td>84.1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>12.8</td>
<td>0.03</td>
<td>86.1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14.1</td>
<td>0.02</td>
<td>83.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12.7</td>
<td>0.02</td>
<td>74.2</td>
<td>-</td>
</tr>
<tr>
<td>Common rice flour (CF)</td>
<td>0</td>
<td>10.6</td>
<td>8.20</td>
<td>77.1</td>
<td>4.72</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>9.8</td>
<td>7.88</td>
<td>74.8</td>
<td>6.10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.4</td>
<td>7.49</td>
<td>77.9</td>
<td>4.77</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.2</td>
<td>6.17</td>
<td>82.0</td>
<td>6.68</td>
</tr>
<tr>
<td>Common rice starch (CS)</td>
<td>0</td>
<td>11.4</td>
<td>1.31</td>
<td>85.5</td>
<td>10.14</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>8.9</td>
<td>0.05</td>
<td>79.7</td>
<td>8.46</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.2</td>
<td>0.04</td>
<td>80.5</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.2</td>
<td>0.04</td>
<td>71.8</td>
<td>-</td>
</tr>
</tbody>
</table>

* Not Detected
Table 3.2. Color characteristics indicated by $L^*$ (brightness), $a^*$ (redness), $b^*$ (yellowness) and whiteness of native and oxidized rice flours and starches.

<table>
<thead>
<tr>
<th>Sample</th>
<th>NaOCl (%)</th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
<th>Whiteness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waxy rice flour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(WF)</td>
<td>0</td>
<td>97.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.44&lt;sup&gt;i&lt;/sup&gt;</td>
<td>4.59&lt;sup&gt;d&lt;/sup&gt;</td>
<td>94.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>90.7&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.9&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>90.2&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>1.20&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.1&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>92.1&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.5&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Waxy rice starch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(WS)</td>
<td>0</td>
<td>100.9&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>-0.11&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>-0.09&lt;sup&gt;h&lt;/sup&gt;</td>
<td>99.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>100.6&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>-0.18&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>0.82&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>98.9&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>100.6&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>-0.19&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>1.09&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>98.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>99.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-0.25&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>1.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>98.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Common rice flour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(CF)</td>
<td>0</td>
<td>95.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.33&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>4.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>93.7&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>91.4&lt;sup&gt;eh&lt;/sup&gt;</td>
<td>0.64&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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<td></td>
<td>2</td>
<td>89.3&lt;sup&gt;i&lt;/sup&gt;</td>
<td>1.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.8&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>90.6&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>1.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.5&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td>Common rice starch</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(CS)</td>
<td>0</td>
<td>100.5&lt;sup&gt;ed&lt;/sup&gt;</td>
<td>-0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.03&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>99.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>101.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.12&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>0.31&lt;sup&gt;fgh&lt;/sup&gt;</td>
<td>98.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>101.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-0.12&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;fgh&lt;/sup&gt;</td>
<td>98.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>100.5&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>-0.22&lt;sup&gt;fgh&lt;/sup&gt;</td>
<td>1.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>98.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-j</sup> Means (n=6) in a column with no common superscripts are significantly different ($P < 0.05$).
Table 3.3. Carbonyl and carboxyl group contents of native and oxidized waxy and common rice flours and starches.

<table>
<thead>
<tr>
<th>Sample</th>
<th>NaOCl (%)</th>
<th>Carbonyl content (%)</th>
<th>Carboxyl content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waxy rice flour (WF)</td>
<td>0</td>
<td>0.02&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.03&lt;sup&gt;def&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.12&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.13&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Waxy rice starch (WS)</td>
<td>0</td>
<td>0.01&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.06&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.22&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Common rice flour (CF)</td>
<td>0</td>
<td>0.01&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.17&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.01&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.30&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>2</td>
<td>0.05&lt;sup&gt;def&lt;/sup&gt;</td>
<td>0.30&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
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<td>5</td>
<td>0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Common rice starch (CS)</td>
<td>0</td>
<td>0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-f</sup> Means (n=6) in a column with no common superscripts are significantly different (<i>P</i> < 0.05).
Table 3.4. Amino acid composition (g/100g sample) of native and oxidized waxy and common rice flours and starches.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Waxy rice flour (WF)</th>
<th>Waxy rice starch (WS)</th>
<th>Common rice flour (CF)</th>
<th>Common rice starch (CS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaOCl (%)</td>
<td>NaOCl (%)</td>
<td>NaOCl (%)</td>
<td>NaOCl (%)</td>
</tr>
<tr>
<td>0</td>
<td>0.8</td>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Asp+Asn</td>
<td>0.13 b</td>
<td>0.16 ab</td>
<td>0.12 b</td>
<td>0.12 b</td>
</tr>
<tr>
<td>Glu+Gln</td>
<td>0.24 b</td>
<td>0.35 a</td>
<td>0.22 b</td>
<td>0.16 b</td>
</tr>
<tr>
<td>Ser</td>
<td>0.23 b</td>
<td>0.20 c</td>
<td>0.18 d</td>
<td>0.15 e</td>
</tr>
<tr>
<td>Gly</td>
<td>0.20 b</td>
<td>0.20 b</td>
<td>0.17 b</td>
<td>0.11 c</td>
</tr>
<tr>
<td>His</td>
<td>0.11 b</td>
<td>0.14 b</td>
<td>0.07 c</td>
<td>0.04 d</td>
</tr>
<tr>
<td>Arg</td>
<td>0.40 bc</td>
<td>0.40 bc</td>
<td>0.33 d</td>
<td>0.17 c</td>
</tr>
<tr>
<td>Thr</td>
<td>0.16 b</td>
<td>0.16 b</td>
<td>0.14 c</td>
<td>0.10 d</td>
</tr>
<tr>
<td>Ala</td>
<td>0.28 ab</td>
<td>0.27 b</td>
<td>0.26 b</td>
<td>0.19 f</td>
</tr>
<tr>
<td>Pro</td>
<td>0.28 ab</td>
<td>0.25 bc</td>
<td>0.25 bc</td>
<td>0.19 d</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.20 b</td>
<td>0.14 c</td>
<td>0.12 c</td>
<td>0.05 d</td>
</tr>
<tr>
<td>Val</td>
<td>0.36 ab</td>
<td>0.36 ab</td>
<td>0.34 ab</td>
<td>0.26 c</td>
</tr>
<tr>
<td>Ile</td>
<td>0.26 a</td>
<td>0.25 a</td>
<td>0.27 a</td>
<td>0.19 b</td>
</tr>
<tr>
<td>Leu</td>
<td>0.49 bc</td>
<td>0.51 ab</td>
<td>0.45 cd</td>
<td>0.37 d</td>
</tr>
<tr>
<td>Phe</td>
<td>0.28 ab</td>
<td>0.28 ab</td>
<td>0.25 b</td>
<td>0.19 f</td>
</tr>
<tr>
<td>Lys</td>
<td>0.13 a</td>
<td>0.10 bc</td>
<td>0.06 d</td>
<td>0.04 e</td>
</tr>
</tbody>
</table>

a-g Means (n=6) in the same row with a different letter are significantly different (P < 0.05).

* Not detected.
Figure 3.1. SDS–PAGE profiles of endosperm proteins extracted with four different solvents (0.1 M NaOH; 2% SDS; 2% SDS, and 6 M urea; and 2% SDS, 6 M urea, and 0.1% DTT) under non-reducing (without β-mercaptoethanol) condition from waxy and common rice flours with different oxidation degrees (0, 0.8, 2, and 5%). Std: molecular weight marker.
Figure 3.2. SDS–PAGE profiles of endosperm proteins extracted with four different solvents (0.1 M NaOH; 2% SDS; 2% SDS, and 6 M urea; and 2% SDS, 6 M urea, and 0.1% DTT) under reducing (with β-mercaptoethanol) condition from waxy and common rice flours with different oxidation degrees (0, 0.8, 2, and 5%). Std: molecular weight marker.
Figure 3.3. *In vitro* starch digestibility of oxidatively modified, non-gelatinized waxy and common rice flours and starches. (A) and (B) represent the waxy rice flour and starch samples, (C) and (D) represent common rice flour and starch samples. Means for the same flour or starch oxidized at different NaOCl concentrations with different letters differ significantly (*P* < 0.05). (a–c) for 20 min digestion; (x–z) for 2 h digestion.
Figure 3.4. *In vitro* starch digestibility of oxidatively modified, gelatinized waxy and common rice flours and starches. (A) and (B) represent the waxy rice flour and starch samples, (C) and (D) represent common rice flour and starch samples. Means for the same flour or starch oxidized at different NaOCl concentrations with different letters differ significantly (*P* < 0.05). (a–c) for 20 min digestion; (x–z) for 2 h digestion.
CHAPTER 4

OXIDATION-INDUCED STRUCTURAL CHARACTERISTICS OF RICE FLOURS AND STARCHES WITH DIFFERENT LEVELS OF AMYLOSE

- Waxy rice flour / starch
- Common rice flour / starch

Oxidized with sodium hypochlorite
- 0, 0.8%, 2%, 5%

Microscopy
- Scanning electron microscopy
- Confocal scanning laser microscopy

Molecular changes
- Fourier transform infrared spectroscopy
- X-ray diffraction

Surface changes
- X-ray photoelectron spectroscopy

Particle size
4.1 Summary

In the present study, we investigated the impact of oxidative stress on microstructure and surface characteristics of native and oxidized (0.8, 2, and 5% NaOCl) waxy and common rice flours (WF, CF) and their starches (WS, CS). The morphological properties of samples were observed by scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). X-ray photoelectron spectroscopy (XPS) was applied to detect the sample powder surface elements and chemical bonds. Further, FT-IR spectra, X-ray diffraction patterns (XRD), and particle size of the samples were obtained. The morphology and surface properties of WF, CF, WS and CS were significantly altered by oxidation as revealed by SEM, CLSM and XPS. WF and CF with 5% NaOCl oxidation showed protein aggregates on the surface of starch granules. The FT-IR results included increased amorphous region peak (1600 cm\(^{-1}\)) in oxidized WS, CF and CS samples. Oxidation did not change rice flour and starch XRD patterns but the crystallinity decreased by 5% NaOCl oxidation. Samples particle size increased by oxidation.
4.2 Introduction

Food processing, including milling, mixing with mechanical forces, heating, as well as physical and chemical modifications can generally influence the microstructure of both starch granules and proteins of cereals (Harbers, 1975; White et al., 2008). The quality of the end product including nutritional value, appearance, texture, taste, stability is highly dependent on the microstructure characteristics (Aguilera, 2005). In addition, the surface characteristics of the particles are considered to be one of most important factors deciding the functional properties of food powders (starches, flours, dairy powder, egg powder, additives, etc.) (Gaiani et al., 2007; Murrieta-Pazos et al., 2012). For example, hydration, dispersion, and dissolution of starch and protein-based dry powders in aqueous solutions are affected by the polarity of surface molecules, microstructure of particles, and the size of pores (air pockets) within the agglomerates (Genovese and Rao, 2003; Halle, 2004; Debet and Gidley, 2007). Therefore, microstructure and surface characteristics are the key parameters in understanding of food materials' behavior.

Endogenous proteins on the starch granule surface have a significant impact on the surface chemistry and physicochemical properties of starch, including swelling, pasting, gelatinization, retrogradation, and enzyme degradation (Baldwin, 2001; Debet and Gidley, 2006; Naguleswaran et al., 2011). Oxidation has been recognized as an important approach to modify both starch and protein to overcome the disadvantages of native biopolymers such as low solubility and retrogradation (Pietrzyk and Fortuna, 2005).

Scanning electron microscopy (SEM) provides an excellent tool in understanding the physical properties of starch granules after chemical and physical modifications (Kaur et al., 2004). Confocal laser scanning microscopy (CLSM) with different staining
chemicals also offers a useful means to analyze protein or lipid distribution and chemical reaction sites on the particle surface (Han and Hamaker, 2002; Lee and BeMiller, 2008). On the other hand, X-ray photoelectron spectroscopy (XPS) serves as a powerful and reliable method to investigate chemical composition and chemical changes resulting from surface modification. A number of studies have applied XPS to observe lipid oxidation in fat particles, and surface property of dairy powders, protein powders, and wheat flours (Kim et al., 2002; Gaiani et al., 2006; Bosquilillon et al., 2004; Saad et al., 2011).

Fourier transform infrared (FT-IR) has been applied to detect molecular changes in oxidized starch to produce information about chemical groups containing highly polar bonds (Kweon et al., 2001; Flores-Morales et al., 2012). A starch granule consists of both crystalline and amorphous regions. Amylose largely comprises the amorphous regions, and amylopectin in starch granule is responsible for crystalline structure that shows a X-ray diffraction (XRD) pattern. There have been conflicting reports on the effect of oxidation on starch granules, some showing oxidation decreasing the relative crystallinity and others showing no effect on the XRD pattern (Kuakpetoon and Wang, 2001; Han and Ahn, 2002). Particle size has been reported to be related to starch digestibility in sorghum and cowpea (Mahasukhonthachat et al., 2010; Tinus et al., 2012). Heaton et al. (1988) demonstrated that reduced particle size of wheat, maize can induce faster digestibility.

The objectives of this study were to investigate the effects of hypochlorite oxidation on the surface structure, composition, crystallinity, and particle size of two types of rice flour and starches, i.e., that comprised of almost homogenous amylose (CF, CS) and that containing a significant portion of amylopectin besides amylose (WF, WS).
4.3 Material and Methods

4.3.1 Materials

Waxy rice and common rice were obtained from Walong Marketing Inc. (Buena Park, CA) and Pacific International Rice Mill, LLC (Woodland, USA). An aqueous solution of sodium hypochlorite (NaOCl) (containing 6% active chlorine) was purchased from RICCA Chemical Co. (Arlington, TX). Fluorescamine was procured from Acros Organics (Morris Plains, NJ).

4.3.2 Preparation of rice flour and starch samples

For details of the preparation procedures of native and oxidized WF, WS, CF and CS, refer to Chapter 3.

4.3.3 Microstructure characterization by microscopy

4.3.3.1 Scanning electron microscopy (SEM)

Morphological properties of native and oxidized samples (WF, CF, WS, and CS) were compared using a Model S-3400N Hitachi scanning electron microscope (Tokyo, Japan). The dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter Coater SPI-Module). The coated sample was placed into vacume chamber. An accelerating potential of 10 kV was used during micrography. All samples were observed under 2000 times magnification.
4.3.3.2 Confocal laser scanning microscopy (CLSM)

The protein distribution of native and 5% NaOCl oxidized samples were characterized by CLSM on the Leica AOBS TCS SP5 inverted laser confocal scanning microscopy system (Leica TCS SP5, Heidelberg, Germany) according the method of Naguleswaran et al. (2011). Briefly, flours and starches were stained according to the procedure of Bantan-Polak et al. (2001). About 10–20 mg were stained in 0.3 mL of 0.1% (w/v) fluorescamine in acetonitrile and 0.15 mL 0.1 M borate buffer (pH 8.0) at room temperature for 1 h and then rinsed 5 times with deionized water and centrifuged to remove extra dye. The stained samples were suspended in 0.5 mL of 50% glycerol for CLSM observation. The stained sample (10 µL) was dropped onto a slide and covered by a glass slip for the observation under 63 x 1.4 oil objective lens. Diode laser used for the excitation at 405 nm with 4% of power capacity and the emission light was detected at the interval wavelength of 406-493 nm.

4.3.4 X-ray photoelectron spectroscopy (XPS)

The surface chemical element and bonds of native and oxidized samples were analyzed by Thermo Scientific K-Alpha XPS system (Thermo Fisher Scientific, FL). The method is based on surface irradiation that causes a compete transfer of photon energy to atomic electrons (Bosquillon et al., 2004). When the electron binding energy is lower than then photon energy, the electron is emitted from atom (Murrieta-Pazos et al., 2012). The energy spectrum of the emitted photoelectrons is determined by means of a high-resolution electron spectrometer.
The sample powder was adhered to carbon tape and outgassed at ambient temperature prior to analysis with a 400 micron beam (Al-Kα X-ray). For each sample a survey spectrum was collected (0–1400 eV) to identify the elements present. Then high resolution spectra were collected for the major line of each element for peak fitting and quantitative analysis.

4.3.5 Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of all samples were obtained by a Nicolet 6700 spectrometer (Madison, WI). Each sample was subjected to 40 scans at a resolution setting of 8 cm⁻¹ for the spectrum collection.

4.3.6 X-ray diffraction (XRD)

The X-ray patterns were analyzed using D5000 X-ray diffractometer instrument (Siemens, Madison, WI) according to the method of Kuakpetoon and Wang (2001) with some modifications. All samples were equilibrated to constant moisture content, and about 1 g powder sample placed into the XRD sample holder. The diffractograms were collected under 40 mA and 40 kV. The scanning region of the diffraction angle (2θ) was from 5° to 45° at the 0.1° step size with count time of 2 s.

4.3.7 Particle size

Particle size analysis was carried out using a LA-950 laser scattering particle size distribution analyzer (Horiba, Japan). About 1 g powder samples were suspended in 200
ml deionized water and stirred at machine agitation speed setting of 10. A 650 nm red laser and a 405 nm blue light emitting diode (LED) were used for particle size analysis.

4.4 Results and Discussions

4.4.1 Morphological properties by SEM and CLSM

The starch granule morphology and the surface features of native and oxidized samples are presented as SEM results in Figure 4.1 (WF, WS) and Figure 4.2 (CF, CS). In native samples, vivid protein bodies in the form of starch-protein agglomerates were observed in flour samples (WF, CF) (Figure 4.1 and 4.2). Starch granules were clustered together and surrounded by protein bodies. The starch granules in WF had more protein bodies distributed outside the starch granule compound, and packed more tightly compared with CF. Moreover, there were some pinholes on the WF starch granule compound surface but not on CF. Similar results have been reported by Evers and Juliano (1976). The surface of native rice starches (WS, CS) appeared to be smooth and more dispersed. Both WS and CS had irregular and regular polygonal shapes with average diameters of 2–8 µm.

After oxidation, there were more starch granules exposed in both WF and CF compared with their native state. Indentations were observed on the oxidized starch granule surface, especially in WS samples. Smaller particles and dents can be found in 20% ozone oxidized rice starch samples (An, 2005). Boruch (1985) showed that oxidation caused distinct granule damage in larger potato granules compared with smaller size granules. However, Kuakpetoon and Wang (2005) and Sandhu et al. (2007) reported
that hypochlorite–oxidized common and waxy corn starch granules had no significant change. Also, there was no apparent change on the corn and potato granules' surfaces by hypochlorite oxidation until at about 8% (Rutenberg and Solarek, 1984). This could explain no significant change observed on the oxidized WS, and CS samples in our present study.

The CLSM results (Figure 4.3) confirmed the presence of protein on the surface of starch granules in both WF and CF, which was indicated by the fluorescent green color from the reaction of fluorescamine with primary amines. The results of CLSM with protein antibody staining showed the storage proteins were largely distributed on the outer layer of the rice starch granules (Furukawa et al., 2003). The 5% NaOCl oxidized WF and RF had higher fluorescence intensity than native samples thus indicating oxidation treatment enhanced the protein cross-linking on the surface of starch granule cluster. In addition, the disappearance and recovery by different solvents of the protein bands on the SDS–PAGE from Chapter 3 supported this conclusion. Fluorescence was also observed in both native starch samples (WS, CS) but not as intense as flour samples (WF, CF). After the oxidation, a very weak fluorescence was captured by the CLSM. This weak fluorescence observed on starch samples could be attributed to SGAPs. Naguleswaran et al. (2011), based on the CLSM with fluorescence staining, reported that SGAPs was predominately distributed on the granule surface of both triticale and corn starches.

The distribution of protein on the starch granule surface revealed by both SEM and CLSM can also explain the lower carboxylic group content in both WF and CF than
WS and CS showed in Chapter 3. Chan et al. (2012) reported that deproteinization significantly increased the oxidation degree of starches from corn, sago, and tapioca.

### 4.4.2 Surface chemical components and modification analyzed by XPS

XPS can measure the relative atomic elemental composition at the surface layer of approximately 5–10 nm of particle (Fälldt et al., 1993). Relative elemental composition can be utilized for the calculation of components according to the atomic ratios. Reasonable agreement results have been proved by comparing the XPS apparent stoichiometry and calculated from theoretical stoichiometry in dairy powders (Kim et al., 2002; Gaiani et al., 2006). Moreover, XPS survey scan peak can be further decomposed at specific binding energies that are attributed to different chemical functional groups (Rouxhet et al., 2008; Saad et al., 2009).

In the native and oxidized WF, CF samples and native WS, the XPS survey scans (Figure 4.4 and 4.5) were dominated by three photoelectron peaks, corresponding to electrons originating in the 1s orbital of the carbon (C1s), nitrogen(N1s), and oxygen (O1s) atoms (Paynter, 2006). Whereas, only C1s and O1s signals showed in the oxidized WS and all the CS samples.

C1s peak decomposed into four subpeaks (C1, C2, C3, and C4) are shown in Figure 4.6 and 4.7 to assign chemical functions. The peak at 284.6 eV (C1) is attributed to C making a single bond (C-O, C-H) in protein side chains. The peak at 286.1 eV (C2) is attributed to C single bond with O and N (C-O, C-N) in alcohol, amine, amide functions in proteins. The peak at 287.5 eV (C3) is attributed to C with two single bonds or one double bond with O and N (O-C-O, O=C-N, O=C-O) in hemiacetal and acetal
functions in polysaccharides or in amide functions in proteins. The peak at 288.7 eV (C4) is attributed to C with double bond with O (O=\text{C-OH}, \text{O=C-OR}) in ester and carboxyl functions in proteins and polysaccharides (Saad et al., 2011).

In all oxidized samples, the atomic percentage of C2 and O increased while C1 and N decreased by the oxidation (Table 4.1 and 4.2). However, the atomic percentage of C4 increased only in CF but decreased in WF, WS, and CS with the oxidation degree. C4 is attributed to carboxyl groups. This conflicted with the results showed in Chapter 3 Table 3.3 where significantly increased carboxylic group contents in oxidized samples were shown. The confliction could be due to that oxidation mainly occurred inside of starch granules.

4.4.3 FT-IR

The IR spectra of flours and starches are shown in Figure 4.8. The intensity of the band at 1022 cm\(^{-1}\) determines the orientation in intermolecular H-bonding of CH and CH\(_2\) in CH\(_2\)OH (Kacurakova and Mathlouthi, 1996). Oxidized WF, CF, and CS had higher intensity of 1022 cm\(^{-1}\) compared with their native samples, whereas there was no significant change in oxidized WS. The band at 1600 cm\(^{-1}\) is associated to the amorphous region of starches (Van Soest et al., 1994). After oxidation, the 1600 cm\(^{-1}\) peak appeared in WS, CF, CS samples. This could be because oxidation disrupted starch granule crystalline structure, therefore more amorphous region band emerged in IR spectra. The region between 3000 and 3400\(^{-1}\) corresponding to the OH stretch in carboxylic acid were augmented in oxidized samples (WF, CF and CS) (Flores-Morales et al., 2012).
4.4.4 X-ray diffraction patterns

Figure 4.9 shows the results of the XRD patterns for native and oxidized rice flours and starches (WF, CF, WS, and CS). Both waxy and common rice showed A-type XRD pattern with peaks at about 15°, 17°, and 23° (2θ) that are typical for cereal starches (Zobel, 1988; Shih et al., 2007). The pattern remained unchanged after oxidation. However, the peak intensity of all the 5% NaOCl samples were decreased, indicating the starch crystallinity was partially disrupted by oxidation.

4.4.5 Particle size

The NaOCl oxidation increased the particle size of all the samples (WF, WS, CF, WS) according to the results shown in Figure 4.10. In general, native flours had larger size compared with native starches due to the presence of endogenous proteins and the protein network, in agreement with the results from SEM and CLSM. Hypochlorite oxidation induced protein cross-linking, starch-protein interaction showed by SDS–PAGE results in Chapter 3 can explain the oxidized flours and starches had larger particle size.

4.5 Conclusions

The microstructure and surface properties of WF, CF, WS and CS were affected by hypochlorite oxidation according to the results from SEM, CLSM and XPS. Higher intense florescence of 5% NaOCl oxidized WF and CF revealed by CLSM further demonstrated that endogenous rice protein had cross-linking and aggregates on the starch granule surface. FT-IR results showed there was increased amorphous region peak (1600
cm\(^{-1}\)) in oxidized WS, CF and CS. Meanwhile, XRD results displayed the decreased crystallinity by 5% NaOCl oxidation. All samples particle size increased by oxidation.
**Table 4.1.** Surface elemental composition of native and oxidized waxy rice flours and starches from X-ray photoelectron spectroscopy.

<table>
<thead>
<tr>
<th>Binding energy (eV)</th>
<th>Element</th>
<th>Functions</th>
<th>Atomic percentage (%)</th>
<th>Waxy rice flour</th>
<th>Waxy rice starch</th>
<th>NaOCl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>284.6</td>
<td>C1</td>
<td>C-O, C-H</td>
<td>36.1</td>
<td>29.0</td>
<td>29.1</td>
<td>26.2</td>
</tr>
<tr>
<td>286.1</td>
<td>C2</td>
<td>C-OH, C-N,C-O-C, C-O-C=O</td>
<td>25.0</td>
<td>28.7</td>
<td>27.9</td>
<td>30.8</td>
</tr>
<tr>
<td>287.5</td>
<td>C3</td>
<td>O-C-O, O=C-N, O=C-O</td>
<td>8.4</td>
<td>8.6</td>
<td>8.1</td>
<td>8.6</td>
</tr>
<tr>
<td>288.7</td>
<td>C4</td>
<td>HO-C=O, O=C-OR</td>
<td>3.0</td>
<td>2.8</td>
<td>3.2</td>
<td>2.5</td>
</tr>
<tr>
<td>399.7</td>
<td>N</td>
<td>NH, NH2, NH3</td>
<td>3.4</td>
<td>2.2</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>532.5</td>
<td>O</td>
<td>C-OH, H2O, O2</td>
<td>24.1</td>
<td>28.3</td>
<td>29.1</td>
<td>29.8</td>
</tr>
</tbody>
</table>
Table 4.2. Surface elemental composition of native and oxidized common rice flours and starches from X-ray photoelectron spectroscopy.

<table>
<thead>
<tr>
<th>Binding energy (eV)</th>
<th>Element</th>
<th>Functions</th>
<th>Atomic percentage (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>5 NaOCl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Common rice flour</td>
<td>0</td>
<td>0.8</td>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>284.6</td>
<td>C1</td>
<td>C-O, C-H</td>
<td>40.7</td>
<td>33.5</td>
<td>35.9</td>
<td>30.7</td>
<td>13.8</td>
<td>21.0</td>
</tr>
<tr>
<td>286.1</td>
<td>C2</td>
<td>C-OH, C-N, C-O-C, C-O-C=O</td>
<td>23.4</td>
<td>26.2</td>
<td>24.4</td>
<td>27.1</td>
<td>36.2</td>
<td>31.9</td>
</tr>
<tr>
<td>287.5</td>
<td>C3</td>
<td>O-C-O, O=C-N, O=C-O</td>
<td>7.3</td>
<td>7.7</td>
<td>6.6</td>
<td>8.7</td>
<td>9.1</td>
<td>7.2</td>
</tr>
<tr>
<td>288.7</td>
<td>C4</td>
<td>HO-C=O, O=C-OR</td>
<td>3.2</td>
<td>3.7</td>
<td>4.3</td>
<td>3.4</td>
<td>2.1</td>
<td>3.7</td>
</tr>
<tr>
<td>399.7</td>
<td>N</td>
<td>NH, NH₂, NH₃</td>
<td>3.1</td>
<td>2.5</td>
<td>1.8</td>
<td>2.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>532.5</td>
<td>O</td>
<td>C-OH, H₂O, O₂</td>
<td>22.4</td>
<td>25.7</td>
<td>25.7</td>
<td>26.5</td>
<td>37.7</td>
<td>34.5</td>
</tr>
</tbody>
</table>
Figure 4.1. Scanning electron microscopy of oxidized (0–5% NaOCl) waxy rice flours (top) and starches (bottom) at 2000 × magnification. P: proteins, I: indentation.
Figure 4.2. Scanning electron microscopy of oxidized (0–5% NaOCl) common rice flours (top) and starches (bottom) at 2000 × magnification. P: proteins, I: indentation.
**Figure 4.3.** Confocal laser scanning microscopy of native and 5% NaOCl oxidized waxy and common rice flours and starches stained with fluorescamine for protein distribution analysis.
Figure 4.4. Survey scans obtained from XPS analysis for native, 0.8, 2, and 5% NaOCl oxidized waxy rice flours and starches.
Figure 4.5. Survey scans obtained from XPS analysis for native, 0.8, 2, and 5% NaOCl oxidized common rice flours and starches.
Figure 4.6. Decomposition of C1s of oxidatively modified (0, 0.8, 2, and 5% NaOCl) waxy rice flours and starches analyzed by X-ray photoelectron (XPS).
Figure 4.7. Decomposition of C1s of oxidatively modified (0, 0.8, 2, and 5% NaOCl) common rice flours and starches analyzed by X-ray photoelectron (XPS).
Figure 4.8. Fourier transform infrared spectroscopy (FT-IR) of oxidatively modified waxy and common rice flours and starches. (A) and (B) represent the waxy rice flour and starch, respectively, (C) and (D) represent common rice flour and starch, respectively.
Diffraction angle ($2\theta$, °)

Counts

Counts

Counts

Counts

Figure 4.9. X-ray diffraction results of oxidatively modified waxy and common rice flours and starches. (A) and (B) represent the waxy rice flour and starch, respectively (C) and (D) represent common rice flour and starch, respectively.
Figure 4.10. Particle sizes of oxidatively modified waxy and common rice flours and starches. (A) and (B) represent the waxy rice flour and starch, respectively, (C) and (D) represent common rice flour and starch, respectively.
CHAPTER 5
EFFECT OF OXIDATIVE MODIFICATION ON THERMAL AND RHEOLOGICAL PROPERTIES OF RICE FLOURS AND STARCHES WITH DIFFERENT LEVELS OF AMYLOSE
5.1 Summary

Oxidative modification is well known to change the functionality of starch; however, the impact of oxidation on the rheology of flours (mixed starch and protein) is much less understood. The objective of the study was to investigate the thermal and rheological properties of waxy (WF) and common (CF) rice flours and their starch fractions (WS, CS) after modification with 0.8, 2, and 5% sodium hypochlorite (NaOCl) at pH 9.0 and 35 °C for 50 min. Thermal transitions and viscoelastic patterns of samples were measured by differential scanning calorimetry (20 °C→100 °C) and oscillatory rheometry (20 °C→80 °C; 80 °C→20 °C). The temperatures corresponding to the onset (To), peak (Tp), and maximum melting rate (Tmr), enthalpy (ΔH), storage modulus (G'), and loss modulus (G'') of gelatinized samples were recorded. Oxidation with 5% NaOCl increased Tp of flours by 3 °C but markedly decreased both Tp (by 8 °C) and To (by 10 °C) of starches (P < 0.05). At 5% NaOCl, Tmr of WS and CS was lowered (P < 0.05) by 10 and 14 °C, respectively, while that of flours was minimally affected. The oxidant had small and inconsistent effects on ΔH. The G', G'', and their peak temperatures rose significantly for flours after oxidation while the opposite trend was observed on starches. Gelatinized CF and CS had 2–5 times greater elastic rigidity than WF and WS due to different amylose:amylopectin ratios, but the discrepancy diminished in oxidized samples.
5.2 Introduction

Starches and proteins, aside from their obvious nutritional values, are the two most important functional ingredients used in food processes for their textural and rheological contributions. Starch granule swelling and protein unfolding occurring during thermal gelatinization are responsible for the rheological behavior, hence, palatability, of starch and flour-based products (Totosaus et al., 2002). A deep insight into the functionality induced by starch and protein structural changes will aid in the application of the appropriate processing conditions in the manufacture of a variety of prepared foods.

The unique properties of rice such as hypoallergenity, and bland taste make it advantageous choice than other cereals (Bao and Bergman, 2004). Waxy and non-waxy (common) rices are mainly differentiated by amylose content and gelatinization properties of the extracted starches (Zhou et al., 2002). Studies have been shown that amylose-amylopectin ratio is correlated with rice starch gel hardness, texture, stickiness, gelatinization temperature, pasting properties (Hibi, 1998; Mariotti et al., 2009; Tran et al., 2001). The endosperm proteins in rice contain storage protein (albumin, globulin, prolamin and glutelin), biosynthetic or degradative enzymes that are most entrapped within the starch granules during starch synthesis (Baldwin, 2001). Although approximately 7% proteins existed in rice, they play an essential role on texture, pasting capacity, palatability of rice products (Frederick, 2004).

In order to satisfy the growth of diversity in food market, various modification methods, including chemical (anionic/cationic substitution, hydroxylpropylation, cross-linking, enzyme hydrolysis, pH shifting) and physical (extrusion, radiation, sonication, pressure, pre-gelatinization) had been developed to receive desirable physicochemical
properties of starch and protein (Bemiller, 1997; Duck-Ki et al., 2008; Feeney, 1977; Jiang et al., 2011). At the same time, these modifications can help food industry to control these food polymer behaviors within complex food process system. Oxidation with sodium hypochlorite is a traditional and useful method due to the conversion C-2, C-3, C-4, and C-6 hydroxyl groups of 1,4-α-D-glucopyranosyl units to dialdehyde and dicarboxylic groups in starch (Floor et al., 1989; Wing, 1994). The introduced dialdehyde group can cross link with amino/imino groups (Ikada et al., 1979; Jane and Kris, 1995). Moreover, oxidative modification either enhances or impairs protein functionalities and alters the textual properties of food. For example, mild oxidative stress improved fresh meat hydration and myofibril swelling capacity (Delles et al., 2011; Liu et al., 2011). Cui et al. (2012) demonstrated that surface activity improved while hydrodynamic behavior diminished by oxidation of whey protein.

Numerous studies have been performed to investigate the thermal and rheological properties of oxidized starch or protein individually (Rosell et al., 2007), however, few studies about how the native protein, amyllose/amyllopectin and oxidation treatment would affect these functionalities has been done. The objective of the study was to elucidate the influence of oxidative modification on the thermal and rheological properties of waxy (WF) and common (CF) rice flours and their starch fractions (WS, CS).
5.3 Material and Methods

5.3.1 Materials

Waxy rice was from Walong Marketing Inc. (Buena Park, CA, USA) and common rice was bought from Pacific International Rice Mill, LLC (Woodland, CA, USA). Sodium hypochlorite containing 6% active chlorine was purchased from RICCA Chemical Co. (Arlington, TX, USA).

5.3.2 Preparation oxidized rice flours and starches

For details of the preparation procedures of native and oxidized WF, WS, CF and CS, refer to Chapter 3.

5.3.4 Thermal stability analysis

Differential scanning calorimeter (DSC, 2920 Modulated DSC, TA Instruments Inc., New Castle, DE) was used for thermal stability analysis. Flour and starch slurries (10%, w/w) were thoroughly dispersed in deionized water. Each slurry was accurately weighed (15–20 mg) into a polymer-coated aluminum pan and hermetically sealed. Samples were heated from 20 to 100 °C at a heating rate of 10 °C/min. An empty sealed pan was used as reference. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures and enthalpy (ΔH) of gelatinization were determined using Universal Analysis version 1.2 N software (TA Instruments Inc., DE). First derivatives of thermal transition results were calculated to indicate the maximum melting rate temperature.
5.3.5 Dynamic rheological analysis

Native and oxidized flours and starches suspensions (10%, w/v) were analyzed by dynamic oscillatory measurement using a CVO Rheometer (Malvern Instruments, Worcestershire, UK) according to Addo et al. (2001). Thoroughly mixed suspensions were loaded in the 1-mm gap with 30mm diameter plate. The over-loaded portion of samples was trimmed off around plate. A thin layer of silicon oil was used to cover the perimeter of the sample to prevent dehydration and the plates were isolated with shell to reduce heat dissipation. Samples were equilibrated at the initial temperature 20 °C for 5 min, then heated up to 80 °C followed by cooled down to initial temperature at a scan rate of 1 °C/min. A fixed shear frequency (0.1 Hz) and strain (0.02) was applied during the heating and cooling process. The storage modulus (G', a measure of elasticity response; solid component of the network) and loss modulus (G", a measure of viscosity response; liquid component) were recorded during the whole dynamic rheological test.

5.3.6 Statistical analysis

All the measurements were carried out into two trials with duplicated. Data were analyzed using the general linear model's procedure of Statistix software 9.0 (Analytical Software, Tallahassee, FL.). Analysis of variance (ANOVA) was performed to determine treatment effects. Significant differences (P < 0.05) were determined using the least significant differences (LSD) procedure.
5.4 Results and Discussions

5.4.1 Thermal properties

Thermal stability of native and oxidized rice flours and starches measured by DSC is summarized in Table 5.1, and their DSC thermograms are presented in Figure 5.1. All samples exhibited one endothermic transition over a similar temperature range (48.9–88.9 °C) which corresponded to the starch gelatinization temperature range (55–85 °C). All the native flours (WF, CF) attained higher \((P < 0.05)\) thermal transition temperatures \((T_o, T_p, T_c)\) and melting enthalpies \((\Delta H)\) than native starches (WS, CS).

After oxidation treatment, similar thermal transition patterns were observed for waxy and common rice flours and their starches, respectively. Significant difference between flours and starches was mainly due to intrinsic proteins presented in flours whereas almost all of proteins were removed of native and oxidized starch samples (Table 3.1). Compared to the slightly increased \(T_o\) of oxidized flours (WF, CF), oxidized WS and CS exhibited lower \((P < 0.05)\) \(T_o\) than native starches, and these changes increased with increased oxidant. The weakened internal granule structure by oxidation rendered starches less stable. Native and oxidized rice flours all have significantly higher \(T_o\) compared to other rice samples with the same oxidized degree except the 0.8% NaOCl oxidized sample. The onset temperature of starch melting was strongly influenced by the amylopectin chains. Higher \(T_o\) is generally associated with a higher amount of B1 chains (DP 13–24) and a lower amount of A chains (DP 6–12), both of which are located in a single crystalline cluster in starch granule (Kuakpetoon and Wang, 2006). Therefore, the
higher amount of B1 chains present in non-oxidized rice flours gave rise to a more compact crystalline structure, hence, higher $T_o$.

The first derivative curves of DSC thermal transitions are shown in Figure 5.1. The peak was indicative of the temperature ($T_{mr}$) corresponding to the crystalline maximum melting rate. The $T_{mr}$ of WF changed little, while CF received a slightly higher value, e.g., was from 68.2 °C to 70.6 °C after 5% NaOCl oxidation. However, both WS and CS $T_{mr}$ decreased significantly after oxidation, and CF received 14 °C lower $T_{mr}$ compared to the native sample. All the first derivative data are consistent with the thermal transition results, i.e., the lower the $T_p$, the lower the $T_{mr}$.

5.4.2 Dynamic rheological properties

The rheological profiles during thermal gelatinization are presented in Figure 5.2 and 5.3, the peak modulus values are displayed in Table 5.3. For all samples, the viscosity (loss modulus, $G''$) and elasticity (storage modulus, $G'$) (Figure 5.2 and 5.3) started to increase at around 57 °C until the their peak temperature then dropped with further heating to 80 °C. During cooling process, all samples, except CS, had a decrease of both $G''$ and $G'$. The native and 0.8% oxidized CS presented substantially increased set back viscosity upon cooling. Several factors contributed to this phenomenon. Amylose free (showed in Table 3.1) waxy rice samples (WF, WS) and protein network slowed down gelation occurred in rice flour samples. Also, carboxylic groups generated by oxidation will lead to higher hydrophilicity to provide steric bulk which will minimize gel network formation of 2% and 5% oxidized CS. These results are similar to the
previous study that amylose rich starches can form rigid, opaque gels on cooling, where amylopectin rich starches form soft gels (Miles et al., 1985).

For all native samples, common rice (CF, CS) required a higher temperature to achieve maximum viscosity and elasticity compared with waxy rice (WF, WS) showed in Table 5.3. This could be mainly due to the 5–10% amylose present in common rice but little in waxy rice (Table 3.1). The peak viscosity and elasticity of both flours (WF, CF) are significantly higher than their starches (WS, CS) which indicated that native proteins contributed to the viscoelasticity in rice. Previously studies demonstrated waxy and common rice flours treated with dithiothreitol to cleave disulfide bonds or proteases to remove protein significantly decreased the rheological peak, breakdown and consistency viscosity values which indicated that protein network linked by disulfide bonds contributed to the higher viscosity (Derycke et al., 2005; Hamaker et al., 1991; Xie et al., 2008). Rice starch granule-associated proteins had been proposed to provide the gelatinized starch granule certain rigidity (Hamaker and Griffin, 1993).

Oxidation at all levels (0.8–5% NaOCl) dramatically altered the viscoelastic pattern (G" and G' curves) of rice flours and starches. Both WF and CF exhibited significantly higher viscoelasticity and peak temperatures with increasing oxidation degree except for CF oxidized by 5% NaOCl. Especially the elasticity of WF increased up to 20 times of 2% oxidative treatment indicating the potential application for solid gel food products such as surimi. Protein disulfide cross linking has been approved to relate with protein gelling, heating stability, thermal rheological properties (Lee et al., 1997; Singh, 1991). Oxidation has been demonstrated to enhance disulfide bonds cross-linking of whey proteins (Wang et al., 2013). Mild protein oxidation induced structure alternation
can facilitate myofibrillar protein cross linking and promote protein network formation (Xiong et al., 2010; Li et al., 2012). Reactive aldehyde groups of oxidized starches are able to react with free amino-or imino groups to form rigid structure (Tharanathan, 2005). This can explain the tremendously increased viscoelasticity by heating of oxidized WF and CF. It has been reported that dialdehyde starches can cross link with protein (zein, or soy protein) at high temperature by reacting with hydroxyls to form hemiacetals, or with amino groups to form schiff base, or with sulfhydryl groups to form thiol cross-linkages (Ikada et al., 1979; Tharanathan, 2005; Jane and Kris, 1995). However, the intense oxidation can cause protein aggregation which was responsible to decreased viscoelasticity of 5% oxidized WF.

Both $G''$ and $G'$ and peak temperatures dramatically dropped by oxidation of starch samples (WS and CS). Little viscosity and non-detectable elasticity of 5% oxidized WS were observed. The oxidation of secondary hydroxyl groups at C2, C3, C4 caused open and cleavage of the monomeric rings which resulted in polymer properties loss and consequently viscoelasticity decay of starches (Chang and Robyt, 1996; Floor et al., 1989) This special properties can be applied to the products have low requirement for the viscosity and also help decrease the energy demanding due to quicker heating transfer during heating and cooling process.

5.5 Conclusions

Thermal and viscoelastic behaviors of rice appeared dependent on amylose/amylopectin ratio, protein content, the oxidation degree. Oxidation increased the gelatinization temperature and rigidity of the protein-starch gel matrix in both WF and
CF due to not only starch-starch association, but protein-protein, protein-starch associations as well. On the other hand, oxidation of protein-free starch resulted in thin starch pastes. These implied that controlled starch and protein oxidation is a potential way to allow the rice flours and starches to be utilized as versatile food ingredients in food products where rheology is considered a primary quality attribute.
### Table 5.1. Temperatures and enthalpies associated with characteristic gelatinization behaviors of oxidatively modified waxy and common rice flours and starches.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>NaOCl (%)</th>
<th>$T_o$ (°C)</th>
<th>$T_p$ (°C)</th>
<th>$T_c$ (°C)</th>
<th>$\Delta H$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waxy rice flour (WF)</td>
<td>0</td>
<td>63.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.5&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>85.2&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>61.1&lt;sup&gt;def&lt;/sup&gt;</td>
<td>70.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85.9&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>61.9&lt;sup&gt;edef&lt;/sup&gt;</td>
<td>71.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>63.2&lt;sup&gt;bed&lt;/sup&gt;</td>
<td>71.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Waxy rice starch (WS)</td>
<td>0</td>
<td>62.5&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>69.6&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>81.9&lt;sup&gt;de&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>62.3&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>69.7&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>81.8&lt;sup&gt;de&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>61.0&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>69.4&lt;sup&gt;f&lt;/sup&gt;</td>
<td>82.5&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>51.3&lt;sup&gt;g&lt;/sup&gt;</td>
<td>61.4&lt;sup&gt;g&lt;/sup&gt;</td>
<td>75.4&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.4&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Common rice flour (CF)</td>
<td>0</td>
<td>65.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>71.9&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>86.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>64.1&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>72.5&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>87.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>65.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>72.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>87.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>66.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Common rice starch (CS)</td>
<td>0</td>
<td>63.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>70.3&lt;sup&gt;def&lt;/sup&gt;</td>
<td>80.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>63.4&lt;sup&gt;bed&lt;/sup&gt;</td>
<td>70.2&lt;sup&gt;def&lt;/sup&gt;</td>
<td>82.8&lt;sup&gt;bede&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>59.9&lt;sup&gt;f&lt;/sup&gt;</td>
<td>68.8&lt;sup&gt;f&lt;/sup&gt;</td>
<td>82.3&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>48.9&lt;sup&gt;h&lt;/sup&gt;</td>
<td>62.1&lt;sup&gt;g&lt;/sup&gt;</td>
<td>80.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* $T_o$, onset temperature; $T_{mr}$, temperature at the maximum melting rate; $T_p$, peak temperature; $T_c$, conclusion temperature; $\Delta H$, enthalpy.

<sup>a-g</sup> Means ($n=3$) in a column with no common superscripts are significantly different ($P < 0.05$).
Table 5.2. Temperature at maximum melting rate ($T_{mr}$, °C) calculated by first derivatives from representative thermal transition results of oxidatively modified waxy and common rice flours and starches.

<table>
<thead>
<tr>
<th>NaOCl (%)</th>
<th>Waxy rice flour</th>
<th>Waxy rice starch</th>
<th>Common rice flour</th>
<th>Common rice starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>68.2</td>
<td>66.0</td>
<td>69.3</td>
<td>67.6</td>
</tr>
<tr>
<td>0.8</td>
<td>66.3</td>
<td>65.8</td>
<td>69.0</td>
<td>66.8</td>
</tr>
<tr>
<td>2</td>
<td>66.4</td>
<td>64.6</td>
<td>67.8</td>
<td>65.1</td>
</tr>
<tr>
<td>5</td>
<td>68.4</td>
<td>55.7</td>
<td>69.3</td>
<td>53.4</td>
</tr>
</tbody>
</table>
Table 5.3. Peak rigidity moduli of oxidatively modified waxy and common rice flours and starches during thermal gelatinization.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>NaOCl (%)</th>
<th>Loss modulus</th>
<th>Storage modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$T_{\text{max}}$ ($^\circ$C)</td>
<td>$G''_{\text{max}}$ (Pa)</td>
</tr>
<tr>
<td>Waxy rice flour (WF)</td>
<td>0</td>
<td>60.6$^{\text{fg}}$</td>
<td>52.3$^{\text{f}}$</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>63.4$^{\text{de}}$</td>
<td>186.8$^{\text{d}}$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>64.2$^{\text{ed}}$</td>
<td>278.6$^{\text{e}}$</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>65.4$^{\text{c}}$</td>
<td>157.0$^{\text{d}}$</td>
</tr>
<tr>
<td>Waxy rice starch (WS)</td>
<td>0</td>
<td>59.6$^{\text{gh}}$</td>
<td>21.0$^{\text{f}}$</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>60.1$^{\text{fg}}$</td>
<td>17.7$^{\text{f}}$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>58.1$^{\text{i}}$</td>
<td>4.9$^{\text{g}}$</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>50.6$^{\text{i}}$</td>
<td>0.2$^{\text{g}}$</td>
</tr>
<tr>
<td>Common rice flour (CF)</td>
<td>0</td>
<td>63.5$^{\text{de}}$</td>
<td>115.4$^{\text{c}}$</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>76.1$^{\text{b}}$</td>
<td>252.6$^{\text{c}}$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>78.2$^{\text{a}}$</td>
<td>374.7$^{\text{a}}$</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>78.1$^{\text{a}}$</td>
<td>338.9$^{\text{b}}$</td>
</tr>
<tr>
<td>Common rice starch (CS)</td>
<td>0</td>
<td>63.6$^{\text{de}}$</td>
<td>32.8$^{\text{fg}}$</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>62.6$^{\text{c}}$</td>
<td>48.6$^{\text{f}}$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>61.1$^{\text{f}}$</td>
<td>20.8$^{\text{fg}}$</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>58.6$^{\text{hi}}$</td>
<td>2.8$^{\text{g}}$</td>
</tr>
</tbody>
</table>

*a–h Means (n=3) in a column with no common superscripts are significantly different ($P < 0.05$).
Figure 5.1. Thermal transitions and first derivative curves of waxy and common rice flours and starches after oxidative modification. (A) and (B) represent waxy rice flour and starch, respectively; (C) and (D) represent common rice flour and starch, respectively.
Figure 5.2. Dynamic rheological properties (loss modulus, $G''$, and storage modulus, $G'$) of oxidatively modified waxy rice flours and starches. (A) and (B) represent flour; (C) and (D) represent starch.
Figure 5.3. Dynamic rheological properties (loss modulus, $G''$, and storage modulus, $G'$) of oxidatively modified common rice flours and starches. (A) and (B) represent flour; (C) and (D) represent starch.
CHAPTER 6

OVERALL CONCLUSION

Both starch and protein were the targets of sodium hypochlorite oxidation in waxy and common rice flours and starches. Carbonyl and carboxyl group contents increased in all samples by oxidation, except for the carboxyl group of WF with no significant change. Amino acid composition was decreased due to oxidation, especially the percentage of Glu+Gln, Gly, His, Arg, Tyr, and Lys. In rice flour samples (WF and CF), protein aggregates through disulfide bond, hydrophobic force, and hydrogen bond were illustrated by SDS–PAGE.

Furthermore, SEM and CLSM results showed that protein existed on the starch granule surface on all rice flours and starches samples. Protein aggregation on the starch granules surface, in terms of higher intense fluorescence, were observed by 5% NaOCl oxidation in flours samples (WF and CF). The protein aggregation on the starch granule surface had a positive correlation with in vitro starch digestibility in rice flour samples. Whereas in rice starch samples, the reduced in vitro starch digestibility was more attributed to the higher contents of starch carbonyl and carboxyl group compared with non-oxidized starch samples.

Thermal and rheological properties were dramatically affected by NaOCl oxidation. Oxidation increased the gelatinization temperature of flours, but decreased in starches. During gelatinization, the viscoelasticity was increased for flour samples but reduced for starch samples by oxidation. Enhanced viscoelasticity of oxidized flour can result from protein cross-linking, while, carbonyl and carboxyl groups were more responsible for the thinner starch paste of oxidized starch samples.
Overall, the results indicated that hypochlorite oxidation can give rice more flexibility to become compatible upon different processing conditions and nutritional requirement.
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