Development and utilization of resistant starch in cooked rice grains and breads

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Development and utilization of resistant starch in cooked rice grains and breads

by

Michael Owen Reed

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
Jay-lin Jane, Major Professor
Terri Boyston
Olga Zabotina

Iowa State University
Ames, Iowa
2012

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DEDICATION

I dedicate this thesis to my parents, Thomas and Sharon, and to my brother, Brian; for your unending support, encouragement, friendship, and love. You have watched as I became the man I am today and never gave up on me. For the times that I fell short, you were always there for me, showing me that there is a better way. I deeply appreciate everything you have done for me and this thesis is a reflection of all that you taught me. I cannot thank you enough for teaching me the lessons that I hold dear and for riding along with me on this journey called Life.
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This thesis consists of two separate studies. The first is titled “Effects of Cooking Methods and Starch Structure on Starch Hydrolysis Rates of Rice”, and the second one is “Application of a Novel Resistant Starch in Bread: Composition, Structure and Sensory Analysis”. With concerns over diabetes, there is a need to find methods of cooking rice to reduce the rate of glucose release after ingesting. Comparing between steamed, stir-fried and pilaf rice, stir-fried rice displayed the slowest starch-hydrolysis rate and the largest resistant starch (RS) content followed by pilaf rice and steamed rice. RS in food is not readily hydrolyzed and is a healthy alternative for use in breads. Breads were made with 20% - 50% type 5 resistant starch (RS5). Sensory results showed no significant differences between bread with 20% RS5 and the control bread for overall opinion, likeliness of purchase, uncharacteristic flavor, and ranking.
CHAPTER 1. GENERAL INTRODUCTION

Starch is the main dietary carbohydrate providing energy for humans. Starch is found in numerous grains and tubers, including corn, wheat, rice, oats, potatoes, and cassava, of which corn, wheat, rice, and potato are the most important staples for humans. Starch has been utilized for both food and nonfood applications. Non-food applications include films, ink, paper, and textiles. Starch is widely used in food applications such as breads, crackers, and pasta. Research has shown that as consumers look for health benefits from the foods they consume, food manufactures are driven to formulate food products with noticeable health benefits. One area of interest is in structural changes of foods resulting from cooking methods used and their impacts on human health.

RS is a portion of starch that is resistant to enzymatic-hydrolysis in the small intestine (SI) but is fermentable by microflora in the colon. RS has been associated with health benefits, such as reducing the incidence of diabetes, obesity, colon cancer, and lowering blood cholesterol. The greater functionality of RS to ferment in the large intestine compared with dietary fiber suggests that increased health benefits can be derived in the use of this ingredient in food products.

People in many countries consume rice as their main energy source. These people use different methods to cook rice depending on their cultural origin. Steamed and stir-fried rice are common cooking methods in China, Japan, Thailand, and Vietnam, whereas people in France and the United States of America commonly cook rice pilaf. Italians use a unique technique called risotto. Each of these cooking methods prepares rice with unique taste and sensory qualities.
Research has been conducted on boiled rice starch and parboiled rice, but studies examining the effect of cooking rice using common methods are not found in literature. An interest in determining if a relationship exists between cooking methods for rice and glycemic index (GI) or starch-hydrolysis rates using in vitro experimental methods led us to develop a study using different cooking techniques with rice grains. A recent study conducted in our laboratory showed that adding lipids to starch reduced starch-hydrolysis rates. To determine if cooking rice with oil such as fried rice could reduce the rate of starch-hydrolysis, we chose stir-fried rice, rice pilaf, and steamed rice methods for an in vitro starch-hydrolysis study. Three different rice varieties, indica, japonica, and waxy rice were selected on the basis of their consumption by different populations. The objectives of this study were to understand how cooking methods and structures of rice starches affect starch-hydrolysis rates and the development of resistant starch (RS).

Incorporation of RS in baked products is challenging because of its limited gelatinization. A recently developed amylose-lipid complexed starch (RS5) shows high resistance to enzymatic-hydrolysis in both in vitro and in vivo studies. The goals of our study were to create formulations for white bread consisting of different levels of RS5, which also had good texture and taste. Studies were focused on improving dough development by adding dough additives and conducting physical studies using mixograph analysis to monitor the structural development of the dough and microscopic study to examine the protein network formation. We also conducted sensory panel studies to determine consumer acceptability of RS5 containing breads.
Thesis Organization

This thesis contains a general introduction and literature review followed by two chapters describing two separate studies. The first chapter, “Effects of Cooking Methods and Starch Structures on Starch Hydrolysis Rates of Rice” will be submitted to the *Journal of Food Science*. The second chapter, “Application of a Novel Resistant Starch in Bread: Analysis, Structural Impacts and Sensory Effects on Bread” will be submitted to *Cereal Chemistry* for publication. General conclusions complete this thesis.
Literature Review

Starch

General starch structure

Starch is synthesized in a granular form in plants and is the major energy storage vehicle for plants. Starch granules have semi-crystalline structures and consist of two major polysaccharides: amylose and amylopectin. Proportions and structures of amylose and amylopectin affect the gelatinization, pasting, and digestibility properties of the starch (Jane et al., 1999). Amylose is a linear molecule comprised of (α-1,4) linked glucose chains and few (α-1,6) linkages. Amylopectin is a highly branched molecule, consisting mainly of (α-1,4) linked glucose chains, and about 5% (α-1,6) linked branches (Takeda et al., 1986, 1993a,b). The structure of starch granules consists of concentric rings, which are organized into alternating amorphous and crystalline regions (Jane, 2006). The amorphous regions are comprised of amylopectin branch points. The crystalline regions are comprised of double helical branch chains of amylopectin while amylose is interspersed within amylopectin molecules. The amylose and amylopectin molecules are known to contribute to the integrity of starch granule (Jane, 2006).

Starch granules

Starch granules display different sizes, shapes, and polymorphs. The sizes of starch granules range from \( \leq 1 \ \mu m \) to \( \geq 100 \ \mu m \). Distributions of granule size for different starches are known to be monomodal or bimodal. Monomodal sized starch granules are found in maize and potatoes, whereas bimodal size distributions consists of large disk-shape and small spherical-shape granules are characteristic of wheat and barley starches (Salesse et al., 2006). Amylose and amylopectin molecules radiate from the center of the granule, called a hilum,
with amylose more concentrated at the periphery of the granule (Blanshard, 1987; Jane & Shen, 1993; Li et al., 2007). In native starch granules, starch molecules form a densely packed structure, which is dependent on the proportions of amylose and amylopectin present in the granule, whereas the hilum displays a loosely packed structure.

**Amylose structure**

Amylose is a linear molecule with α-1,4 linked glucose chains and a few branches of α-1,6 linkages. Amylose content has been reported to comprise 0 - 30% in most starches. Some high-amylose mutant starch has been reported to consist of more than 80% amylose (Jiang et al., 2010). Amylose in starch occurs in a non-crystalline amorphous form, which is interspersed with amylopectin (Jane et al., 1992; Kasemsuwan & Jane, 1994). Amylose is concentrated at the periphery of the granule, whereas the amylose and amylopectin is more loosely packed around the hilum (Jane & Shen, 1993; Pan & Jane, 2000; Jane, 2006; Li et al., 2007). The structure of amylose plays an important role on enzymatic-hydrolysis rates of starches by increasing stability in the starch granule, which can reduce enzymatic attack (Lu et al., 1997; Kim et al., 2004; Jane, 2006). Concentration of amylose in the starch determines the pasting properties and starch-hydrolysis rates of the starch and thus is important to the properties of the starch.

**Amylopectin structure**

Amylopectin is a highly branched molecule consisting mainly of α-1,4 linked glucose and about 5% α-1,6 linkages. Amylopectin makes up about 70% of normal starches with amylose as the remaining and 100% for waxy starch. Amylopectin molecules are composed of three types of chains: A-chains, B-chains, and C-chain. A-chains are short branch-chains (DP ~ 15), which are attached to the B-chains or the C-chains. The B-chains are connected to
C-chains that comprise the amylopectin backbone. The C-chains contain the reducing end of the molecule (Oates, 1997; Jane et al., 1999). Branch-chains of amylopectin molecules form double helices, which contribute to starch crystallinity. The A and B₁ chains are known to form double helices extended within one cluster of the amylopectin (Nakamura et al., 2002). The crystalline structure relates to the dense packing of the starch molecules.

**Gelatinization**

Gelatinization of starch granules occurs during heating in excess water. The process of gelatinization results in the disassociation of amylopectin double helical branch-chains, resulting in a loss of crystallinity. This decrease in crystallinity is attributed to the dissociation of double helices, disruption of granular structure, and loss of birefringence when viewed under polarized light (Jane, 2004). Gelatinization is associated with greater starch-hydrolysis rates compared with raw starch (Nakamura et al., 2002). Differential scanning colorimetry (DSC) is used to determine gelatinization temperatures of starch, which shows the amount of thermal energy needed to dissociate the crystalline structures of amylopectin and the amylose-lipid complex (Zhu & Corke, 2011). DSC parameters include onset, peak, conclusion temperatures, enthalpy changes, and the percentage retrogradation on the starch (Vandeputte et al., 2003).

**Retrogradation**

Retrogradation is the reassociation of starch chains into partial crystalline structures during storage after gelatinization of the starch granule (Englyst & Cummings, 1987; Eerlingen et al., 1994). Amylose retrogradation occurs rapidly, whereas amylopectin retrogradation occurs over days (Miles et al., 1985). Storage temperature, amylose contents, and proportion of short branch-chains of amylopectin play a primary role in the development
rate of retrograded starch. Amylose and short-chain amylopectin are known to form partial crystalline structures at 5°C, while higher storage temperatures slow the rate of retrogradation (Lu et al., 1997).

Retrograded starch is reported to reduce enzymatic-hydrolysis of glycosidic bonds of starch and increases the relative melting enthalpy change (Riva et al., 2000; Chung et al., 2006). Thermal properties of retrograded starches displayed lower gelatinization temperatures and enthalpy changes compared to native starches because of the weaker crystalline structures formed during reassociation of amylopectin and amylose linear chains (Sasaki et al., 2000).

**Resistant Starch**

Resistant starch (RS) is defined as the starch fraction resistant to starch hydrolysis in the small intestine but is fermented by microflora in the colon, producing short-chain fatty acids, which have been shown to provide health benefits (Englyst and Macfarlane, 1986; Englyst et al., 2003). Resistant starch (RS) has been investigated for its beneficial effects in preventing colon cancer, diabetes, obesity, and cardiovascular disease (Topping and Clifton, 1999; Behall et al., 2006, Fuentes-Zaragoza et al., 2010). Five types of RS have been reported. They correspond to physically inaccessible starch (type 1 resistant starch, RS1), the B- and C-type crystalline structures of native uncooked starch granule (RS2), retrograded amylose (RS3), chemically modified or cross-linked starch (RS4), and amylose-lipid complex (RS5) (Englyst et al., 1992; Eerlingen et al., 1994; Hasjim et al., 2010). It is reported that RS3, the reassociation of the amylose and some amylopectin into semi-crystalline double-helical structures, lowers the enzymatic-hydrolysis rate by reducing the susceptibility of starch to enzyme attack (Englyst and Cummings, 1987; Eerlingen et al.,
Amylose-lipid complexes (RS5) resist enzyme hydrolysis and are obtained from lipid interactions with gelatinized starch (Hasji et al., 2010).

RS is of interest in the production of food products because of its health benefits. Studies have investigated the use of RS in foods, including spaghetti, tortilla, and breads (Goni et al., 1996; Juarez-Garcia et al., 2006; Rohlfing et al., 2010). Use of RS in baked products is limited because of adverse quality effects resulting from RS properties. Studies have been reported on the natural formation of RS3 and its use as an ingredient in food products (Riva et al., 2000; Solzer et al., 2007). Addition of RS3 resulted in decreases in pliability, rollability, and cohesiveness to flour tortillas (Rohlfing et al., 2010). High concentrations of RS3 led to reduced structural integrity and thus, decrease in quality of the product. Formation of RS in processed foods has been extensively studied (Wang et al., 2002; Hung et al., 2005; Korus et al., 2009).

Investigations on the beneficial impacts of RS on glycemic index and insulin responses have been carried out in the past decade. Some research shows that RS must make up at least 14% of the total starch intake to have a significant positive impact (Behall and Hallfrisch, 2002; Brown et al., 2003; Higgins, 2004). Namratha et al. (2002) reported that RS contents in processed foods are dependent on many factors, including types of processing, storage conditions, and lipid and protein contents. The use of RS in foods may help decrease the calorie content of prepared food products, increase prebiotic effects, and perhaps increase satiety (Brown, 2004; Elia and Cummings, 2007; Willis et al., 2009). Effects of RS on increasing satiety in humans have been inconclusive and not been widely accepted (Rabab et al., 1994; Barkeling et al., 1995; Willis et al., 2009).
Studies have been conducted on resistant starch with regard to clinical studies and applications in food. Baixauli et al., (2008) reported the effect of different concentrations of RS on sensory attributes in muffins. The investigators reported that increased concentrations of RS (5% to 20%) had reduced quality effects on attributes, such as, chewiness, moisture, and cohesiveness. Behall et al., (2006b) reported that medium to higher levels of RS (2.51 g/100 g muffin and 5.06 g/100 g muffin, respectively) reduced blood glucose concentrations and insulin response in women during clinical trials.

Rice

General

Rice is an important staple crop worldwide for many centuries. Approximately 90% of rice is consumed by the populations of six countries: China, Japan, Vietnam, India, Bangladesh, and Indonesia (Khush, 2004). As the main dietary carbohydrate for many people, rice is their main energy intake, and preparations of rice have primarily involved steaming or stir-frying.

Cooked rice is rapidly converted to glucose after enzymatic-hydrolysis of the starch. Although white rice grains and parboiled rice grains have been primarily consumed, brown rice has increasingly been perceived as a healthier alternative. Zhang et al. (2010) found that consumer acceptability increased for brown rice compared with white rice after learning about health benefits. Brown rice is rich in nutrient content compared with white rice due to its limited processing (USDA, 2008).

Structures and properties of rice

Rice starch has lower amylose contents than other grain starches, such as, corn, wheat, and oats. White rice has little to no dietary fiber and higher amylopectin contents,
which is believed to be responsible for its rapid starch-hydrolysis rate. Amylose contents of rice starch range from (0-2%) for waxy varieties to (25%) for normal rice varieties (Juliano, 1979; Sagum & Arcot, 2000). The relative amounts of amylose and amylopectin have a profound effect on the thermal properties, pasting properties, and enzyme hydrolysis rates of rice starch. The fine amylopectin with a greater proportion of branch-chains DP 12-24 enhances the stability of the crystalline structure, whereas amylopectin with more short branch-chains of DP 6-12 decreases the stability of the crystalline structure (Vandeputte & Delcour, 2004). The long branch-chains can form longer double helixes that help stabilize the starch granule by requiring a higher temperature to disassociate the crystalline lamellar structure. Amylopectin branch-chain lengths of rice are composed of more short branch-chains and fewer long branch-chains (Jane et al., 1999) than that of other grains. These structures of rice amylopectin further increase the starch hydrolysis rates of rice, which lead to higher postprandial blood glucose concentrations.

Rice starch can form inclusion compounds with lipids known as amylose-lipid complexes (Morrrison et al., 1993; Guraya, 1997), which are responsible for lowering starch-hydrolysis rates. Reduction in starch hydrolysis rates in rice is associated with increased health benefits by lowering postprandial glucose and insulin responses (Goddard, 1984).

Amylose and amylopectin contribute to the structural integrity of the starch granule, which affects its gelatinization and pasting properties (Vandeputte et al., 2003). These properties are affected by other components in the rice, such as lipids and proteins. Lipids may form amylose-lipid complexes, whereas proteins provide a barrier for enzymes to hydrolyze (Hamaker & Griffin, 1993; Kitahara et al., 1996; Kitahara et al., 1997; Kaur & Singh, 2000).
Cooking methods

Many cooking methods are used to prepare rice for human consumption. Depending on the country of origin and cultural backgrounds, these methods vary widely, but all involve a heat moisture process. Rice cooking methods include parboiling, boiling, steaming, stir-frying, baking, pilaf, and risotto (Conway, 1991). These methods impart different textures to rice and are used to create different food eating properties. These different methods also impact starch properties, such as pasting, thermal, starch-hydrolysis rates, and resistant starch contents (Ong & Blanshard, 1995; Patindol et al., 2008; Kaur & Singh, 2008).

Parboiling is a processing treatment that is conducted before milling and is reported to make up to 15% of the world’s milled rice supply (Bhattacharya, 2004). The parboiled rice requires further treatment before it can be eaten. Boiled rice is prepared by heating rice in excess water until the starch has gelatinized and starch granules become swollen. Boiling is a common preparation in most countries and results in a fluffy and dry grain with textural qualities favored by many cultures. Steaming and boiling are used interchangeably although differences exist between the two methods. In traditional steaming, grains are held above boiling water in a closed container, or the rice is boiled for a short period of time and then steamed in a closed container. The pilaf method first cooks rice in oil before adding water. This imparts a nutty flavor due to the initial toasting of the rice grains and results in a firmer texture. Risotto is an Italian-style of cooking rice; wherein rice is continuously stirred while slowly adding water. This process results in slow release of rice starch, which develops a creamy consistency (Conway, 1991). Stir-frying is primarily used in the Asian countries, although this method is also currently used in the U.S. and European countries due to globalization of culinary trends. The traditional stir-frying method involves steaming or
boiling the rice and storing it at 4°C for 24 h before stir-frying in oil. Stir-frying results in high quality eating rice and is highly accepted by consumers.

**Starch-hydrolysis rates**

Rice starch is known to be quickly digested in the human digestion tract. The ability of the digestive enzymes to break down glycosidic bonds to produce glucose for energy use in the body is central to metabolism. Amylose-lipid complex formation has been shown to decrease starch-hydrolysis rate (Jane, 2006, 2007; Li et al., 2008). The amylose-lipid complex, which is resistant to enzyme-hydrolysis (Jane & Robyt, 1984), interacts with amylopectin and further restricts the swelling of the starch granule, leading to a reduction in the enzymatic-hydrolysis rate (Morrison et al., 1993c; Morrison, 2000). This rate reduction is also attributed to interactions between amylose molecules and amylopectin, which restricts starch granule swelling and reduces enzyme accessibility to starch molecules (Case et al., 1998; Shi et al., 1998). Both endogenous and exogenous lipids are known to restrict the ability of the enzyme to hydrolyze starch (Tester & Morrison, 1990). Lipid coating of the starch granule may further restrict the accessibility of the starch granule to digestion amylases (Morrison, 1981, 1995).

**Bread**

**Types of breads**

Numerous kinds of breads have been made reflecting the cultural, taste, and traditional attributes of human societies. These vary in shape, ingredients, baking processes, and types of flour, which provide unique attributes to the bread. Bread types are associated with certain characteristics, which are categorized by specific names: French baguette (French), ciabatta (Italian), focaccia (Italian), Pullman loaves (American), Kaiser rolls
(Germany), croissants (French), and pita (Middle East). The use of similar ingredients including flour, yeast, and water to make many different types of breads indicates that bread is a very versatile product (Conway, 1991).

**Baking methods**

Baking techniques vary in the methods used to create bread dough. These include straight-dough, sponge-dough, sourdough, and puff pastry dough (Conway, 1991). A straight-dough method combines all ingredients simultaneously; the dough is allowed to rise once or twice and then baked. The sponge-dough method is a two-step process: the first step is to create a sponge using specific ratios of water, flour, sugar, and all the yeast and is allowed to ferment for a specific period of time. In the second stage, the sponge is combined with the remaining ingredients to make the final dough. The sponge method is usually reserved for breads with high sugar content and results in a soft, tender crumb. Sourdough is produced by longer dough fermentation using naturally occurring yeasts (known as wild yeasts) and *lactobacilli*. Sourdough consists of a starter (a flour and water mixture) allowed to ferment over days and added to the remaining ingredients. This extensive fermentation results in highly sour bread (Friberg, 1996). Puff pastry dough uses a higher percentage of fat, in this case butter, than normal bread dough. The dough is folded multiple times to create layers of butter and dough. This process produces layers of a flaky crust formed by the rapid conversion of water into steam. The layering of the butter and dough traps steam and puffs up the crust, which results in flaky, thin layers.

**Structure of bread**

The structure of bread relies upon five main components: protein, starch, lipid, yeast, and water. Dough development begins with the addition of water to flour, which is attracted
to the water-binding proteins and wets the starch granules. Wheat proteins develop into a cohesive protein network called gluten (Patient & Ainsworth, 1994) that entraps CO₂ produced by the yeast (Saccharomyces cerevisiae) fermentation of carbohydrates. The protein network increases the stability of the dough during expansion of gas cells during rising resulting in a more tender bread crumb. The gelatinized starch forms a continuous gel during baking that firm during cooling and is largely responsible for bread crumb texture and volume. Gluten acts as a stable structure for gelatinized starch granules. Lipid is known to coat gluten proteins and prevents over-association of gluten proteins, thereby increasing loaf volume and tenderness. Water is used to form the dough and for gelatinization of the starch granules. High temperature denatures the protein during baking, which releases bound water. The starch then absorbs this excess water and begins to gelatinize (Mondale & Datta 2008).

**Flour/starch**

Wheat flour provides the principal structural component in baked bread. It has been suggested that starch in flour is filler for the protein structure, although this has been disputed (Bloksma, 1990). Starch has been shown to absorb up to 46% of water in bread dough with the remaining moisture bound to proteins (Goesaert et al., 2005). Some amylose is leached from starch granules during baking and may form inclusion complexes with wheat flour lipids, which is confirmed by the V type crystalline patterns in baked bread. The cooling of bread is accompanied by rapid amylose retrogradation, which is responsible for the initial firming of the crumb (Eliasson & Larsson, 1993).

**Gluten**

Proteins in wheat grains are the backbone of bread, which develop into a cohesive network called gluten. For acceptable dough formation, protein should comprise about 11%-
13% of flour weight (Park et al., 2006). There are two main classifications of wheat proteins: non-gluten (15-20% of total wheat proteins) and gluten proteins (80-85% of total wheat proteins). Gluten proteins play the major role in bread structure. Gluten is comprised of two types of water insoluble proteins: gliadin and glutenin. Gliadins are polymorphic monomeric protein subunits, which form disulfide bond bridges between glutenin high molecular weight subunits. Glutenin subunits (HMW-GS) are long-chain like polymer molecules that are responsible for the dough’s strength and elasticity (Goesaert et al., 2005). Glutenin protein molecular weights range between 500,000 to over 10 million and are thought to be the largest proteins reported in nature (Wrigley, 1996; Wieser et al., 2006).

The gluten network gives bread its characteristic volume, texture, tenderness, and softness. Gliadin is responsible for the cross-linking of the glutenin and adds stability and structure to the dough. These proteins are crucial in the formation of dough and bread quality (Ewart, 1972; Patient and Ainsworth, 1994; Khatkar, Bell & Schofield, 1995; Belton, 1999). Research has shown that the composition of glutenin plays an important role in the formation of the visco-elastic structure of the dough (Singh & MacRichie, 2001; Veraverbeke & Delcour, 2002; Dobraszcyk & Morganstern, 2003).

**Lipid**

The development of gluten network in breads is affected by lipids through coating individual protein strands. Shortening in bread acts as a plasticizer, lubricant, and increases dough rise, oven spring, and final volume (Smith & Johansson, 2004; Fu et al., 1997; Ghotra et al., 2002; Chin et al., 2010). Shortening acts as a tenderizer on the gluten proteins and results in a softer crumb by shortening the protein strands (Crowley, et al., 2000; Smith & Johansson, 2004). The shortened strands form a gluten network that incorporates more
gliadin per gliadin proteins, which has a direct impact on viscoelastic properties of bread (Wieser, 2007).

Lipids contribute to stability of the gas bubbles formed by aeration during kneading and CO₂ production by yeast. During dough expansion, fat crystals in shortening stabilize gas bubbles by migrating to the gas-liquid interface. This localization of lipid allows gas cells to expand without breaking, which increases volume and results in a fine crumb structure (Bell et al., 1977; Brooker, 1994). If too much lipid is incorporated in bread dough, excess lubrication diminishes the protein network formation and results in decreased loaf volume. A lipid content of about 3%-5% by weight is generally accepted as producing the greatest loaf volume (Stauffer, 1996a). In addition to their effects on leavening, fats serve to preserve freshness, and impart favorable mouth feel.

**Dough conditioner and additives**

Industrial use of dough conditioner is widespread (Barrett et al., 2002). The primary use of dough conditioners is to reduce loss of freshness in the form of staling resulting from storage by reducing amylose retrogradation (Szczodrak & Pomeranz, 1992). Amylose recrystallization was long thought to be primarily responsible for staling, but recent research has shown that retrogradation of amylose occurs rapidly during the cooling period after baking and is responsible for the initial loaf firming (Eliasson & Larsson, 1993; Zobel & Kulp, 1996; Gray & BeMiler, 2003). Research has shown that retrograded amyllopectin is the main cause for bread staling (Zobel & Kulp, 1996, Hug-Iten et al, 2003). Amylopectin retrogrades slowly over time during which the recrystalization of its branch-chains occur.

Emulsifiers or surfactants, for example mono and di-glycerides, are used for their ability to complex with starch and to be absorbed on the starch granule surface. Both starch-
inclusion complex formation and coating on the surface of starch granules increased moisture migration in the crumb due to the inability of the starch granule to absorb excess water released from gluten during baking (Pisesookbunterng & D’Appolonis, 1983). The use of mono- and diglycerides inhibits staling of bread during storage and is common in industry.

Dough additives are used in bread baking to improve functional characteristics and overcome undesirable attributes, such as color, off flavors, texture, and softness issues that result from processing, use of different flours, and addition of dietary fibers (Ravi et al., 1999). While colorants, flavorants, and flavor maskers are commonly used to increase the quality of the taste and appearance of breads, dough conditioners are most commonly used for their effects on texture and softness (Stampfli & Nersten, 1995; Ranhotra et al., 1995). These conditioners are further separated into different classes: strengtheners, softeners, and emulsifiers, and antistaling, oxidizing, and reducing agents (Tamstorf, 1983; Krog, 1984).

The use of dough strengtheners, oxidizers, and reducing agents to increase bread quality functionality is based primarily on their modification of the gluten network structure. Increases in thiol-disulfide bonds between glutenin and gliadin that make up the gluten structure, result in greater loaf volume, softness, and tenderness (Stampfli & Nersten, 1995). Thiol-disulfide bonds are affected by oxidizing and reducing agents and result in changes in the dough structural properties. Changes in the development of disulfide bonds or breaking the disulfide bonds of glutenin affect the functionality of the bread and lead to softer crumb and greater loaf volume (Fitchett & Frazier, 1986).

**Staling**

Storage of bread leads to staling, which imparts increased firmness, toughness of the crust, decreased flavor, and insoluble crystalline starch from the retrogradation of the
amylopectin. The effect of fats or shortening on bread volume and tenderness are well documented (Smith & Johansson, 2004). Shortening is known to decrease the development of firmness during staling (Rogers et al. 1988) by forming starch-lipid complexes, which slows crystallization of amylopectin branches.

Enzymes such as α-amylase are commonly used to decrease the staling effect of storage. There is debate regarding the exact mechanism that amylases play in retarding staling (Zobell & Senti, 1995; Bowles, 1996; Defloor & Delcour, 1999). These enzymes are thought to prevent the recrystallization of amyllose and amylopectin structures and thereby reduce retrogradation. Amylase partially hydrolyzes amylopectin and reduces the reassociation of the branch-chains that lead to a firming of the crumb structure. Amylases are thought to play a less important role in amyllose retrogradation than that of amylopectin. Retrogradation of amyllose during storage is proposed to be inhibited by amylases by reducing the development of crystalline structures occurring immediately after baking (Hug-Iten et al., 2001).

**Resistant Starch in Breads/ and Development of Healthy Breads**

The rapid digestibility of white bread has led to research in developing low glycemic index (GI) breads. GI is a method used to rank foods by the incremental blood glucose response after ingesting a given amount of carbohydrate (Jenkins et al., 1981). Current daily dietary-fiber recommendations for adequate intake (AI) are 14 g/1000 kcal; for men and women these are approximately 36 and 28 g/day, respectively (USDA, 2005). Current estimates of dietary fiber intakes for Americans are about half of AI levels. Dietary fibers are currently utilized in fiber-enriched breads although a focus on starch-based ingredients is becoming important to food manufacturers.
High amylose starch and dietary fiber have been investigated for its RS content and beneficial impacts on the GI of starchy foods, gut microbial profiles, satiety, obesity, cardiovascular disease and diabetes (Hoebler et al., 1999; Wang et al., 2002; Brennan, 2005). The development of retrograded amylose (RS3) in breads is known and current interest in adding high-amylose starch to promote RS content, maintain bread quality, and reduce starch hydrolysis rates are the focus of many studies (Liljeberg et al., 1995; Hung et al., 2005).

Research on RS in bread focuses on the formation of RS3 during baking and storage, which is influenced by baking time, baking temperature, storage time, and component of the flour (Asp et al., 1987; Eerlingen et al., 1994; Niba, 2003; Hung et al., 2005). Amylose in baked breads easily reassociates and develops into retrograded starch; thereby further increasing RS3 content during cooling and storage (Hung et al., 2005). Although RS3 is developed in cooled and stored breads, use of amylose-lipid complex, RS5, is less well known. Investigations conducted on RS5 in bread products have shown positive results in reducing starch-hydrolysis rates and GI response during human feeding studies (Hasjim et al., 2010).

The use of RS in breads presents formulation challenges (Korus et al., 2009). Texture, softness, gas cell size, and gluten network formation are issues that were identified when using RS (Wang et al., 2002; Hung et al., 2004). Different types of RS have been investigated on their effects on bread structure, and RS3 is the main starch currently used in bread formulations.

Reported RS contents has shown that most RS is sustainable after baking and leads to lower starch-hydrolysis rates (Eerlinger et al., 1994; Juarez-Garcia et al., 2006; Hasjim et al., 2005).
Studies have also demonstrated that added RS in bread decreased slightly after baking, thus, baking processes may slightly to moderately degrade RS (Eerlinger et al, 1994).

**Evaluation of Sensory Attributes**

**Sensory methodology**

Breads, wafers, and snack foods are types of foods that are tested by sensory methodology (Duizer et al., 1998; Gambano et al., 2004; Hardacre et al., 2006). The importance of sensory methods in determining consumer perceptions has been demonstrated in producing quality products, such as virgin olive oil, cheddar cheese, and breads (Bogue et al., 1999; Caporale et al., 2006; Gellynck et al., 2008). Breads are known to have specific quality attributes, which are important to consumers. These attributes include loaf volume, tenderness, flavor, firmness, low staling rate, and color. Sensory methods are utilized in the food industry to identify impacts of additives as functional ingredients in formulations (Shalini et al., 2007). Instrumental tests identify physical changes to a product resulting from changes in formulations, storage, and processing (Zhang & Moore, 1999; Flander et al, 2007; Meilgaard et al., 2007). Gambaro et al. (2002) demonstrated that instrumental and sensory measurements can be correlated, and, thus, instrumental analysis results may be used to determine food attributes. Furthermore, sensory panels may identify aspects of the formulation that consumers find acceptable or unsatisfactory (Zielinski et al 2008).

**Instrumental analysis of sensory attributes**

Instrumental methods commonly used to evaluate sensory attributes of breads include: texture profile analysis (TPA), colorimetry, and rapeseed volume displacement (Wang et al., 2002; Wang, et al., 2007). These methods yield quantitative results that may be compared with those generated by a human sensory panel (Brady & Mayer, 1985). Texture
profile analysis (TPA) measures hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience, which are related to sensory attributes (Gambaro et al., 2002; Meilgaard et al., 2007). Hunter colorimetry measures the color of a sample: \( L^* a^* b^* \) values, where \( L^* \) represents whiteness (value 100) or blackness (value 0), \( a^* \) represents red (+a) or green (−a), and \( b^* \) represents yellow (+b) or blue (−b). Rapeseed displacement tests give a precise measurement of bread loaf volume.

Investigation of non-digestible components (dietary fiber) in bread on loaf volume, TPA parameters, and quality attributes (aroma, flavor, crumb softness) showed that some dietary fibers resulted in better consumer acceptability (Wang, 2002), whereas other dietary fibers decreased quality attributes of bread (Sangnark et al., 2004). Wang (2002) investigated the effect of different commercial dietary fibers (3%) on the visco-elastic, proofing, and baking quality attributes when added to bread. Sangnark et al. (2004) investigated effects of sugarcane bagasse, a type of dietary fiber, at increased concentrations on bread quality. Sucrose esters, used as an emulsifier, were then added to improve quality attributes. TA-XT2 parameters of stickiness, firmness, and springiness, and rapeseed displacement measurement for loaf volume evaluated for increased dietary fiber contents (0 - 15 g / 100 g wheat flour) showed reduced loaf volume, firmness, and springiness (Sangnark et al., 2004). Masoodi & Chauhan (1998) also demonstrated that supplemented apple pomace used as a dietary fiber resulted in a decrease in bread volume.

**Sensory evaluation**

Sensory evaluations are conducted by sensory panelists to identify attributes of products, which is of importance to manufacturers. These tests can be divided into trained and untrained methods depending on the desired outcome of the product developers (Murray et
Trained panelists are presented with reference materials to define the expected range of the sensation of an attribute and to evaluate the intensity of specific attributes to better identify sensory changes in a product. The panelist’s objective is to give detailed, specific information on the attributes using a highly developed perception range. Untrained panelists lack the training to identify specific attributes, such as flavors or aromas. Just About Right (JAR), acceptance, and descriptive analysis tests are a few of the methods used to determine human perception of a product (Meilgaard et al., 2007). Each test provides specific evidence and knowledge about how consumers identify a perceived change in product formulation. These tests are used to determine how well a formulation matches a competitor’s product or a consumer’s perception of a reformulation of an existing product.

Just About Right (JAR) tests identify whether the level of the intensity of an attribute is above at or below an optimum amount (Lawless and Hayman, 1998). JAR uses a 5-7 point scale, although usually only a 5 point scale is used with scales labeled “Much too little” to “Much too much” with “Just about right” in the middle (Anon, 2003). JAR has been used to optimize texture, flavor, appearance, and acceptability attributes in formulations of baked products such as bread and muffins (Bordi et al., 2001; Charoenthaikij et al., 2010).

Acceptance tests yield data on consumer liking of a product. Panelist’s responses are recorded using a 7 to 9 point hedonic scale on a product's attribute and overall acceptability. Acceptance tests are usually based on a scale with extremes labeled “dislike very much” and “like very much” (Kihlberg et al., 2005). Lazaridou et al. (2007) reported that acceptance tests showed positive responses when hydrocolloids were used in gluten-free breads. Acceptance tests have been used to evaluate bread freshness and influences of descriptive sensory attributes on consumer judgment (Heenan et al., 2008).
Descriptive analysis methods “involve the detection and descriptions of both qualitative and quantitative sensory aspects of a product” (Meilgaard et al., 2007). These methods describe specific attributes, such as color, aroma, appearance, taste, flavor, and texture of a product evaluated by a trained panel (Schwartz 1975).

Reference


CHAPTER 2. EFFECTS OF COOKING METHODS AND STARCH STRUCTURES ON STARCH HYDROLYSIS RATES OF RICE

A paper to be submitted to the Journal of Food Science

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Abstract

This study aimed to understand the effects of different cooking methods using rice, steamed, pilaf, and traditional stir-fried, on starch-hydrolysis rates. Rice grains of three varieties, japonica, indica and waxy, were used for the study. Rice starch was isolated from the grain and characterized. Apparent amylose contents of starches from japonica, indica, and waxy rice were 13.2\%, 18.0\%, and 0.9\%, respectively. The onset gelatinization-temperature of indica starch (77.5°C) was higher than that of the japonica and waxy starch (55.6°C and 56.3°C, respectively). The difference was attributed to longer amylpectin branch-chains of the indica starch. After cooking each rice variety using different methods, starch-hydrolysis rates and resistant-starch (RS) contents of the rice differed. Stir-fried rice displayed the slowest starch-hydrolysis rate followed by pilaf rice and steamed rice for each rice variety. RS contents of freshly steamed japonica, indica and waxy rice were 0.0\%, 6.6\%, and 0.0\%, respectively; that of rice pilaf were 12.2\%, 13.1\%, and 3.4\%, respectively; and the stir-fried rice displayed the largest RS contents of 15.9\%, 16.6\%, and 12.2\%, respectively.
Mechanisms of the large RS contents of the stir-fried rice were investigated. RS contents of stir-fried rice was positively correlated with amylose contents of starch \( r = 0.96, p < 0.002 \).

With the least starch-hydrolysis rate and the largest RS content, stir-fried rice would be a desirable way of preparing rice to reduce postprandial blood glucose and insulin response and improve colon health.

**Key words:** hydrolysis, rice, starch, cooking, resistant starch

**Practical Application**

After rice was cooked using different methods, (steamed, pilaf, and stir fried) the stir-fried indica rice, prepared by stir-frying steamed rice that was held at 4°C for 24 h, displayed the slowest starch-hydrolysis rate and the largest resistant-starch content. These results showed that cold-storage of steamed normal rice at 4°C for 24 h followed by stir-frying with corn oil (3 min) reduced the rate of starch-hydrolysis and increased the RS content. Ingesting stir-fried rice, therefore, could reduce the postprandial blood glucose concentration and insulin response, which could benefit the health of diabetics and prediabetics. The large RS content of the stir-fried normal rice could also provide health benefits to the colon.

**Introduction**

Rice is an important staple worldwide, and food preparations of rice primarily involve steaming and sometimes followed by stir-frying or preparing rice pilaf. Rice starch is known to be hydrolyzed fast by amylases after ingesting and is considered as a high-glycemic-index food (Frei and others 2003). With increasing concerns over diabetes development worldwide, there is a pressing need to find methods of cooking rice to reduce the hydrolysis rate of starch after ingesting rice. Starch in steamed rice is readily hydrolyzed by salivary and pancreatic \( \alpha-\)
Amylase in the digestive tract, resulting in a high postprandial blood-glucose concentration (Juliano and others 1986). This is detrimental to populations who need to control the blood-glucose level, such as diabetics and prediabetics. Human feeding studies have been conducted on foods of the same carbohydrate load but prepared by different cooking methods, such as, french fries and boiled potatoes. Glycemic responses of the subjects that consumed french fries were significantly lower than the subjects that consumed boiled potatoes, and the results could not be explained by the fat content of the foods (Leeman and others 2008).

Amylose and amylopectin are the two major polysaccharides of starch, and their proportions and structures determine the gelatinization, pasting, and enzymatic-hydrolysis properties of the starch (Lu and others 1997; Kim and others 2004; Jane 2006). Amylose is a primarily linear molecule that comprises α-1,4 linked glucose chains and a few α-1,6 linkages. Amylopectin is a highly branched molecule consisting mainly of α-1,4 linked glucose chains and about 5% α-1,6 linked branches. Enzymatic-hydrolysis of starch is affected by the amylose content and the interactions of starch with proteins and cellulosic material in food (Goni and others 1997; Jane and others 2006). Slowly digestible starch in food can provide a steady blood-glucose concentration after ingesting the food without causing hyper- and hypo-glycemic and insulinemic responses (O'Dea and others 1981; Hasjim and others 2010).

Resistant starch (RS) is defined as a portion of starch that is resistant to enzymatic-hydrolysis in the small intestine (SI) but is fermentable by microflora in the colon (Englyst and Macfarlane 1986; Englyst and Cummings 1987). RS has been reported for its health benefits of preventing colon cancer, hyperglycemia, hyperinsulinemia, diabetes, and obesity.
There are five types of RS, physically inaccessible starch (type 1 resistant starch, RS1), the B- and C-type crystalline starch with native or uncooked starch granules (RS2), retrograded amylose (RS3), chemically cross-linked or modified starch (RS4), and amylose-lipid complex (RS5) (Englyst and others 1992; Eerlingen and others 1994; Hasjim and others 2010).

Japonica, indica, and waxy rice are three commonly used rice grains for food. These varieties have different physical properties, including gelatinization and pasting properties. The japonica rice has a lower onset gelatinization-temperature than the indica rice, resulting from its shorter amylopectin branch-chain lengths (Okuda and others 2005). The starch synthase IIa (SSIIa), responsible for elongation of amylopectin branch-chains from DP ≤ 11 to DP 12-25, is missing in the japonica rice (Umemoto and others 1999, 2002). The SSIIa gene, however, is present in the indica rice, resulting in longer branch-chain length and a higher gelatinization-temperature (Umemoto and others 2002). Waxy rice is missing the granular-bound starch synthase 1 (GBSSI) gene, which is responsible for the biosynthesis of amylose (Sano 1984). Thus, waxy rice does not have amylose.

Steamed, pilaf, and stir-fried rice are the three most common ways of preparing rice for human consumption. While much research has been conducted on starch-hydrolysis rates of boiled rice flour and steamed rice, effects of different cooking methods on starch-hydrolysis rates of cooked rice and their mechanisms have not been thoroughly studied and reported. The objectives of this study were to understand effects of the cooking methods, steamed, pilaf, and stir-fried, and starch structures of japonica, indica, and waxy rice on starch-hydrolysis rates in cooked rice. The results of this study will enable us to understand
effects of the cooking methods of foods on the properties of starch and, in turn, the glycemic responses of humans after ingesting the foods.

**Materials and Methods**

**Materials**

Two normal rice varieties, japonica (Nomura and Company, Burlingame, CA) and indica (Riceland, Stuttgart, AK), one waxy rice variety (Oriental Mascot Brand, CA), and the corn oil used for the study were purchased from a local grocery store. Amyloglucosidase from *Aspergillus niger* (200 U/mL), porcine pancreatic α-amylase (PPA), and porcine pancreatin were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. The Total Starch Assay Kit (AA/AMG) and D-Glucose assay kit (glucose oxidase/peroxide, GOPOD) were purchased from Megazyme International Ireland Ltd. Co. (Wicklow, Ireland).

**Grinding and Composition of Rice Grains**

Rice grains were ground using a cyclone mill (UDY Corp., Fort Collins, CO) with a sieve of 0.5-mm opening, and the ground rice was used for compositional analysis and starch-hydrolysis studies. Starch content was determined using the Total Starch Assay Kit (AA/AMG, Megazyme, Co. Wicklow, Ireland) following the procedure provided by the manufacturer. Lipid content was determined using the Goldfisch Fat Extractors (Labconco Corp., Kansas City, MO) with hexanes following the AACC Method 30-25. Protein content was determined using a CN Analyzer (Vario MAX , Elementar 107 Analysensysteme, Hanau, Germany) and calculated by multiplying the nitrogen content with a conversion factor of 5.95 (AACC, 2000). The above analyses were performed in duplicate.

**Starch Isolation**
Rice starch was isolated from rice grains by wet milling, following the method of Yang and others (1984) with modifications. Rice grains (~50 g) were soaked in a sodium hydroxide solution (NaOH, 0.05%, w/w) at room temperature for 24 h. Rice grains were then ground using a blender (Osterizer 14 speed blender, US), and the slurry was filtered through a filter cloth with openings of 53-µm. Rice starch precipitated was collected and resuspended in a sodium chloride solution (0.1 M, 450 mL) with 50 mL toluene and stirred for 1 h to remove protein and lipids. This treatment was repeated until the toluene layer became clear and contained no protein. The purified starch was washed three times with water, twice with absolute ethanol, and dried at 37°C for 48 h.

**Amylose Content of Rice Starch**

The amylose content of rice starch was determined using an iodine potentiometric method (Yoo and Jane 2000). Starch (0.1 mg) was defatted and dispersed in 90% DMSO (10 mL). Amylose content was calculated by dividing the iodine affinity of the starch by 0.20 (Takeda and others 1987). The analysis was done in duplicate.

**Thermal Properties of Starch**

Thermal properties of isolated starch were determined in triplicate following the procedure reported by Ai and others (2011). Starch samples (~2.5 mg, db, precisely weighed) with (3X) water were heated at 10°C/min from 10 to 110°C in a sealed aluminum pan after being equilibrated at 25°C for 2 h. An empty pan was used as the reference. Onset, peak, and conclusion temperatures (T_o, T_p, and T_c, respectively) and enthalpy-changes (ΔH J/g) of the endotherm were calculated using the Pyrus Software (Perkin-Elmer, Norwalk, CT). Retrograded starch was prepared by storing the gelatinized starch in the DSC pan at 4°C for 7 days. Thermal properties of the retrograded starch were analyzed using the same parameters.
and the percentage retrogradation (R%) was calculated as follows: \( R\% = 100\% \times (\Delta H \text{ of dissociation of retrograded starch})/(\Delta H \text{ of starch gelatinization}) \).

**Pasting Properties of Starch**

Pasting properties of isolated starch were analyzed in duplicate using a Rapid Visco-Analyser (RVA, RVA-4, Newport Scientific, Sidney, Australia). A starch suspension (8%, w/w, db; 28 g of total weight) was equilibrated at 50°C for 1 min, heated at a rate of 6°C/min to 95°C, maintained at 95°C for 5 min, and then cooled to 50°C at a rate of 6°C/min. The paddle-rotating speed was 960 rpm for the first 10 s followed by 160 rpm for the remainder of the analysis (Ao and Jane 2007).

**Branch Chain-Length Distribution of Amylopectin**

Amylopectin was separated from amylose using sepharose Cl-2B gel-permeation chromatography (Li and others 2008). Purified amylopectin was collected and then debranched using isoamylase. Debranched amylopectin chains were labeled with 8-amino-1,3,6-pyrenetrisulfonic acid, and the branch-chain-length (BCL) distribution was analyzed using a fluorophore-assisted capillary electrophoresis (P/ACEMDQ, Beckman Courter, Fullerton, CA) (Hasjim and others 2009).

**Cooking Methods of Rice Grains**

Steamed rice was prepared by cooking rice grains (100 g) in water (250 g for japonica and indica, and 200 g for waxy rice) using a rice cooker (Aroma Rice Cooker, model: ARC – 914SB, San Diego, CA, 2011). Rice was boiled for 20 min using the cooking setting of the rice cooker and held at the warming setting for 12 min. Stir-fried rice was prepared using the steamed rice after being held at 4°C for 24 h (cold-stored). The cold-stored steamed rice was then stir-fried in a pan with corn oil (10%, db) for 3 min.
Pilaf rice was prepared by pre-cooking rice grains (100 g) with corn oil (10%, db) to coat the surface of the rice grains for 2 min. Water (200 g) was added to the oil pre-cooked rice. The mixture in the pan was covered, boiled on a stove, and then placed in an oven at 350°F for 18 min or until water was absorbed. After removing from the oven, the rice was allowed to sit, with lid covered, for 10 min (Conway, 1991).

Step-wise cooking methods were conducted to understand the mechanism of changing starch-hydrolysis rates of the stir-fried rice. To test the effect of cold-storage, the streamed rice after being cold-stored at 4°C for 24 h was stir-cooked in a pan for 3 min without adding corn oil. To test the effect of oil, freshly steamed rice, without prior cold-storage was stir-fried in a pan with corn oil (10%, db) for 3 min.

**Enzymatic-Hydrolysis Rate of Starch in Cooked Rice Grains and Ground Rice Powders**

Starch-hydrolysis rates of cooked ground rice and rice grains prepared using different cooking methods were analyzed in duplicate following the method of Ai and others (2012) with modifications. Ground rice (containing 300 mg starch, db) was suspended in deionized water (15.0 mL), and cooked in boiling water for 10 min. The samples were equilibrated in a water bath at 37°C with shaking (80 rpm) for 30 min, and PPA (32 units), in a phosphate buffer solution (5.0 mL, 0.40 M, pH 6.9, containing 0.25 mM CaCl$_2$, and 0.02% w/v NaN$_3$), was added to the rice sample to hydrolyze starch for 30, 60, and 120 min. The supernatant (0.4 mL) was collected at each time interval and was further hydrolyzed to glucose using glucoamylase. The concentration of glucose was quantified using a GOPOD method (Setiawan and others 2010).

Cooked rice grains (containing 300 mg starch, db) were transferred to a 50-mL tube with a phosphate buffer solution (15 mL, 0.10 M, pH 6.9, containing 0.25 mM CaCl$_2$,
and 0.02% w/v NaN₃) and homogenized using a homogenizer (T25 Digital Ultra-Turrex®, IKA® Works Inc., Wilmington, NC) for 20 s. The same starch-hydrolysis procedures described above was used for the cooked rice grains. The percentage starch-hydrolysis was calculated using the equation: % starch-hydrolysis = 100% × total mass of glucose released/initial dry mass of starch × (162/180).

**Resistant Starch Content**

Proportions of rapidly digested starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) contents of the cooked rice samples were analyzed following the method of Englyst and others (1992) with modification as described by Li and others (2008). Cooked rice (containing 1.0 g starch, db) was homogenized for 20 s in a sodium acetate buffer (20 mL, 0.1 M, pH 5.2). The analysis was done in duplicate.

**Statistical Analysis**

Mean values were analyzed using Analysis of Variance (ANOVA). The analysis was in SAS version 9.2 (SAS Institute, Inc., Cary, NC). Pearson correlation coefficients were calculated using Microsoft Excel Version 12.0 to determine associations between amylose content and RS content. Differences were evaluated by t-test using Tukey’s adjustment. The significance level was set at \( p < 0.05 \).

**Results and Discussion**

**Compositions of Rice Grains and Structures of Starches**

Rice grain compositions and starch structures of rice varieties are summarized in Table 1. Starch contents of the rice grains ranged from 80.1% (waxy rice) to 83.7% (japonica rice) for the varieties. Lipid contents of the grains varied between 0.6% and 0.9%, and protein contents ranged from 5.4% to 6.9% (Table 2.1). For normal rice varieties, the
indica rice starch had a larger amylose-content (18.0%) than the japonica rice starch (13.6%). The waxy starch, however, had little amylose (0.9%).

Branch-chain-length (BCL) distributions of amylopectin are summarized in Table 2.1. The japonica rice and waxy rice showed larger proportions of short branch-chains with DP 6-12 (37.7% and 38.3%, respectively) and smaller proportions of branch chains with DP 13-24 (42.4% and 44.9%, respectively) than indica rice (20.9% and 58.4%, respectively). The average amylopectin BCL of the indica starch (DP 21.3) was longer than that of the japonica and waxy starch (DP 19.4 and 18.4, respectively). The large proportion of the short branch-chains with DP 6-12 of the waxy rice starch indicated that the waxy starch had a japonica rice background, lacking the SSIIa gene. These results were in agreement with literature results that amylopectin of indica rice displayed longer average BCL than that of japonica rice (Lu and others 1997).

**Thermal Properties of Isolated Starch**

Thermal properties of japonica, indica, and waxy rice starches are shown in Table 2.2. The indica rice starch displayed a higher gelatinization-temperature ($T_o$, 71.6°C) than the japonica and waxy rice starch (55.6°C and 56.8°C, respectively). The gelatinization enthalpy-change of the indica starch was 13.8 J/g, which was larger than that of the japonica and waxy rice (12.0 J/g and 13.3 J/g, respectively). The difference in the enthalpy-change between the indica and japonica starch were attributed to the larger proportion of branch-chains with DP 13-24 of the indica rice starch, which formed stable crystalline structures and displayed higher gelatinization-temperature and a larger enthalpy-change (Vandeputte and others 2003a). The indica rice starch also displayed a substantially greater percentage-retrogradation (52.9%) than the japonica (29.1%) and waxy (20.0%) rice starch after being stored at 4°C for
7 days. These differences could be explained by the larger amylose-content and fewer short-branch-chains (DP 6-12) of the indica rice starch. The thermograms of japonica and indica starches showed an amylose-lipid dissociation peak (96°C-105°C), whereas that of the waxy rice starch showed no such a peak at the temperature range because of lacking amylose. The enthalpy-change (0.4 J/g) of the amylose-lipid dissociation peak of the indica starch was larger than that of the japonica starch (0.3 J/g), which agreed with the results of greater amylose-content of the indica starch than the indica starch.

**Pasting Properties of Isolated Starch**

Pasting profiles of rice starches analyzed using an RVA are shown in Figure 2.1, and the results are summarized in Table 2.3. The waxy rice starch displayed the lowest pasting temperature (63.7°C), the greatest peak viscosity (215.5 RVU) and breakdown viscosity (150.3 RVU), but the least setback viscosity (22.4 RVU), which were attributed to the low amylose content of the starch (0.9%). The japonica rice starch displayed lower peak viscosity (130.6 RVU) than the indica starch (159.3 RVU), which could be attributed to the short amylopectin BCL of the japonica starch (Jane and others 1999). It is known that amylopectin is primarily responsible for the swelling power and viscosity of the starch. The setback viscosity correlates with the content of amylose that develops a network and increases the viscosity upon cooling (Singh and others 2006).

**Enzymatic-Hydrolysis Rate of Starch in Cooked Ground Rice**

The percentage starch-hydrolysis of cooked ground waxy rice (58.1%) using PPA for 30 min was greater than that of the japonica and indica rice counterparts (48.3% and 52.9%, respectively). This resulted from the lack of amylose and greater swelling and viscosity of the waxy rice starch (Figure 1.1), which was the most susceptible to the enzyme-hydrolysis.
Although the indica rice had the largest amylose-content (18.0%) and protein content (6.9%) (Table 2.1), it displayed a significantly greater percentage starch-hydrolysis ($p < 0.05$) than the japonica rice (Figure 2.2). This was contrary to literature results showing that rice starch with larger amylose content displayed a slower starch-hydrolysis rate (Okuda and others 2005). The greater percentage starch-hydrolysis of the indica rice could be a result of its higher viscosity (peak viscosity, 159.3 RVU) than the japonica rice starch (130.6 RVU) and a lower pasting temperature (79.5°C) than the japonica rice starch (83.1°C) (Figure 2.1). Because the indica rice starch was swollen to a greater extent (Table 2.3), the starch was more susceptible to enzyme-hydrolysis.

**Enzymatic-Hydrolysis Rate of Starch in Cooked Rice Grains**

Starch-hydrolysis rates of rice grains cooked using different methods are shown in Figure 2.3. The stir-fried rice displayed the slowest starch-hydrolysis rates (42.3%, 39.9%, and 49.4% for japonica, indica, and waxy rice varieties, respectively) after incubation with PPA for 30 min compared with the pilaf rice (45.7%, 47.9%, and 52.9%, respectively) and the steamed rice (62.2%, 55.1%, and 57.8%, respectively). The slow hydrolysis rates of the stir-fried rice were in agreement with the lower glycemic responses after ingesting French fries (GI = 54) than boiled potatoes (GI = 78) (Leeman and others 2008). Stir-fried indica rice also showed a significantly slower starch-hydrolysis rate ($p < 0.05$) than the stir-fried japonica and waxy rice. The slower starch-hydrolysis rates of the stir-fried and pilaf rice compared with that of the steamed rice suggested that the amylose-lipid complex formation (Type 5 resistant starch) (Ai and others, 2013) and lipid coating on the surface of the rice starch granules (Type 1 resistant starch), resulted from cooking rice with lipids, and this may protect starch from enzyme-hydrolysis.
To understand the mechanism of the slow hydrolysis-rate of starch in the stir-fried rice, we conducted step-wise studies to reveal the effects of the cold-storage and stir-frying with oil on the rate of starch-hydrolysis. Starch-hydrolysis rates of steamed rice varieties followed by stir-cooking with and without cold-storage and oil are shown in Table 2.4. Stir-frying freshly steamed rice without cold-storage showed rates of starch-hydrolysis slower than the steamed rice but faster than the stir-fried rice. The effects of cold-storage of steamed rice on the reduction in the starch-hydrolysis rate of stir-fried rice were more pronounced in the indica and japonica varieties than in the waxy variety. These results agreed with the concept that cold-storage of gelatinized normal rice starches displayed greater extents of retrogradation than the waxy rice starch (Table 2.2). Retrograded starch is known to be resistant to enzymatic-hydrolysis (Type 3 resistant starch) (Englyst and Cummings 1987, Eerlingen and others 1994).

Japonica and indica rice after being steamed, cold-stored for 24 h, and stir-cooked without adding corn oil showed starch-hydrolysis rates (at 30 min) of 52.6% and 50.4%, respectively, which were faster than the stir-fried rice (38.2% and 42.3%, respectively) but slower than the steamed rice (62.2% and 55.1%, respectively). The waxy rice showed less impacts by stir-frying with oil than the normal rice of japonica and indica, confirming the lack of an amylose-lipid complex formation (RS5) (Ai and others 2013).

Resistant Starch Content

The RDS, SDS, and RS contents of different rice varieties cooked using different methods are shown in Table 2.5. Among steamed rice, indica rice was the only variety to have RS (6.6%), which was attributed to its larger amylose content (18%). Preparation of the pilaf rice method displayed RS contents of 12.2%, 13.1%, and 3.4%, for japonica, indica and
waxy rice respectively. Stir-frying the steamed rice, without cold-storage increased RS contents to 4.5%, 7.2%, and 4.3%, respectively. After cold-storage of the steamed rice at 4°C for 24 h followed by stir-cooking for 3 min without corn oil, RS contents of japonica, indica, and waxy rice increased to 13.6%, 12.2%, and 7.9%, respectively. Stir-frying the cold-stored rice with corn oil further increased RS contents to 15.9%, 16.6%, and 12.1%, respectively. Stir-fried indica and japonica rice showed significantly less RDS ($p < 0.05$) than the waxy counterpart. Stir-fried rice of all varieties displayed significantly larger RS contents ($p < 0.05$) than steamed and pilaf rice. The larger RS contents of pilaf rice compared with steamed rice suggest the formations of amyllose-lipid complexes formed during cooking.

The largest RS content of the stir-fried indica rice (16.6%) was a result of the greatest amyllose content of the indica rice starch, which developed the most retrograded starch after cold-storage and formed an amyllose-lipid complex after stir-frying with oil. The amyllose content of the starch positively correlated with the RS content of the stir-fried rice ($r = 0.96$, $p < 0.002$). Different cooking methods demonstrated impacts on the starch-hydrolysis rate, and the RDS, SDS, and RS contents (Rashmi and Urool 2003).

**Conclusions**

Results of the present study clearly showed that stir-fried rice displayed the slowest starch-hydrolysis rates compared with steamed and pilaf rice. The differences were attributed to the formation of retrograded starch after cold-storage, development of amyllose-lipid complexes, and lipid coating of the starch after stir-frying with corn oil. Indica rice displayed the greatest levels of RS contents because of its largest amyllose contents. The slower starch-hydrolysis rate and larger RS contents of stir-fried rice make it a better choice to maintain a stable postprandial blood glucose level and prevent hyper-and hypoglycemic responses.
Acknowledgments

The authors thank the Plant Science Institute, Iowa State University, for the funding support for this project. The authors thank Ignacio Alvarez for assistance in statistical analysis and Jovin Hasjim for discussions and providing encouragements.

References


Table 2.1. Rice grain composition and amylose content of the starch$^1$

<table>
<thead>
<tr>
<th>Variety</th>
<th>Starch</th>
<th>Lipid</th>
<th>Protein</th>
<th>Amylose</th>
<th>Branch-chain length distribution of amylopectin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DP 6-12</td>
</tr>
<tr>
<td>Japonica</td>
<td>83.7±0.8$^a$</td>
<td>0.6±0.0$^a$</td>
<td>5.7±0.1$^b$</td>
<td>13.6±0.2$^b$</td>
<td>37.7±0.4$^a$</td>
</tr>
<tr>
<td>Indica</td>
<td>82.7±0.3$^a$</td>
<td>0.6±0.0$^a$</td>
<td>6.9±0.0$^a$</td>
<td>18.0±0.3$^a$</td>
<td>20.9±1.2$^b$</td>
</tr>
<tr>
<td>Waxy</td>
<td>80.1±0.8$^b$</td>
<td>0.9±0.0$^b$</td>
<td>5.4±0.0$^c$</td>
<td>0.9±0.1$^c$</td>
<td>38.3±1.4$^a$</td>
</tr>
</tbody>
</table>

$^1$ Means ± standard deviations. Values with the same letter in a column are not significantly different at $p < 0.05$.

$^2$ CL = chain length; DP = degree of polymerization
Table 2.2. Thermal properties for isolated starch

<table>
<thead>
<tr>
<th>Variety</th>
<th>Gelatinization of starch</th>
<th>Amylose-lipid dissociation</th>
<th>Dissociation of retrograded starch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_o$ (°C)</td>
<td>$T_p$ (°C)</td>
<td>$T_c$ (°C)</td>
</tr>
<tr>
<td>Japonic</td>
<td>55.6±0.1</td>
<td>63.2±0.1</td>
<td>70.0±0.0</td>
</tr>
<tr>
<td>Indica</td>
<td>71.6±0.1</td>
<td>77.5±0.0</td>
<td>83.3±0.1</td>
</tr>
<tr>
<td>Waxy</td>
<td>56.8±0.1</td>
<td>65.1±0.0</td>
<td>74.6±0.1</td>
</tr>
</tbody>
</table>

1 Means ± standard deviations. $T_o$ = onset temperature, $T_p$ = peak temperature, $T_c$ = conclusion temperature, and ΔH = enthalpy change, and R (%) = percentage of retrogradation.
Table 2.3 Pasting profiles of isolated rice starches (8% w/w, db)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Peak</th>
<th>Trough</th>
<th>Breakdown&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Final</th>
<th>Setback&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pasting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japonica</td>
<td>130.6</td>
<td>83.1</td>
<td>47.5</td>
<td>163.0</td>
<td>79.9</td>
<td>83.1</td>
</tr>
<tr>
<td>Indica</td>
<td>159.3</td>
<td>95.4</td>
<td>63.8</td>
<td>173.5</td>
<td>78.1</td>
<td>79.5</td>
</tr>
<tr>
<td>Waxy</td>
<td>215.5</td>
<td>65.3</td>
<td>150.3</td>
<td>87.7</td>
<td>22.4</td>
<td>63.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Breakdown is the difference between peak viscosity and trough.

<sup>b</sup>Setback is the difference final viscosity and trough.
<table>
<thead>
<tr>
<th>Variety</th>
<th>Cooking Method</th>
<th>Time Point (min)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>60</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Japonica</td>
<td>Steamed</td>
<td>62.2±0.6</td>
<td>69.3±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.6±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stir-frying, (oil, no cold-storage)</td>
<td>50.1±0.1</td>
<td>60.9±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.9±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stir-cooking, (cold-stored, no oil)</td>
<td>52.6±0.9</td>
<td>64.7±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.1±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stir-fried (cold-stored, oil)</td>
<td>38.2±0.5</td>
<td>53.9±0.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>61.7±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Indica</td>
<td>Steamed</td>
<td>55.1±1.0</td>
<td>68.9±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.3±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stir-frying, (oil, no cold-storage)</td>
<td>49.6±0.4</td>
<td>60.7±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>67.6±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stir-cooking, (cold-stored, no oil)</td>
<td>50.4±0.6</td>
<td>66.3±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.6±0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stir-fried (cold-stored, oil)</td>
<td>42.3±0.3</td>
<td>55.1±0.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>64.4±1.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Waxy</td>
<td>Steamed</td>
<td>57.8±1.0</td>
<td>67.5±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.7±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stir-frying, (oil, no cold-storage)</td>
<td>56.5±0.7</td>
<td>66.0±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.2±0.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stir-cooking, (cold-stored, no oil)</td>
<td>53.3±0.2</td>
<td>68.7±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.6±0.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stir-fried (cold-stored, oil)</td>
<td>49.4±0.6</td>
<td>58.5±0.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.2±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Means ± standard deviations. Values with the same letter in a column are not significantly different at $p < 0.05$. 
Table 2.5 Rapid digestible (RDS), slowly digestible (SDS) and resistant starch (RS) of rice grains cooked using different methods\textsuperscript{1}

<table>
<thead>
<tr>
<th>Variety</th>
<th>Cooking method</th>
<th>RDS (%)</th>
<th>SDS (%)</th>
<th>RS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japonica</td>
<td>Steamed</td>
<td>84.8±1.8\textsuperscript{b}</td>
<td>16.5±0.1\textsuperscript{a}</td>
<td>0.0±1.5\textsuperscript{c}</td>
</tr>
<tr>
<td></td>
<td>Stir-frying, (oil, no cold-storage)</td>
<td>88.8±0.7\textsuperscript{a}</td>
<td>6.8±0.3\textsuperscript{b}</td>
<td>4.5±0.4\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Stir-cooking, (cold-stored, no oil)</td>
<td>81.2±1.2\textsuperscript{c}</td>
<td>5.2±0.6\textsuperscript{b}</td>
<td>13.6±0.5\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Stir-fried (cold-stored, oil)</td>
<td>75.9±1.1\textsuperscript{c}</td>
<td>8.2±0.7\textsuperscript{b}</td>
<td>15.9±0.4\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Pilaf</td>
<td>80.0±0.9\textsuperscript{c}</td>
<td>7.9±1.4\textsuperscript{b}</td>
<td>12.2±0.5\textsuperscript{a}</td>
</tr>
<tr>
<td>Indica</td>
<td>Steamed</td>
<td>85.4±0.7\textsuperscript{c}</td>
<td>8.1±1.1\textsuperscript{b}</td>
<td>6.6±0.3\textsuperscript{c}</td>
</tr>
<tr>
<td></td>
<td>Stir-frying, (oil, no cold-storage)</td>
<td>84.0±1.0\textsuperscript{b}</td>
<td>8.8±0.9\textsuperscript{b}</td>
<td>7.2±0.1\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Stir-cooking, (cold-stored, no oil)</td>
<td>70.2±0.6\textsuperscript{a}</td>
<td>17.7±0.7\textsuperscript{a}</td>
<td>12.2±0.3\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Stir-fried (cold-stored, oil)</td>
<td>66.8±0.8\textsuperscript{a}</td>
<td>16.5±1.7\textsuperscript{a}</td>
<td>16.6±1.0\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Pilaf</td>
<td>85.4±2.0\textsuperscript{c}</td>
<td>1.6±0.3\textsuperscript{c}</td>
<td>13.1±1.7\textsuperscript{a}</td>
</tr>
<tr>
<td>Waxy</td>
<td>Steamed</td>
<td>86.0±0.1\textsuperscript{a}</td>
<td>14.3±1.1\textsuperscript{a}</td>
<td>0.0±1.1\textsuperscript{d}</td>
</tr>
<tr>
<td></td>
<td>Stir-frying, (oil, no cold-storage)</td>
<td>90.7±0.3\textsuperscript{b}</td>
<td>4.9±0.6\textsuperscript{b}</td>
<td>4.3±0.3\textsuperscript{c}</td>
</tr>
<tr>
<td></td>
<td>Stir-cooking, (cold-stored, no oil)</td>
<td>83.5±1.1\textsuperscript{a}</td>
<td>8.6±1.9\textsuperscript{a}</td>
<td>7.9±0.8\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Stir-fried (cold-stored, oil)</td>
<td>80.2±0.2\textsuperscript{a}</td>
<td>7.7±0.6\textsuperscript{a}</td>
<td>12.1±0.4\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Pilaf</td>
<td>86.1±0.0\textsuperscript{a}</td>
<td>10.4±0.1\textsuperscript{a}</td>
<td>3.4±0.1\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}RDS = rapid digestible starch, SDS = slow digestible starch, and RS = resistant starch.

Means ± standard deviations. Values with the same letter in a column are not significantly different at \( p < 0.05 \).
Figure 2.1. Pasting profiles of isolated starches (8%, w/w, db, 28 g total weight) using a rapid Visco-Analyzer (RVA).
Figure 2.2 Starch hydrolysis profiles of cooked ground rice samples using porcine pancreatic α-amylase. The reactions were conducted in a shaker water-bath at 37°C and 80 RPM.
Figure 2.3 Starch hydrolysis profiles of cooked rice using different methods. Different rice varieties were used for the study (A) japonica, (B) indica, and (C) waxy rice grains.
CHAPTER 3. APPLICATION OF A NOVEL RESISTANT STARCH IN BREAD: EFFECTS ON COMPOSITION, STRUCTURE, AND SENSORY ATTRIBUTES

A paper to be submitted to Cereal Chemistry

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Abstract

The objectives of this study were to determine the effects of a recently developed type-5 amylose-lipid resistant starch (RS5) on the composition, structure, and consumer acceptability of white bread. Breads were substituted with RS5 at different concentrations: 0%, 20% (RN-20), 30% (RN-30), 40% (RN-40), and 50% (RN-50). Vital wheat gluten (V WG) was added to treatments with RS5 to standardize total protein content. Resistant starch (RS) contents of breads were 3.1%, 11.5%, 17.8%, 22.2%, and 31.9%, respectively. Microscopy demonstrated increased birefringence and decreased gluten network formation with increased RS5 and VWG, indicating resistance to gelatinization and structural effects. Dough conditioner, colorant, and flavor masker were used to improve quality attributes for the 20% (RA-20) and 30% (RA-30) RS5 breads. Mixograph results for the control and RS5
breads showed decreases in peak time (4.0 to 2.7 min), which confirmed reduced gluten network development observed in micrographs while addition of dough conditioner increased peak time. Sensory results showed no significant differences (p < 0.05) existed between RN-20 and the control for overall opinion, likeliness of purchase, uncharacteristic flavor, and ranking. These results suggest that RS5 can be added to bread formulations for improved health benefits and increased consumer acceptability.

**Introduction**

Bread is a common starchy food product consumed primarily in the United States and Europe. Bread contains a concentrated energy source in the form of starch, which undergoes enzymatic hydrolysis during digestion and results in rapid bioavailability of glucose. Many types of whole wheat breads have been produced to limit the rapid digestion of starch by increasing the dietary fiber (DF) contents through incorporating whole grains and/or supplemental exogenous DF into the formulations (Yousif et al 2012). DF is defined as a non-starch polysaccharide (NSP) that is resistant to digestion within the small intestine and is completely or partially fermented in the large intestine (AACC 2001). DF from whole grains is known to reduce incidence of coronary heart disease, hypertension, diabetes, and obesity (Liu et al 1999; Montonen et al 2003; Lairon et al 2005; Whelton et al 2005). Although whole grain bread is consumed to obtain these beneficial effects, many consumers regularly consume white bread, which is deficient in DF. While dietary fibers are currently used in many food products, research has been conducted to identify starch fractions which behave similarly to dietary fiber (Englyst and Cummings 1985; Englyst and Macfarlane 1986).

Resistant starch (RS) is the starch fraction that is not digested in the small intestine and is thus, fermented in the colon (Englyst and Macfarlane 1986; Wyatt and Horn 1988;
Englyst et al 2003). Five types of RS have been reported: physically inaccessible starch (RS1), the B- and C-type crystalline native, uncooked starch granule (RS2), retrograded amylose (RS3), chemically modified or cross-linked starch (RS4), and amylose-lipid complex (RS5) (Englyst et al 1992; Eerlingen et al 1994; Hasjim et al 2010). RS5 is a starch developed by complexing high-amylose starch (HA7) with lipid. RS5 displayed a higher RS content, higher peak gelatinization temperature, and lower onset gelatinization temperature compared with high-amylose maize starch (Hasjim et al 2010). The effects of RS5 on postprandial blood glucose levels in clinical trials has been investigated, however there has been no research on the sensory attributes of RS5 in food products (Hasjim et al 2010).

RS has been used to prepare foods with slower starch digestive rates (Jenkins et al 1987b). Commercial grade RS3, produced by the reassociation of amylose and some amylopectin into semi-crystalline double-helical structures following gelatinization, is primarily used in bread formulations. RS3 also develops naturally through retrogradation in cold-stored breads. The development of RS3 in these breads depends on many factors, including baking temperature, amylose content, and storage conditions (Liljeberg et al 1996; Hung et al 2005; Yadav 2011).

RS is shown to have a high resistance to starch hydrolysis and may be a healthy alternative to DF (Sanz et al 2008b). DF use may be reduced in bread by developing bread products with RS5, which has been shown to have better functionality. This is through greater fermentation in the large intestine, which is known to increase health benefits (Silvester et al 1995; Topping and Clifton 2001). By incorporating RS5 into bread products, resistant starch fractions may be utilized as a prebiotic substrate (Scholz-Ahrens 2007).
Concentrations of different types RS (23% - 40%) have been shown to improve the appearance, texture, and mouthfeel of food products (Sajilata et al 2006), whereas DF is known to adversely affect bread quality. The effects of DF on breads include decreased gas retention, reduced loaf volume, increased crumb firmness, and darker crust color (Wang et al 2002; Anil 2007). The effects of lower concentrations (20%) of RS on quality attributes of breads were reported to have minimal effects on loaf volume, gas cell development, and crumb texture, whereas higher concentrations (30%) displayed slight decreases in quality attributes (Hung et al 2004; Korus et al 2009; Mario Sanz-Panella et al 2010).

The objective of this study was to determine the effects of RS5 on the structure, texture and consumer acceptability of RS breads. Furthermore, investigations were conducted to determine the effect of additives to improve color, flavor, and texture on RS5 bread quality and consumer acceptability.

**Materials and Methods**

**Materials**

Bread flour (Pillsbury Best, Orrville, OH), table salt, yeast, sugar, vital wheat gluten (VWG, Arrowhead Mills, Melville, NY) were purchased from a local market in Ames, IA. High-amylose maize starch VII (HA7, AmyloGel 03003, Cargill, Hammond, IN), dough conditioner (SWF 125, Brolite, Streamwood, IL), flavor masker (natural bread flavor, Bell Flavor, Northbrook, IL), and colorant (celestial yellow, GNT, Tarrytown, NY) were gifts from the manufacturers. Amyloglucosidase from *Aspergillus niger* (200 U/mL), porcine pancreatic α-amylase (PPA), pancreatin from porcine pancreas, and stearic acid were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. The Total Starch Assay
Kit (containing AA/AMG) and D-Glucose Assay Kit (glucose oxidase/peroxide, GOPOD) were purchased from Megazyme International Ireland Ltd. (Co. Wicklow, Ireland).

**Bread Flour Composition**

Analysis of the composition of bread flour was conducted following standard methods. Moisture content of ingredients and bread were determined using standard methods. Starch content was determined using a Total Starch Assay Kit. Lipid content was determined using hexanes and Goldfisch fat extractors (Labconco Corp., Kansas City, MO) following the AACC Method 30-25. Protein content was determined using a Vario MAX CN Analyzer (Elementar 107 Analysensysteme, Hanau, Germany). The protein content of the bread flour was calculated by multiplying the nitrogen content by a conversion factor of 5.33 (AACC, 2000). The starch and protein content analyses were performed in duplicate.

**Resistant Starch**

Resistant starch (RS5) was produced using the method of Hasjin et al. (1999) with modification. Stearic acid was used to complex with the HA7 starch. RS analysis of RS5 was conducted and was approximately (65%).

**Bread Making**

Formulations for the control and RS5 breads are presented in **Table 2.1**. Yeast and sugar were suspended in water (40.5°C) for 10 min, oil was added, and this mixture was added to the dry ingredients. After mixing in a commercial mixer (Kitchen Aid, NJ) at speed 1 until the dough formed (~ 1.5 min), the dough was kneaded for 3.5 min, allowed to rise for 40 min, formed into pup loaves (125 g) and allowed to rise again for 20 min before baking in a convection oven (Blodgett duel flow) at 190°C for 20 min.
RS5 bread was made by substituting bread flour with RS5 at 20% (RN-20), 30% (RN-30), 40% (RN-40), and 50% (RN-50, db) while other ingredients were kept the same as those used in the control bread (Table 3.1). The control bread and the RS5 breads had the same proportions of starch and protein, which was achieved by adding VWG to the RS5 breads (Table 3.2). Calculated starch and protein contents of the formulations were similar in all bread formulations (69.1% - 71.6% and 10.9% - 11.1%, respectively) for optimal bread structure and gluten network formation (Bushuk 1975; Park et al 2006). Additional water was added to the RS5 dough so the texture of the dough was comparable to that of the control (Table 3.1). Bread flour composition of starch, lipid, and protein contents were 75.3%, 1.2%, and 11.1%, respectively. Bread was formulated (Table 3.1) with a reduced lipid content (0.9%) compared with standard amounts (~3% by weight) to decrease amylose-lipid complex formation during baking. RS5 contents of breads were expressed on db. Breads were baked in small batches (1/2 formulation) in the same proportion as the formulation for all breads. Dough conditioner (0.25% and 0.35%, w/w, wb), colorant (0.0008% and 0.0012%, w/w, wb) and flavor masker (0.3% and 0.4% w/w, wb) were added to the 20% (RA-20) and 30% (RA-30) formulations, respectively. Batch size was increased (4X) for the sensory panel study in the same proportions of the formulations.

Resistant Starch (RS) Content

The RS content of the bread samples were analyzed using AOAC Method 991.43 for dietary fiber (AOAC 2000) with modification (Li et al., 2008). The undigested protein content of the residue was determined using a Vario MAX CN Analyzer with a protein conversion factor of 5.33 (AACC 2000). The analysis was performed in duplicate. Percent
resistant starch (RS%, db) was calculated as follows: RS% = % total resistant residue – % undigested protein.

**Mixograph**

Mixing behavior of the control (flour), RN-20 and RN-30 (flour, VWG, and RS5), and RA-20 and RA-30 (flour, VWF, RS5, and dough conditioner) dough were evaluated using the 10 g Mixograph Procedure (Method AACC 54-40A, AACC, 1983). Peak time, peak height, development angle, weakening angle, mixing tolerance angle, and tail width were measured by the Mixograph (National Mfg. Co, Lincoln, NE). The analyses were performed in duplicate.

**Light Microscopy**

Dough taken immediately after mixing (prior to any rising) and baked bread samples were analyzed using light microscopy to determine effects of RS5 on the bread structure. The 0.5 cm balls of dough and cubes of breads were placed in cassettes with OTC (Optimal Cutting Temperature) compound and frozen with liquid nitrogen. Samples were sliced with a Leica CM1900 microtome to 10 µm and stained individually with Hematoxylin and Eosin, Oil Red O, and Periodic Acid Schiff with Hematoxylin to highlight protein, lipid, and carbohydrate content, respectively. Slides were then evaluated by light microscopy (BX60, Olympus America, Center Valley, Pennsylvania) using standard and polarized light. Micrographs were obtained at 40X and 100X magnifications.

**Sample Preparation for Sensory and Instrumental Analysis**

Bread samples were evaluated after storing in the freezer for 24 h and then thawed at ambient temperature (25°C) for 4 h. The center part of the bread loaf was cut into 3 slices of 15 mm thickness using a slicer (Model # 7 70969, The Hobart MFG Co., Troy, OH). The
analysis was performed by taking three readings on one bread slice taken from three different
breads for texture and color analyses. Sensory panel analysis was determined using one bread
slice taken randomly from 24 loaves that were sliced into six slices from each loaf

**Texture Profile Analysis (TPA)**

Texture analysis was performed using a TA.XT2i Texture Analyzer (Stable
Microsystems, Surrey, U.K.). A texture profile was performed on bread slices (15 mm
thickness) compressed to 50% of their original height at 1.0 mm/s using an aluminum probe
(32 mm x 12.7 mm, diameter flat contact surface plate) with elapsed time between
compressions being 5 s (Hug-Iten et al 2003). Hardness, cohesiveness, adhesiveness,
springiness, chewiness, and resilience of the bread samples, as defined by Bourne (2002),
were calculated by using a texture analysis program (version V1.22), which was coupled to
the texture analyzer.

**Color Analysis**

Baked crumb color was determined using the Hunter LAB Colorimeter, (UltraScan
XE, Hunter Associates Laboratory, Reston, VA). Analysis of the data was determined by the
CIELAB system, using D65 as the reference illuminate and a standard observer of 10°. The
instrument used a standard white tile for calibration prior to color measurements and a black
tile was used for reflectance calibration. In the Hunter-Lab colorimeter, the color of a sample
is denoted by the three dimensions as the $L^*a^*b^*$ values, where $L^*$ represents whiteness (value
100) or blackness (value 0), $a^*$ represents red (+$a^*$) or green ($-a^*$), and $b^*$ represents yellow
($+b^*$) or blue ($-b^*$). Hue angle and saturation index were also calculated.
Experimental Design of Sensory Evaluation

Untrained (100) panelists were recruited from the faculty, staff and students of Iowa State University. Five samples, control, RN-20, RN-30, RA-20, and RA-30, were assigned a 3-digit random number and presented separately in a randomized order. Sensory evaluation of bread was conducted using JAR (Just About Right), attribute intensity, and forced ranking tests (Lawless and Heymann, 1999; Meilgaard et al 1999). Attributes of the bread (Table 3.3) were selected according to a list of a standardized lexicon of terms for bread evaluation (Meilgaard et al 1999). After panelists evaluated all attributes of the individual samples, all 5 samples were returned to the panelists who then ranked the samples based on preference. Panelists were provided with plain water and sliced apples to remove any residual taste between samples. All sensory sessions were carried out in individual booths equipped with white lighting, and a computerized system with sensory evaluation software for statistical analysis (Compusense Five, V 5.0, Guelph, Ontario, Canada). Procedures for sensory evaluation were approved by Iowa State University Institutional Review board (IRB) in accordance with Office for Human Research Protections (OHRP) guidelines and Health Insurance Portability and Accountability Act (HIPAA) regulations.

Statistical Analysis

Analysis of Variance (ANOVA) with Statistix V 9.0 (Analytical Software, Tallahassee, FL) was used to determine the effects of RS5 and additives on the quality attributes of breads. Two-way ANOVA was used for TPA and Hunter colorimeter analysis to determine effects of treatments and large batch size. Interaction (significant) and main effects (no significant interaction) means were reported. Main factors for each experiment were concentration of RS5, additives, and small and large batch and their interaction. Mean values
were analyzed to determine effects of RS5 and additives on RS content and Mixograph. Pearson correlation coefficients were calculated using Microsoft Excel Version 12.0 to determine associations between TPA hardness parameters and sensory panel softness scores. Attributes in which treatment effects were significantly different based on ANOVA were further analyzed using Tukey’s adjustment to identify significant differences between treatments. The significance level was set at \( p < 0.05 \).

**Results and Discussion**

**Baking**

The control and RS5 breads showed differences in both rising time (40 and 45 min, respectively) and baking times (20 and 25 min, respectively). Moisture contents of RN-20, RN-30, RN-40, and RN-50 breads (36.5%, 38.6%, 37.5%, and 40.1%) were greater than the control (34.7%) after baking. This could be attributed to the greater amounts of water added to the RS5 dough and the lower water binding affinity of RS5 granules. Gluten binds water during proofing but denatures and unfolds during baking to release bound water. Water released from gluten is absorbed by the wheat starch granules during starch gelatinization (Fessas and Schiralda 2001). RS5 has shown to be only partially gelatinized by heating (Hasjim et al. 2010) and, thus, the partial gelatinized RS5 in breads might result in less water absorbed by starch leading to greater moisture contents.

Bread loaves displayed different loaf volumes (A) and bread crumb structure (B) in small and large batch sizes (Figures 3.1 and Figure 3.2, respectively). Figure 3.1A showed a decrease in volume in the RN-20 and RN-30 breads compared with the control and RA-20 and RA30 breads. The large batch size RN-20 and RN-30 breads displayed a lower volume compared with the control and RA-20 and RA-30 breads (Figure 3.2A). The addition of
dough conditioner increased loaf volume, which was similar to the control. Bread crumb of the RN-30 breads displayed decreases in gas cell and structure attributes whereas RA-30 breads showed lesser cracking on the crust and crumb. The inclusion of dough conditioner in treatment breads showed a more homogenous crumb structure compared with breads without conditioner. Furthermore, Small batch RN-20 breads displayed a reduced loaf volume, and this was more pronounced in the large batch RN-20 breads. This is in agreement with reported literature that showed that dough conditioner with enzymes increased loaf volume (Shogren et al 1981). These results suggest that dough conditioner may have a positive effect on bread structure and volume.

**Resistant Starch Content**

RS contents of the RS5 breads are shown in Table 3.4. The effects of dough conditioner on the RA-20 and RA-30 breads were not significantly different ($p < 0.05$) from the RN-20 and RN-30 breads, respectively. This confirms that the use of a dough conditioner in the RS5 breads did not alter RS contents of the baked breads. The proportion of RS5 that remained was less than the calculated amount used in the breads (Table 3.4). This result is in agreement with literature showing a decrease in RS content of RS-enriched products compared with theoretical values (Aravind et al 2013). This slight decrease may suggest that RS5 largely survived the baking process and enzymatic hydrolysis of the starch.

**Microscopy**

To analyze crumb structure of the control and RS5 (RN-20, RA-20, RN-30, and RA-30), doughs (Figure 3.3) and baked breads (Figure 3.4) of each were stained and viewed under a light microscope using bright field and polarized light. Protein structure appears as pink and nucleic acids, from yeast, appear as blue in the Hematoxylin and Eosin stained
samples (Figures 3.3A and Figure 3.4A). The control dough displayed a more linear gluten network formation, with thick strands of protein, compared with the RN-20 and RN-30 dough, which displayed a dispersed to fragmented gluten structure. Figure 3.4A showed that the gluten network in the control bread displayed a linear and well-formed structure compared with the RN-20 and RN-30 breads, which are dispersed and displayed a non-linear and random structure. This suggested that the exogenous VWG did not form a homogenous gluten network with the endogenous wheat gluten, and instead it was dispersed within the bread (Jenkins et al 1987). The RA-20 and RA-30 breads displayed well-defined structures of the gluten network, which might reflect the effect of the dough conditioner on improving the development of the gluten network.

Figures 3.3B and 3.4B show the doughs and baked breads under polarized light, respectively. As is typical of expected morphology of different starches, wheat starch consisted of bimodal disk-shaped starch granules, whereas the RS5 appeared as small spherical starch granules. Figure 3.3B shows the presence of birefringence in all doughs, consistent with the presence of non-gelatinized starch granules. RS5 starch granules displayed birefringence in the RS5 bread (Figure 3.4B), but wheat starch granules showed no birefringence, confirming that the RS starch granules were not gelatinized after baking. The RN-20 and RN-30 breads showed non-linear random alignment of gluten protein and starch granules, compared with the control, which displayed a dense gluten network that caused separation and alignment of starch granules. This suggests that the exogenous VWG did not form a homogenous gluten network with the endogenous wheat proteins and instead may be dispersed within the bread. This was in contrast to the normal wheat starches in the control formulation that became gelatinized during the baking process.
Dough and baked breads stained with Oil Red O for lipid are shown in Figure 3.5. Myriad, minute, red droplets were observed on the surface of the ungelatinized starch granules, consistent with micelles of lipid coating RS5 granules, as seen in the RA-20 dough (Figure 3.5A) and RN-20 bread (Figure 3.5B). This observation suggests that ungelatinized starch granules were RS5, which is complexed with stearic acid and would be stained red, whereas the larger dark red droplets are likely exogenous lipid dispersed within the bread structure.

Figure 3.6 shows dough (A) and baked (B) breads stained with periodic acid-schiff regent-hematoxylin stain that stains starch granules bright magenta pink. The control dough and breads displayed a compact packing structure of wheat starch granules, whereas RS5 dough and breads displayed a more loosely packed structure with the RS5 starch granules dispersed among the wheat starch granules. Structures consistent with gelatinized wheat starch granules and the ungelatinized RS5 granules were present in sample breads after baking.

The use of VWG in the RS5 breads may have resulted in increased dispersion of gluten proteins throughout the bread, preventing the formation of a gluten network. The inability of VWG to form a continuous network with endogenous wheat proteins is thought to result from structural differences between these proteins and intact RS5 granules interfering with cross-linked gluten network formation (Hung et al 2007). The effect of dough conditioner on the RA-20 and RA-30 breads suggests the shortening of the protein strands enhanced gluten network formation leading to increased loaf volume (Figure 3.4) (Stampfli & Nersten 1995). The improved gluten network of RA-20 and RA-30 breads that developed in breads with dough conditioner resulted in greater loaf volume compared with
RN-20 and RN-30 breads (Figures 3.1 and 3.2). Further research is needed to better understand the interaction of VWG and RS5 and their impact on bread structure.

**Mixing Behavior of Dough**

Gluten network development of the control and RS5 bread dough with substituted RS5 and VWG are shown in Table 3.5. Peak time is expressed as the time to achieve optimum gluten network formation in the dough. Increased peak heights (increased dough viscosity) and larger tail widths (increased dough elasticity) in the RS5 dough with VWG confirmed that the dough increased in viscosity and elasticity compared with the control. However, when dough conditioner was added to RA-20 and RA-30 doughs, tail width decreased suggesting that dough elasticity was reduced. Increased weakening angle of the doughs indicated greater resistance to deformation during oven spring, which resulted in cracking in the crust and crumb after baking as shown in the RN-30 bread (Figure 3.1B). These results suggested that adding RS5 and exogenous VWG affected texture and crumb quality by preventing optimal gluten network formation.

Peak time for RA-20 and RA-30 (3.7 and 4.4, respectively) increased compared to the RN-20 and RN-30 (3.1 and 2.8, respectively) dough but were not significantly different ($p < 0.05$) from the control (4.0). Peak time results suggested that the optimum gluten formation occurred earlier in the RS5 doughs than the control dough, which indicated weaker gluten network in the RS5 dough (Bonnand-Ducasse et al 2010). Mixing tolerance angle decreased from 160.0° to 129.8° as the RS5 concentration and VWG increased in the dough. These results suggest that with increased concentrations of VWG and RS5, the doughs exhibited less tolerance to over-mixing.
Mixograph of the RS5 dough with VWG results showed a decrease in peak time and mixing tolerance, which demonstrated a weakening of the gluten network, confirming the microscopy results (Table 3.5). The gluten network development for the RA-20 bread showed improved alignment of network structures compared with RN-20, which displayed a dispersed gluten structure (Figure 3.4A). Micrographs of RN-20 and RN-30 doughs (Figure 3.3) confirmed that the gluten was more dispersed in those doughs than that of the control, showing a typical gluten network. This data suggested that the gluten network in the RA-20 and RA-30 dough were improved by the use of the dough conditioner. There are differences in the numerical data but these trends were not significantly different based on statistical analysis. This may result from greater standard deviation in some of the attributes.

**Instrumental Analysis of Sensory Attributes**

Texture Profile Analyzer results for the control and RS5 breads are shown in Table 3.6. Small and large batch size was compared to better understand effects of scale-up on quality attributes. Small batch size breads were formulated with additives that were observed to increase texture, color, and taste quality attributes. To understand if comparable amounts of additives in large batch size breads for use in sensory panels had the same effects on quality as the small batch size, instrumental analysis was performed on these breads.

Small batch size RS5 treatment breads with hardness, resilience, chewiness, and springiness attributes were significantly different ($p < 0.05$) than the control. Large batch size RS5 breads with hardness, chewiness, and springiness attributes were significantly different ($p < 0.05$) than the control. Although the chewiness attribute of small batch size breads increased in the RA-20 and RA-30 breads (121.7 and 109.4, respectively) compared with the RN-20 and RN-30 breads (118.6 and 89.9, respectively), these breads displayed lower
chewiness attributes compared with the control (214.2). The RS5 treatment breads were not significantly different \((p < 0.05)\) from each other. These results suggest that dough conditioner did not improve the RN-20 and RN-30 breads.

Interactions effects were not significant for RS5 and batch size for adhesiveness. Mean effects for adhesiveness showed RN-20 (-0.0421) was significantly different \((p < 0.05)\) from the control (-0.028). Mean effects for hardness and chewiness were not significantly different \((p < 0.05)\) between small and large batch size for all RS5 treatments suggesting that these could be scaled-up without effects on quality attributes. TPA results of large batch samples displayed significant differences \((p < 0.05)\) in hardness, cohesiveness, springiness, chewiness and resilience compared with that of the small batch samples. This may result from structural change that occurs in bread during scale-up.

Table 3.7 shows Hunter colorimeter results for the control and RS5 breads. Use of a colorant in RS5 breads changed the color profile of the bread, and the color was more closely aligned with that of the control bread. The L*, a*, b*results for small batch size showed that the L* value (76.9) for RA-20 was not significantly different \((p < 0.05)\) from the control (76.9), whereas RN-20, RN-30, and RA-30 were significantly different (78.9, 78.6, and 79.2, respectively) from the control. Saturation index for large batch size displayed RA-20 breads were not significantly different from the control, although these breads were not significantly different \((p < 0.05)\) from RN-30 and RA-30 breads.

\(\text{L*a*b*}\) results for RA-30 breads were significantly different \((p < 0.05)\) from the control. These results suggest that using RS5 at different concentrations had impacts to the crumb color. Although the TPA and Hunter results showed positive effects of additives in small batch sizes, these were not evident in the large batch scale-up samples. This suggested
that reformulations of breads were needed to obtain quality results during a commercial scale-up.

**Sensory Evaluation**

Sensory results of panel evaluations are shown in Table 3.8. Results indicated that RS5 treatments and additives had significant effects on bread quality compared to the control (Table 3.8). Sensory scores were lower at increased levels of additives, which caused a decrease in preference of RS5 treatments and poor panel response. Panels judged additives worse than the RS5 breads alone. Most sensory attributes were not significantly different ($p < 0.05$) between breads without additives and those with additives. This indicates that additives did not perceptibly improve quality attributes of RS5 breads at higher levels.

Panelists assigned higher values for uncharacteristic flavor in RA-20 and RA-30 compared with RN-20 and RN-30 breads. Uncharacteristic flavor of RN-20 was not significantly different ($p < 0.05$) from the control, whereas RA-20, RN-30, and RA-30 were significantly different. Lower levels of RS5 in breads were not perceived as significantly different in the presence of uncharacteristic flavor compared with the control.

Breads containing 30% RS5 were rated less acceptable than the control bread. RN-30 was not significantly different ($p < 0.05$) than RA-30 breads for the uncharacteristic flavor attribute. Attribute scores were significantly higher ($p < 0.05$) from the RN-30 and RA-30 breads compared to the control. These results indicate that RN-30 and RA-30 breads are not acceptable due to uncharacteristic flavor.

Major characteristic attributes of the control bread were comparable to RN-20 (Table 3.9). Panel preference did not differ between RN-20 and control breads for flavor of crumb, likeliness of purchase, chewiness of interior and bitterness attributes. Overall opinion,
likeliness of purchase and ranking attributes were not statistically different ($p < 0.05$) from the control. These results indicated that substitution of 20% RS5 into a product is comparable to the control.

Panel preference for the control, RN-20, and RA-20 breads were ranked higher than the RN-30 and RA-30 breads. These breads were not significantly ($p < 0.05$) different from each other. Ranking showed that RN-20 and RA-20 were comparable to the control, which was supported by likeliness of purchase scores.

Sensory TPA instrumental results for hardness attribute was positively correlated ($r = 0.98, p < 0.05$) with sensory panel softness attribute in large batch breads. Sensory instrumental results for chewiness attribute was positively correlated ($r = 0.88, p < 0.05$) with sensory panel chewiness of the interior attribute. This suggests that TPA may be a good indicator for softness and chewiness responses for human panelists.

**Conclusion**

This study demonstrated that RS5 had significant impacts on physical and sensory qualities of unbaked dough and baked bread formulations. RS5 breads displayed decreases in loaf volume and color with increased density with addition of RS5 and VWG. Results of the present study showed that RA-20 displayed attributes most similar to the control, compared with the RN-20 bread. These differences were attributed to the use of dough conditioners that improved the development of a gluten network, which is a critical factor in bread quality. RS contents increased in treatment breads making it a better choice to deliver dietary fiber to consumers. RA-20 showed improvements in instrumental attributes but not by sensory panels. The RN-20 breads were judged to be comparable to the control by sensory panels. The RN-20 bread was comparable in quality attributes for overall opinion, likelihood of
purchase and ranking for control bread. This study showed that a recently developed RS5 can successfully be incorporated into breads. RN-20 breads may meet the needs of consumers looking for healthy white breads that have the same texture and eating qualities as commercial breads.

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Literature Cited


Aravind, N., Sissons, M., Fellows, C. M., Blazek, J., Gilbert, E. P. 2013. Optimisation of resistant starch II and III levels in durum wheat pasta to reduce in vitro digestibility


Yadav, B. S. (2011),"Effect of frying, baking and storage conditions on resistant starch content of foods", *Br Food J.* 113 6 710-719.

Table 3.1. Formulation of control and resistant starch bread

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<th>RN-30</th>
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<td>0</td>
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<td>4.68</td>
</tr>
<tr>
<td>Total weight</td>
<td>723</td>
<td>760</td>
<td>788</td>
<td>823</td>
<td>838</td>
<td>763</td>
<td>794</td>
</tr>
</tbody>
</table>

<sup>a</sup> Starch, protein, and other contents of bread ingredients provided by suppliers.

<sup>b</sup> HA7+ISO+SA was preheated HA7 debranched using ISO and complexed with SA as RS source.
Table 3.2 Percentage of starch and protein on dry basis of the total weight

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>RN-20</th>
<th>RN-30</th>
<th>RN-40</th>
<th>RN-50</th>
<th>RA-20</th>
<th>RA-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch(^a)</td>
<td>69.1</td>
<td>69.85</td>
<td>70.6</td>
<td>71.1</td>
<td>71.6</td>
<td>69.4</td>
<td>69.6</td>
</tr>
<tr>
<td>Protein(^a)</td>
<td>11.0</td>
<td>11.1</td>
<td>11.1</td>
<td>11.1</td>
<td>11.1</td>
<td>11.1</td>
<td>11.0</td>
</tr>
<tr>
<td>Other(^a)</td>
<td>19.9</td>
<td>19.0</td>
<td>18.3</td>
<td>17.8</td>
<td>17.3</td>
<td>19.7</td>
<td>19.4</td>
</tr>
</tbody>
</table>

\(^a\)Starch, protein, and other contents of bread ingredients provided by suppliers.
Table 3.3 Scale of attributes for sensory panel

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Scale</th>
<th>Low Intensity</th>
<th>High Intensity</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crumb Color</td>
<td>1-5</td>
<td>Much Too Light</td>
<td>Much Too Dark</td>
<td>JAR</td>
</tr>
<tr>
<td>Crumb Appearance</td>
<td>1-5</td>
<td>Not Enough Gas Cells</td>
<td>Too Many Gas Cells</td>
<td>JAR</td>
</tr>
<tr>
<td>Denseness</td>
<td>1-5</td>
<td>Not Dense Enough</td>
<td>Much Too Dense</td>
<td>JAR</td>
</tr>
<tr>
<td>Overall Aroma</td>
<td>1-9</td>
<td>Dislike extremely</td>
<td>Like extremely</td>
<td>Attribute</td>
</tr>
<tr>
<td>Overall Opinion</td>
<td>1-9</td>
<td>Dislike extremely</td>
<td>Like extremely</td>
<td>Attribute</td>
</tr>
<tr>
<td>Moistness of interior</td>
<td>1-5</td>
<td>Much Too Dry</td>
<td>Much Too Moist</td>
<td>JAR</td>
</tr>
<tr>
<td>Chewiness of interior</td>
<td>1-5</td>
<td>Not Nearly Chewy</td>
<td>Much Too Chewy</td>
<td>JAR</td>
</tr>
<tr>
<td>Softness</td>
<td>1-5</td>
<td>Much Too Soft</td>
<td>Much Too Firm</td>
<td>JAR</td>
</tr>
<tr>
<td>Flavor of crumb</td>
<td>1-9</td>
<td>Dislike extremely</td>
<td>Like extremely</td>
<td>Attribute</td>
</tr>
<tr>
<td>Uncharacteristic flavor</td>
<td>1-9</td>
<td>None</td>
<td>Strong</td>
<td>Attribute</td>
</tr>
<tr>
<td>Overall Bitterness</td>
<td>1-9</td>
<td>None</td>
<td>Strong</td>
<td>Attribute</td>
</tr>
<tr>
<td>Likelihood of purchase</td>
<td>1-5</td>
<td>Would not purchase</td>
<td>Would purchase</td>
<td>Ranking</td>
</tr>
</tbody>
</table>
Table 3.4 RS content of bread\(^1\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Calculated Resistant Starch (%)</th>
<th>Resistant Starch (%)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>3.1±0.1(^a)</td>
</tr>
<tr>
<td>RN-20</td>
<td>14.1</td>
<td>11.5±0.3(^b)</td>
</tr>
<tr>
<td>RA-20</td>
<td>14.0</td>
<td>12.2±0.1(^b)</td>
</tr>
<tr>
<td>RN-30</td>
<td>22.0</td>
<td>17.8±0.0(^c)</td>
</tr>
<tr>
<td>RA-30</td>
<td>21.3</td>
<td>18.4±0.2(^c)</td>
</tr>
<tr>
<td>RN-40</td>
<td>29.1</td>
<td>22.2±0.2(^d)</td>
</tr>
<tr>
<td>RN-50</td>
<td>36.1</td>
<td>31.9±0.4(^e)</td>
</tr>
</tbody>
</table>

\(^a\) Total resistant residue was analyzed using AOAC method 991.43 for total dietary fiber (Horwithz 2003). Mean ± standard deviation from duplicate.

\(^1\) Means ± standard deviations. Values with the same letter in a column are not significantly different at \(p < 0.05\).
Table 3.5 Effect of RS concentration on flour mixing at constant water absorption

<table>
<thead>
<tr>
<th>RS (%)</th>
<th>Peak Time</th>
<th>Peak Height X 10^2</th>
<th>Development Angle (°)</th>
<th>Weakening Angle (°)</th>
<th>Mixing Tolerance Angle (°)</th>
<th>Tail Width x 10^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.0±0.3^{abc}</td>
<td>5.0±0.4^{a}</td>
<td>14.3±5.1^{a}</td>
<td>5.8±0.9^{a}</td>
<td>160.0±6.0^{a}</td>
<td>2.2±0.1^{cd}</td>
</tr>
<tr>
<td>RN-20</td>
<td>3.1±0.1^{bc}</td>
<td>5.1±0.1^{a}</td>
<td>18.3±0.9^{a}</td>
<td>3.8±1.2^{a}</td>
<td>158.0±0.4^{ab}</td>
<td>2.2±0.1^{cd}</td>
</tr>
<tr>
<td>RA-20</td>
<td>5.2±0.2^{ab}</td>
<td>5.5±0.2^{a}</td>
<td>9.0±2.8^{a}</td>
<td>4.0±0.0^{a}</td>
<td>167.0±2.8^{a}</td>
<td>2.1±0.1^{d}</td>
</tr>
<tr>
<td>RN-30</td>
<td>2.8±0.2^{c}</td>
<td>6.1±0.0^{a}</td>
<td>29.0±7.1^{a}</td>
<td>8.5±1.1^{a}</td>
<td>142.5±6.6^{ab}</td>
<td>3.2±0.1^{ab}</td>
</tr>
<tr>
<td>RA-30</td>
<td>4.4±0.1^{abc}</td>
<td>5.1±0.4^{a}</td>
<td>17.0±4.2^{a}</td>
<td>5.5±0.7^{a}</td>
<td>157.5±0.1^{ab}</td>
<td>2.6±0.2^{bcd}</td>
</tr>
<tr>
<td>RN-40</td>
<td>2.7±0.2^{c}</td>
<td>5.7±0.0^{a}</td>
<td>31.0±0.7^{a}</td>
<td>7.0±0.7^{a}</td>
<td>142.0±1.4^{ab}</td>
<td>3.0±0.1^{abc}</td>
</tr>
<tr>
<td>RN-50</td>
<td>5.4±0.6^{a}</td>
<td>6.3±0.3^{a}</td>
<td>39.5±6.7^{a}</td>
<td>10.8±2.7^{a}</td>
<td>129.8±4.1^{b}</td>
<td>3.6±0.1^{a}</td>
</tr>
</tbody>
</table>

\[1\] Means ± standard deviations. Values with the same letter in a column are not significantly different at \( p < 0.05 \).
Table 3.6 TPA for frozen-thawed bread. Small batch and large batch scale-up¹

<table>
<thead>
<tr>
<th>Batch</th>
<th>Control</th>
<th>RN-20</th>
<th>RA-20</th>
<th>RN-30</th>
<th>RA-30</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hardness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>548.0±29.7²ay</td>
<td>399.6±6.7³bx</td>
<td>400.5±1.4³bx</td>
<td>309.0±23.4³bx</td>
<td>401.1±10.7³bx</td>
<td>410.91</td>
</tr>
<tr>
<td>Large</td>
<td>962.1±88.4³ax</td>
<td>429.3±18.4³bx</td>
<td>370.7±51.1³bx</td>
<td>403.4±15.3³bx</td>
<td>324.0±14.9³bx</td>
<td>497.89</td>
</tr>
<tr>
<td>Mean</td>
<td>755</td>
<td>414.4</td>
<td>385.6</td>
<td>356.2</td>
<td>360.2</td>
<td></td>
</tr>
<tr>
<td><strong>Cohesiveness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>0.477±0.009³ax</td>
<td>0.394±0.005³bx</td>
<td>0.431±0.001³bx</td>
<td>0.392±0.004³cx</td>
<td>0.402±0.004³bx</td>
<td>0.419</td>
</tr>
<tr>
<td>Large</td>
<td>0.377±0.021³aby</td>
<td>0.391±0.017³bx</td>
<td>0.356±0.015³bx</td>
<td>0.359±0.025³bx</td>
<td>0.346±0.009³by</td>
<td>0.366</td>
</tr>
<tr>
<td>Mean</td>
<td>0.427</td>
<td>0.392</td>
<td>0.393</td>
<td>0.376</td>
<td>0.374</td>
<td></td>
</tr>
<tr>
<td><strong>Adhesiveness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>-0.0031±0.0002</td>
<td>-0.0684±0.0637</td>
<td>-0.0037±0.0011</td>
<td>-0.0077±0.0072</td>
<td>-0.0022±0.0002</td>
<td>-0.0167³x</td>
</tr>
<tr>
<td>Large</td>
<td>-0.0024±0.0012</td>
<td>-0.0159±0.0096</td>
<td>-0.0133±0.0177</td>
<td>-0.0096±0.0088</td>
<td>-0.0145±0.0068</td>
<td>-0.0111³x</td>
</tr>
<tr>
<td>Mean</td>
<td>-0.0028⁰³a</td>
<td>-0.0421⁰³b</td>
<td>-0.0085⁰³ab</td>
<td>-0.0080⁰³ab</td>
<td>-0.0083⁰³ab</td>
<td></td>
</tr>
<tr>
<td><strong>Springiness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>0.820±0.016³ax</td>
<td>0.754±0.022³abx</td>
<td>0.709±0.037³bx</td>
<td>0.727±0.023³bx</td>
<td>0.676±0.021³bx</td>
<td>0.7398</td>
</tr>
<tr>
<td>Large</td>
<td>0.874±0.055³ax</td>
<td>0.757±0.001³bx</td>
<td>0.698±0.017³bx</td>
<td>0.667±0.036³cy</td>
<td>0.656±0.023³cy</td>
<td>0.7304</td>
</tr>
<tr>
<td>Mean</td>
<td>0.847</td>
<td>0.756</td>
<td>0.704</td>
<td>0.697</td>
<td>0.672</td>
<td></td>
</tr>
<tr>
<td><strong>Chewiness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>214.2±13.0³ay</td>
<td>118.6±1.8³bx</td>
<td>121.7±9.7³bx</td>
<td>89.9±10.3³bx</td>
<td>109.4±3.0³bx</td>
<td>131.03</td>
</tr>
<tr>
<td>Large</td>
<td>323.2±48.2³ax</td>
<td>126.7±3.1³bx</td>
<td>93.5±13.2³bx</td>
<td>95.8±3.8³bx</td>
<td>74.0±7.6³cx</td>
<td>142.63</td>
</tr>
<tr>
<td>Mean</td>
<td>268.7</td>
<td>122.62</td>
<td>107.58</td>
<td>92.85</td>
<td>92.36</td>
<td></td>
</tr>
<tr>
<td><strong>Resilience</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>0.190±0.007³ax</td>
<td>0.129±0.003³bx</td>
<td>0.136±0.007³bx</td>
<td>0.129±0.001³bx</td>
<td>0.129±0.001³bx</td>
<td>0.1425</td>
</tr>
<tr>
<td>Large</td>
<td>0.131±0.011³ay</td>
<td>0.121±0.006³abx</td>
<td>0.105±0.008³by</td>
<td>0.110±0.011³bx</td>
<td>0.107±0.011³by</td>
<td>0.1146</td>
</tr>
<tr>
<td>Mean</td>
<td>0.16</td>
<td>0.125</td>
<td>0.121</td>
<td>0.119</td>
<td>0.118</td>
<td></td>
</tr>
</tbody>
</table>

¹Results are the main effects (no interaction was observed). For each attribute, means in a row followed by a different letter (a-c) are significantly different (p < 0.05). An interaction between RS treatments and batch size for TPA attributes were noted; therefore interaction means were reported. For the effects of RS, means with different letters (a-c) within the same row are significantly different (p < 0.05). For the effects of batch size, means with different letters (x-y) within the same column are significantly different (p < 0.05).
Table 3.7 Colorimetric analysis of bread samples. Small and large batch scale-up

<table>
<thead>
<tr>
<th>Batch</th>
<th>Parameter</th>
<th>Control</th>
<th>RN-20</th>
<th>RA-20</th>
<th>RN-30</th>
<th>RA-30</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>76.86±0.23&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>78.97±0.70&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>76.91±1.17&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>78.62±0.27&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>79.17±0.51&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>78.10</td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>72.60±0.82&lt;sup&gt;by&lt;/sup&gt;</td>
<td>78.73±0.40&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>77.55±0.24&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>79.17±0.05&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>78.53±0.02&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>77.16</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>74.74</td>
<td>78.86</td>
<td>77.23</td>
<td>78.56</td>
<td>78.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>-0.34±0.02&lt;sup&gt;dx&lt;/sup&gt;</td>
<td>0.18±0.06&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>1.01±0.06&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>0.17±0.06&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>0.60±0.17&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>0.42±0.17&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>-0.11±0.02&lt;sup&gt;by&lt;/sup&gt;</td>
<td>0.38±0.06&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>0.60±0.05&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>0.60±0.03&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-0.39</td>
<td>0.03</td>
<td>0.69</td>
<td>0.28</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>20.76±0.14&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>16.49±0.28&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>23.44±0.23&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>16.07±0.25&lt;sup&gt;dy&lt;/sup&gt;</td>
<td>20.72±0.09&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>19.85</td>
<td></td>
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<tr>
<td>Large</td>
<td>19.80±0.47&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>16.17±0.44&lt;sup&gt;cy&lt;/sup&gt;</td>
<td>19.00±0.24&lt;sup&gt;by&lt;/sup&gt;</td>
<td>20.72±0.10&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>18.48±0.12&lt;sup&gt;by&lt;/sup&gt;</td>
<td>18.51</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>20.31</td>
<td>17.20</td>
<td>17.23</td>
<td>17.43</td>
<td>19.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>1.571±0.000</td>
<td>1.571±0.000</td>
<td>1.569±0.000</td>
<td>1.571±0.006</td>
<td>1.570±0.000</td>
<td>1.570&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>1.570±0.029</td>
<td>1.571±0.000</td>
<td>1.570±0.000</td>
<td>1.570±0.000</td>
<td>1.570±0.000</td>
<td>1.571&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.570&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.571&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.570&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.569&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.570&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saturation</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>20.76±0.14&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>16.49±0.28&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>23.46±0.23&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>16.07±0.25&lt;sup&gt;dy&lt;/sup&gt;</td>
<td>20.73±0.09&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>19.85</td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>19.80±0.48&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>16.17±0.43&lt;sup&gt;cy&lt;/sup&gt;</td>
<td>19.00±0.62&lt;sup&gt;aby&lt;/sup&gt;</td>
<td>20.73±0.29&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>18.48±0.48&lt;sup&gt;by&lt;/sup&gt;</td>
<td>18.41</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>20.32</td>
<td>17.20</td>
<td>21.24</td>
<td>17.29</td>
<td>19.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Results are the main effects (no interaction was observed). For each attribute, means in a row followed by a different letter (a-d) are significantly different (p < 0.05). An interaction between RS treatments and batch size for TPA attributes were noted; therefore interaction means were reported. For the effects of RS, means with different letters (a-d) within the same row are significantly different (p < 0.05). For the effects of batch size, means with different letters (x-y) within the same column are significantly different (p < 0.05).
### Table 3.8 Sensory panel (n = 100)

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Control</th>
<th>RN-20</th>
<th>RA-20</th>
<th>RN-30</th>
<th>RA-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crumb Color</td>
<td>5.12a</td>
<td>3.31d</td>
<td>4.29b</td>
<td>3.09d</td>
<td>3.78c</td>
</tr>
<tr>
<td>Crumb Appearance</td>
<td>2.86a</td>
<td>2.96a</td>
<td>3.08a</td>
<td>2.97a</td>
<td>3.04a</td>
</tr>
<tr>
<td>Denseness</td>
<td>3.70a</td>
<td>3.33b</td>
<td>3.11b</td>
<td>3.33b</td>
<td>3.25b</td>
</tr>
<tr>
<td>Overall Aroma</td>
<td>5.93a</td>
<td>5.60a</td>
<td>5.03bc</td>
<td>5.40ab</td>
<td>4.47c</td>
</tr>
<tr>
<td>Overall Opinion</td>
<td>5.36a</td>
<td>5.31a</td>
<td>4.89a</td>
<td>4.14b</td>
<td>4.01b</td>
</tr>
<tr>
<td>Moistness of interior</td>
<td>2.27b</td>
<td>2.69a</td>
<td>2.72a</td>
<td>2.61a</td>
<td>2.68a</td>
</tr>
<tr>
<td>Chewiness of interior</td>
<td>3.38a</td>
<td>3.22ab</td>
<td>3.13ab</td>
<td>2.99b</td>
<td>3.02b</td>
</tr>
<tr>
<td>Softness</td>
<td>3.71a</td>
<td>3.11b</td>
<td>3.04bc</td>
<td>3.11b</td>
<td>2.84c</td>
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<tr>
<td>Flavor of crumb</td>
<td>5.78a</td>
<td>5.22ab</td>
<td>4.70b</td>
<td>4.17c</td>
<td>3.97c</td>
</tr>
<tr>
<td>Uncharacteristic flavor</td>
<td>3.18c</td>
<td>3.69bc</td>
<td>4.16ab</td>
<td>4.58a</td>
<td>4.77a</td>
</tr>
<tr>
<td>Overall Bitterness</td>
<td>2.65d</td>
<td>3.31c</td>
<td>3.46bc</td>
<td>3.98ab</td>
<td>4.12a</td>
</tr>
<tr>
<td>Likelihood of purchase</td>
<td>2.99a</td>
<td>2.95a</td>
<td>2.83a</td>
<td>2.22b</td>
<td>2.18b</td>
</tr>
<tr>
<td>Ranking</td>
<td>242b</td>
<td>239b</td>
<td>276b</td>
<td>356a</td>
<td>357a</td>
</tr>
</tbody>
</table>

Values with the same letter in a column are not significantly different at $p < 0.05$.
Refer to Table 3 for term discussions.
Figure 3.1 Small-batch breads. Whole (A) and sliced (B) breads. RN-20/30 = Breads with 20% - 30% RS% without additives; RA-20/30 = Breads with 20% - 30% RS% with additives.
Figure 3.2 Large-batch breads. Whole (A) and sliced (B) breads. RN-20/30 = Breads with 20% - 30% RS% without additives; RA-20/30 = Breads with 20% - 30% RS% with additives.
Figure 3.3 Light micrographs of bread dough. (A) Hematoxylin and Eosin staining and (B) polarized light microscopy. All at 100X. RN-20/30 = Breads with 20% - 30% RS% without additives; RA-20/30 = Breads with 20% - 30% RS% with additives. Hematoxylin and Eosin stains for proteins.
Figure 3.4 Light micrographs of baked bread. (A) Hematoxylin and Eosin staining and (B) polarized light microscopy. All at 100X. RN-20/30 = Breads with 20% - 30% RS% without additives; RA-20/30 = Breads with 20% - 30% RS% with additives. Hematoxylin and Eosin stains for proteins.
Figure 3.5 Light micrographs using Oil Red O staining of (A) dough and (B) baked at 100X. RN-20/30 = Breads with 20% - 30% RS% without additives; RA-20/30 = Breads with 20% - 30% RS% with additives. Oil Red O stains for lipid.
Figure 3.6 Light micrographs using PASH (Periodic Acid Schiff and Hematoxylin) staining of (A) dough and (B) baked bread at 100X. RN-20/30 = Breads with 20% - 30% RS% without additives; RA-20/30 = Breads with 20% - 30% RS% with additives. PASH stains for carbohydrates.
CHAPTER 4. GENERAL CONCLUSIONS

The overall objective of this research was to understand the development and utilization of resistant starch in food products. The first paper investigated effects of cooking methods and rice structures on starch-hydrolysis rates. The japonica rice and waxy rice showed larger proportions of short branch-chains with DP 6-12 (37.7% and 38.3%, respectively) and smaller proportions of branch chains with DP 13-24 (42.4% and 44.9%, respectively) than indica rice (20.9% and 58.4%, respectively). Step-wise studies results agreed with that cold-storage of gelatinized normal rice starches displayed greater extents of retrogradation than the waxy rice starch. Stir-fried indica rice displayed greater (16.6%) RS contents than japonica (15.9%) and waxy (12.1%) rice varieties. Stir-fried rice demonstrated the slowest starch-hydrolysis rate and the largest resistant-starch content.

The second paper evaluated effects of a novel type-5 resistant starch (RS5) on the analysis and structure of bread and investigated the consumer acceptability of bread made with the RS5 and those reformulated with food additives. Treatments of the bread were developed with 20%, 30%, 40%, and 50%, db, RS5 substituted for the bread flour. The analysis of RS content demonstrated that RS5 resisted baking processing and enzyme-hydrolysis for all breads made in the study. Introduction of food additives to the 20% and 30% RS5 breads displayed improve quality attributes in small batch, but large batch scale-up did not demonstrate improvement in bread quality. Sensory results showed that RN-20 breads were not significantly different ($p < 0.05$) from the control and thus could be deemed acceptable by consumers.
Overall, these studies demonstrated that RS could be increased in rice dishes by using proper cooking methods and selecting rice variety. Adding RS5 to food products, such as breads, increased the RS content. The quality of the breads made with RS5 could be improved by adding proper additives.

**Future Work**

Having completed this research, questions arose based on the results of the two studies. It is of interest to researchers to conduct further studies in understanding these questions and determining scientific outcomes. These questions were interpreted to advance knowledge by developing studies in the following ways:

The rice study allowed us to obtain valuable insight into the effects of cooking methods on rice grains. During this research, questions were asked that would help better understand effects of specific ingredients, methods, and mechanisms on the rice.

**Rice**

- RS formation: Investigate ratio of RS1, RS3, and RS5 that occurs in the cooked rice
- RS3 content: Effects of cold-storage over 24 hrs in RS3 development
- Lipid mechanism: Effects of exogenous lipids in pilaf rice and stir-fried rice and its effect on starch-hydrolysis rates and its ability to complex with the starch to increase RS content.
- Lipid coating: Investigate coating of lipids on rice grains during stir-frying of steamed, cold-stored rice and impact on RS development

**Bread**

The RS5 bread research demonstrated that developing a consumer acceptable bread using RS5 could be successfully completed. The results of this research furthered questions on the
structural formation, quality, and gluten network development of this product. Listed below are some of the issues that need to be investigated:

- Freeze-thaw stability: Effects of freeze-thaw cycles on RS5 bread quality attributes
- Scale-up effects: Effective levels of additives and bread quality issues
- Lipid content: Increase lipid content to match industry standards (2%-3%) and investigate effects on quality and loaf volume.
- Lipid type: Effects of fat or oil on gas cell size and gluten network development
- Dough conditioners: Effects of different additives on bread texture and RS content
- Gluten network formation: Extent of disulfide bond formation between endogenous wheat proteins and vital wheat gluten; effects of RS5 on gluten network strength
- Water-holding capacity of RS5: Effects on moisture content of breads and crumb quality after baking
APPENDIX: SENSORY BALLOT

Consumer Evaluation of White Bread

Please answer all of the questions. Your name is not on the questionnaire and you will not be identified with your answers. Read the informed consent form and, if you agree to participate, sign it and pass it through the door at the front of the booth. Please register using the Registration Code provided to you in the booth. An attendant will provide your first sample. You will be evaluating five white breads. After you have evaluated each of the five kinds of white bread you will be asked which one you preferred and the reason for your preference.

Registration Code ____________

What is your age group?

18-24  25-34  35-44  45-54  55-64  65 or older
☐  ☐  ☐  ☐  ☐  ☐  ☐

What is your gender?

Male  Female
☐  ☐

What is your household income?

$0-$25,000  $25,001-$50,000  $50,001-$75,000  $75,001-$100,000  over $100,000
☐  ☐  ☐  ☐  ☐  ☐  ☐
How often do you consume commercial brands of bread?

More than once per day  Once per day  Once every other day  Once per week  Once every other week

☐ ☐ ☐ ☐ ☐

How often do you consume breads with improved nutritional value (e.g. whole wheat, high fiber)?

More than once per day  Once per day  Once per week  Once every other week  Once per month

☐ ☐ ☐ ☐ ☐

Code number for the bread sample_______

Look at the sample and indicate how you feel about the following Appearance attributes.

Color of Crumb

☐ ☐ ☐ ☐

☐ ☐ ☐ ☐ ☐

Much too light  Just about right  Much too dark

Crumb

☐ ☐ ☐ ☐ ☐

Not nearly enough gas cells  Just about right  Way too many gas cells
**Denseness**

- Not nearly dense
- Just about right
- Much too dense

Break the bread slice in half and sniff the bread. Evaluate how much you like or dislike the overall aroma.

**Overall Aroma**

- Dislike
- Neither
- Like

- extremely dislike
- like nor extremely dislike

Please take a bite of apple before starting and between samples.

Now take a bite of the bread and indicate your overall opinion about this sample.

**Overall Opinion**

- Dislike
- Neither
- Like

- extremely dislike
- like nor extremely dislike

Please re-taste the bread as often as needed and indicate how you feel about the following texture, taste and flavor attributes.
### Moistness of the Interior

- [ ] Much to dry
- [ ] Just about right
- [ ] Much too moist

### Chewiness of the Interior

- [ ] Not nearly chewy enough
- [ ] Just about right
- [ ] Much too chewy

### Softness

- [ ] Much too soft
- [ ] Just about right
- [ ] Much too firm

### Flavor of the Crumb

- [ ] Dislike
- [ ] Neither like nor dislike
- [ ] Like
- [ ] Dislike extremely
- [ ] Like extremely

### Uncharacteristic Flavor of the Crumb

- [ ] None
- [ ] Moderate
- [ ] Strong
Overall Bitterness

None Moderate Strong

How likely would you be to purchase this bread if it were available at a reasonable price in your area?

Likelihood of Purchasing

Definitely would  Probably would  May or may not  Probably would  Definitely would

not buy  not buy  buy  buy  buy

The Bread sample codes are listed below in the order you evaluated them from left to right. Please rank the samples according to how much you liked them. Please write #1 under the sample you liked the most and #2 under the sample you liked second best, a #3 under the sample you liked third best and a #4 under the sample you liked fourth best.

Please indicate the reason for your preference.
ACKNOWLEDGEMENT

First and foremost, I wish to extend my extreme gratitude and appreciation to my faculty advisor, Dr. Jay-lin Jane. It goes without saying that without your guidance, motivation, deep passion, unending knowledge, and desire to advance science, I would not have achieved the level of educational expertise that I now possess nor the ability to watch my dreams come true.

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