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Rice Fortification by Parboiling in Limited-Water Soaking to Alleviate Mineral and Vitamin Deficiency

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Rice Fortification by Parboiling in Limited-Water Soaking to Alleviate Mineral and Vitamin Deficiency

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by

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Abstract

Fortification of rice by parboiling is considered a potential alternative to currently available fortification technologies to produce rice with higher mineral and vitamin content significantly contributing to nutrient intake in populations with high prevalence of micronutrient deficiencies. Higher nutrient retention rates and improved sensory characteristics are advantages of fortified parboiled rice compared to fortified rice obtained by currently used fortification technologies including dusting, coating, and extrusion. However, conventional parboiling processes employ excess water that presents an environmental hazard if discarded without treatment. Thus, the objective of this study was to evaluate a limited-water soaking method for the fortification of rice with calcium, iron, folic acid, β-carotene, and vitamin A. Excess- and limited-water soaking method were compared for fortified rice quality attributes including head rice yield and kernel color, mineral and vitamin contents, the amount of wastewater, total solids in wastewater. Limited-water parboiling utilizes only 75% of water and fortificant that is used in the excess-water parboiling process, thus reduced the amount of effluent and solids in wastewater significantly without affecting rice quality attributes. Fortification with lipophilic β-carotene and vitamin A was evaluated and optimized by comparing two different fortificant types, pure β-carotene and vitamin A dissolved in Tween® 80 and water-soluble forms of the respective vitamins. Water-soluble forms of β-carotene and vitamin A were shown to be more feasible fortificants due to higher vitamin uptake and time-efficiency. The limited-water parboiling method obtained fortified rice that contributes to about 15% of the recommended dietary allowances (RDAs) of calcium, 72% (male) and 32% (female) for iron, 75% for folic acid, and 58% (male) and 45% (female) for vitamin A. Thus, limited-water parboiling is a cost-efficient and environmentally friendly alternative to the conventional excess-water parboiling for the fortification of rice with minerals and vitamins.

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I. Introduction

Rice is considered a highly effective tool for food fortification and has great potential to complement other interventions, such as supplementation and dietary diversification, whereas changes in a population's eating habits are not necessary (Patindol, Fragallo, Wang, & Durand-Morat, 2017). Rice can be fortified by various technologies, including dusting, coating, and extrusion (Steiger, Müller-Fischer, Cori, & Conde-Petit, 2014). However, the success of rice fortification has remained a technological challenge due to poor nutrient retention rates and high cost (Atungulu, & Pan, 2014). As an alternative to the currently available fortification technologies, fortification of rice by parboiling has been evaluated and is considered an excellent method to produce rice with higher nutrient content and to prevent micronutrient deficiencies worldwide (Patindol et al., 2017).

Parboiling is a traditional way to improve the nutritional value of rice via a three-step hydrothermal treatment that involves the soaking, heating, and drying of rough rice (Bhattacharya, 2004). Inward diffusion of micronutrients from the bran into the endosperm during parboiling contribute to significant higher mineral and vitamin contents of parboiled rice when compared with non-parboiled milled rice (Bhattacharya, 2004; Atungulu & Pan, 2014). By adding fortificants such as minerals and vitamins to the soaking water, nutrient content of rice can be further increased by the parboiling method (Prom-u-thai, Fukai, Godwin, Rerkasem, & Huang, 2008).

However, the conventional parboiling process employs excess water in the soaking step and generates about 1-1.2 L of effluent per kg of paddy (Rajesh, Bandyopadhyay, & Das, 1999). In rice fortification by parboiling, if micronutrients are added to the soaking water, most of the nutrients would enter the water effluent. The wastewater would lead to nutrient overload in soil and therefore present an environmental hazard if discarded into the environment without treatments (Kim, Im, Park, Lee, Benham, & Jang, 2008; Alderson, Santos, & Filho, 2008). The objective of the study was to develop and evaluate a limited water soaking condition for the fortification of rice with minerals and vitamins by parboiling to reduce amount of effluent generated during the parboiling fortification process.

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II. Literature Review

Rice (*Oryza sativa* L.) is one of the most important food crops for over 3.5 billion people worldwide and provides more than 20% of their daily calorie intake (Juliano, 1985; Khush, 2013). However, in its milled form, rice does not contribute significantly to dietary intake of micronutrients due to the removal of micronutrient rich germ and bran layers during milling (Steiger, Müller-Fischer, Cori, & Conde-Petit, 2014). Rice fortification can prevent micronutrient deficiencies in populations that subsist on rice as a dietary staple. Fortification of rice by the parboiling method was shown to produce rice with higher mineral and vitamin contents and can deliver nutrients to a large number of people (Patindol, Fragallo, Wang, & Durand-Morat, 2017).

2.1 Rice Kernel Structure

Kernel structure, an important characteristic of rice, determines physical and chemical properties of the rice grain (Bhattacharya, 2004). Rice is harvested as paddy (rough rice), in which the caryopsis is encased by a tough siliceous hull (husk) (Figure II-1). The caryopsis is a single-seeded fruit, whereas the pericarp, composed of seed coat, nucellus, endosperm, and embryo, is fused to the seed. The hull composes of two modified leaves (lemmae), the palea and the larger lemma. Composition and function of various tissues differ drastically among cultivars, and the cells show compartmentalization of nutrients. (Juliano & Bechtel, 1985).

2.1.1 Hull

The hull plays an important role in protecting the rice caryopsis from pests. It is made of the lemma and palea, which are held together by two hook-like structures (Figure II-1). The lemma is a boat-shaped structure larger than the palea, and largely encloses the caryopsis. Lemma and the palea overlap and interlock with each other at the edges of both structures, which protects the caryopsis from pests before dehulling (Juliano & Bechtel, 1985). The characteristics of the hull such as thickness, tightness of interlocking between lemma and palea, pore size and concentration of silica are important factors in water and nutrient diffusion during soaking of rice (Bhattacharya, 2004). Some paddy varieties have hairy hulls that further impose a barrier for diffusion of water and steam (Luh & Mickus, 1991). Long-grain hybrid varieties are mostly pubescent (covered with hair), while the purelines are non-pubescent (Figure II-2) (Bhattacharya, 2004). The water diffusion coefficient of de-husked brown rice is at least two times greater than that of paddy, which suggests that the hull is one of the major barriers for water diffusion (Oli, Ward, Adhikari, & Torley, 2014).

The outer cells of the hull have thick cell walls due to the presence of silica and other components such as 17.7 to 18.4% pentosans, 31.4% to 36.3% cellulose and 9.5 to 18.4% lignin (Juliano and Bechtel, 1985). Calcium ions were shown to interact with lignin, cellulose and pectin, whereas lignin exhibited a higher capacity for calcium retention, and the mechanism for interaction of lignin and calcium was elucidated to be the formation of stoichiometric bond between them, i.e., one Ca^{2+} ion is bound by two CO_2^- groups in lignin irrespective of intra or inter molecular bond (Torre, Rodriguez, & Saura-Calixto, 1992). The exact stoichiometry was observed in other divalent metal cations with oligomeric fragments of pectin (Kohn, 1987). The large amount of lignin in the husk could be the reason for maximum retention of the minerals during fortification. This was observed in a study where paddy was fortified with zinc and iron during the parboiling process, and most of the iron and zinc were retained in the hull (Prom-u-thai, Huang, Cakmak, & Rerkasem, 2011).

2.2.2 Caryopsis

Brown rice is botanically known as the caryopsis and consists of the seed and the tightly adherent pericarp. The seed consists of a seed coat, nucleus, embryo, and endosperm (Figure II-1). The seed coat, which is also known as testa, is located below the pericarp (Juliano & Bechtel, 1985). These thin layers of pericarp, seed coat and aleurone layer combined is called the bran layer (Champagne, Wood, Juliano, & Bechtel, 2004). Brown rice constitutes 7.2% protein, 2.8% fiber, 2.9% fat, 0.78% ash and 66.4% starch based on 14% moisture and the distribution of these nutrients in bran is 11%, 70%, 51%, 42% and 1%, respectively (Juliano & Bechtel, 1985). Parboiling is a method to naturally improve nutrient content of rice as a result of the inward diffusion of micronutrients from the bran into the starchy endosperm (Juliano, 1993). In rice fortification by parboiling, the nutrients added to the soaking water diffused into the endosperm and were retained even after simultaneous washing, which suggests that the bran layer helps to retain the nutrients inside the rice (Prom-u-thai et al., 2011). However, the bran layer contains phytic acid (5.94 to 6.04 g/100 g) that can bind with multivalent mineral ions (Champagne, 1985), which may be the reason for increased mineral retention in the bran layer and therefore a decrease in mineral content when brown rice is milled to obtain white rice.

The embryo constitutes 1-3% weight of the total kernel (Juliano & Betchel, 1985) and is located at the basal end of the kernel. The starchy endosperm is the largest portion of the rice kernel tightly bound together with the aleurone layer and serves as a reservoir for the germinating seedling and as a food source for humans and animals (Krishnan, Ebenezer, Dayanandan, 2001). The bulk of the endosperm consists of 77-89% starch, 4.5-10.5% protein and 0.3-0.5% lipid (Juliano & Bechtel, 1985).

2.3 Rice Chemical Composition

2.3.1 Starch

Removal of the bran layer results in milled rice that is composed of approximately 90% of starch on a dry weight basis. Starch in rice occurs as compound granules of 3 to 8 μm in size and angular in shape. The amylose content of starch may vary from 8 to 37% for non-waxy rice and 0.8 to 1.3% for waxy rice (Champagne et al., 2004). Generally, a higher amylose content of rice is associated with a firmer cooked rice texture; rice with a medium amylose content cooks softer and stickier (Ong & Blanshard, 1995).

Five stages that occur during heating of starch in excess water include glass transition, gelatinization, swelling, pasting and retrogradation (Patindol, Newton, & Wang, 2008). During glass transition the amorphous lamellae changes from glassy to rubbery in the presence of water (Fitzgerald, 2004). Water lowers the glass transition temperature, hence in the presence of high moisture content, glass transition occurs at lower temperature (Biliaderis, Page, Maurice, & Juliano, 1986). The melting of the crystalline lamellae is called gelatinization, which is an important property of rice due to its strong correlation with cooked rice texture and time required for cooking (Maningat & Juliano, 1979). Rice with a lower gelatinization temperature takes less time to cook and is usually softer than rice with a higher gelatinization temperature. Once gelatinized, starch granules begin to swell and increase in volume to cause an increase in viscosity, which is termed pasting and accompanied by leaching of amylose and amylopectin into the continuous phase (Mizukami & Takeda, 2000; Cameron & Wang, 2005).

Cooked rice texture is one of the most important quality attributes of rice and is mainly affected by amylose content (Juliano, 1981; Juliano, Onate, & del Mundo, 1972; Sowbhagya, Ramesh, & Bhattacharya, 1987; Champagne et al. 1999) and the cooking method (Juliano & Perez, 1984; Leelayuthsoontorn & Thipayarat, 2006; Srisawas & Jindal, 2007). Cameron & Wang (2005) found that the more the leaching of amylose during cooking the greater the hardness and lower the adhesiveness of the cooked rice as a result of reassociation of the leached amylose, also known as starch retrogradation. The amount of amylose content in the cooking water was significantly dependent on milling duration and cultivar but there was no significant increase in amylose content in the cooking water when water to rice ratio was increased (Altheide, Morawicki, & Hager, 2011). It was observed that during fortification of rice with minerals by soaking, the fortified rice obtained harder texture compared to non-fortified rice. This may be caused by the entrapment of the mineral and formation of complexes with the amylose polymers (Lee, Hettiarachchy, McNew, & Gnanasambandam, 1995).

2.3.2 Protein

Proteins in rice are predominantly present in the endosperm in the form of protein bodies. The protein content ranges from 4.3 to 18.2 % in brown rice and 4.5 to 10.5 % in milled rice (Juliano & Bechtel, 1985). The protein content in rice significantly influences the structural and functional properties such as texture, pasting property and sensory characteristic (Shih, 2004). Proteins act as barriers for starch swelling due to disulfide bonds resulting in higher pasting viscosities (Hamaker & Griffin, 1990). While cooking rice in excess water, there were losses of protein in the cooking water, however the loss was not dependent on the water-to-rice ratio (Altheide et al., 2011). The loss of protein in rice during fortification process has not been reported. There are mainly two types of protein present in rice, rice-endosperm protein (REP) and rice-bran protein (RBP) with REP comprising four amino acids including globulin (40%), albumin (31%), prolamin (21%), and glutelin (5%) (Helm & Burks, 1996; Shih, 2004). When minerals such as calcium, iron and zinc where added to rice bran slurry it was noted that the minerals interacted with albumin to yield insoluble mineral-albumin complexes. It was also found that the addition of these minerals to the bran slurry decreased the protein solubility (Champagne, 1985).

2.3.3 Lipids

Lipids in rice are generally classified into non-starch lipids or surface lipids, which are mainly present in the bran layer, and starch lipids present in starch granules (Juliano & Bechtel, 1985). The starch lipids constitute a relatively small proportion of the total lipid content but play a major role in starch synthesis and functionality (Morrison, 1995). Lipids form complexes with amylose, which increase gelatinization temperature and cooking duration but decrease viscosity (Kaur & Singh, 2000). The rice milling process removes the bran and the outer layer of rice kernels and therefore lowers the lipid content of the remaining kernel (Perdon, Siebenmorgen, Mauromoustakos, Griffin, & Johnson, 2001). Therefore, the degree of milling (DOM) determines the shelf life of milled rice based on the content of non-starch lipids present on the surface, which undergoes hydrolytic and oxidative rancidity after milling (Chen, Marks, & Siebenmorgen, 1997).

2.4 Micronutrient Deficiencies Worldwide

Micronutrients are essential vitamins and minerals that are needed in small quantities and play an important role in physical growth and mental development. Inadequate intake of micronutrients has adverse effects on human body function and can result in many diseases and contribute to high morbidity and mortality rates (Black, 2003; World Health Organization (WHO), 2006). In poorer regions of the world, people rely on monotonous diets based on cereals, roots, and tubers, whereas micronutrient-rich foods are only consumed in small amounts (WHO, 2006). Micronutrient malnutrition, also referred to as “hidden hunger”, due to lack of micronutrients in the diet affects more than 3 billion people worldwide and therefore remains a major public health problem in the industrialized nations and even more in developing countries (WHO, 2006; Atungulu & Pan, 2014). In developing countries, approximately 800 million people experience starvation leading to undernourishment and multiple micronutrient deficiency disorders (Díaz, de las Cagigas, & Rodríguez, 2003; Khush 2005). Although all age groups can be affected by micronutrient deficiency, pregnant women and children are at greatest risk of developing deficiencies (Ritchie & Roser, 2017). In every African country, approximately 20% of all women of reproductive age suffer from anemia which is highly associated with iron deficiency, and despite noteworthy progress, about 58 million children are stunted due to malnutrition (World Food Programme (WFP), 2018) Globally, iron, vitamin A and iodine deficiency are the most prevalent micronutrient deficiencies affecting at least one third of the world's population. Furthermore, zinc, folate and vitamin D deficiency contribute significantly to the global burden of disease and calcium deficiency due to lack of dairy intake has become of public concern in developing as well as affluent countries (WHO, 2018).

2.4.1 Iron Deficiency

A diet based on cereals, legumes, roots or tubers leads to insufficient iron intake as iron is predominantly present in animal sources (meat, poultry and fish); and high intake of phytate and phenolic compounds through consumption of legumes, cereals, coffee, tea, sorghum and millet lead to poor iron absorption in the body (WHO, 2018). Iron deficiency is the most common micronutrient deficiency widespread in both industrialized and non-industrialized countries leading to microcytic anemia, decreased cognitive capacity for work, and impaired immune and endocrine function (WHO, 2006, 2018; Bailey, West Jr., & Black, 2015). Iron is an essential component of hemoglobin, myoglobin, enzymes, and cytochromes, thus plays an important role in the transport of oxygen and cellular respiration and is essential for optimal growth and cognitive function (Food and Nutrition Board, 2001). Iron deficiency as a result of a long-term negative iron balance causes anemia and about 40% of the world's population is estimated to suffer from iron-deficiency anemia (WHO, 2018). Particularly pregnant women and pre-school children are at high risk of iron deficiency due to increased requirements for fetal growth and development and is associated with low birth weight, premature delivery, and perinatal complications (de Benoist, McLean, Egli, & Cogswell, 2008). To prevent and cure symptoms of iron deficiency, fortification programs using flours, dairy products, condiments, sugar, and salt as food vehicles, have been implemented in many countries (Bailey et al., 2015). In the United States, iron supplementation during pregnancy reduced the number of preterm deliveries and low birth-weight infants significantly (Cogswell, Parvanta, Ickes, Yip, & Brittenham, 2003).

2.4.2 Calcium Deficiency

Calcium is the most abundant mineral in the body, about 99% (1000-1200 g) of which is located in the skeleton (Dietary Reference Intakes, 2010). It plays a major role in maintaining rigidity and strength of the skeleton and is involved in several metabolic processes including blood clotting, cell adhesion, muscle contraction, hormone and neurotransmitter release, glycogen metabolism and cell proliferation and differentiation (WHO, 2006, 2018). Recommended dietary allowances (RDAs) support a daily dietary intake of 1,000 mg of calcium (Dietary Reference Intakes, 2010). Dairy products including milk, yogurt, and cheese are rich sources of calcium making up the majority of calcium intake in the general diet in the United States and Canada (Dietary Reference Intakes, 2010). However, many countries in Asia, Africa and South America have average dietary calcium intakes as low as 400-700 mg/day (Balk et al., 2017). Low calcium intake is mostly associated with osteoporosis, a disease characterized by increased skeletal fragility and susceptibility to fractures. Therefore, adequate calcium intake is important throughout the whole life span, especially during periods of rapid skeletal growth in childhood and adolescence and for post-menopausal women and the elderly (WHO, 2006). Furthermore, lactose-intolerant individuals are at high risk of calcium deficiency because they avoid dairy products (Suchy et al., 2010). Thus, increasing calcium intake through supplementation and food fortification, especially in countries were the consumption of dairy products is low, is essential (WHO, 2018).

2.4.3 Folic Acid Deficiency

Folic acid (vitamin B_9) is a water-soluble B vitamin naturally present in a wide variety of foods including green leafy vegetables, fruits, nuts, beans, peas, seafood, eggs, dairy products, meat, poultry, and grains (Carmel, 2005; U.S. Department of Agriculture, 2019). Folic acid is necessary for the synthesis and methylation of nucleotides involved in cell multiplication and tissue growth. Inadequate folate intakes are primarily linked to high risk of giving birth to infants with neural tube defects along with other birth defects. Moreover, folic acid deficiency can result in cardiovascular diseases, cancer, and impaired cognitive function in adults (WHO, 2006). Most neural tube defects, however, can be prevented if women consume sufficient amounts of folic acid, a synthetic form of folate, prior to and during the pregnancy (De-Regil, Peña-Rosas, Fernàndez-Gaxiola, & Rayco-Solon, 2015; Zimmerman, 2011). Fortification of staple foods and supplementation for target groups have been implemented in many countries to increase folic acid intake (Rogers et al., 2018).

2.4.4 Vitamin A Deficiency

Vitamin A (retinol), a fat-soluble vitamin, is required by humans for the functioning of the visual system, the maintenance of cell function for growth, epithelial cellular integrity, immune function, and reproduction. Foods from animal sources are rich in vitamin A and foods from vegetable origin contain provitamin A carotenoids, such as β-carotene, which are converted into retinol by the intestinal mucosa and the liver (WHO, 2006). Whereas these foods typically provide dietary requirements for vitamin A, changing lifestyles and diet patterns shifting from the consumption of fresh meat, nuts, fruits and leafy greens (characteristics of early humans) towards higher intake of cereals and grains have resulted in a decreased intake of bioavailable vitamin A (Underwood, 2000). Symptoms of vitamin A deficiency are non-specific but include night blindness and xerophthalmia (WHO, 1996). Vitamin A deficiency is also associated with an increased risk of child mortality as a result of diarrhea and measles (WHO, 2006). Worldwide, about 3 million preschool-aged children were diagnosed with impaired visual function due to vitamin A deficiency, and the WHO estimates that 254 million preschool-aged children have low serum retinol levels (WHO, 2006). Vitamin A supplementation or fortification decreased all-cause mortality in children aged between 6 months and 5 years by 23% (Beaton et al., 1992). Maternal mortality was reduced by 40% and 49%, when vitamin A-deficient pregnant women received vitamin A or β-carotene, respectively (West et al., 1999). Synthetic β-carotene in oil and other synthetic forms of β-carotene commonly used to fortify foods have conversion rates to retinol of 2:1 and 6:1, respectively (Institute of Medicine, 2001; WHO, 2004).

2.5 Fortification of Rice

Producing foods rich in micronutrients is a common strategy to combat micronutrient deficiencies (de Pee et al., 2000). To increase levels of certain nutrients and/or to restore nutrients lost during food manufacturing such as washing or milling, food is fortified by adding micronutrients in certain processing steps (Piccoli et al., 2012). The fortification of staple foods, such as wheat flour, maize, or rice, has been considered a very cost-effective way to provide more nutritious food to a large number of people and is recommended because they are consumed in large quantities (WHO, 2006; Bezanson & Isenman, 2010). Therefore, fortified staple crops can improve nutrient intake without the need for changes in the eating behavior (Prom-u-thai, Fukai, Godwin, Rerkasem, & Huang, 2008; Piccoli et al., 2012).

White rice is the number one staple food in countries of southeast and northeast Asia, and in several African and South American countries and contributes to up to 30% of the daily calories consumed in most developing countries, which can increase to more than 70% in some low-income countries (FAO, 2017). Thus, rice is considered a potentially excellent vehicle for delivering micronutrients to a large section of the population to significantly alleviate micronutrient deficiencies (Steiger et al., 2014). Industrial fortification of rice with vitamins and minerals has been done in several countries where rice is a staple (WHO, 2018), however, it remains a technological challenge (Muthayya et al., 2012). Four main technologies for rice fortification, including dusting, coating, hot extrusion, and cold extrusion, have been developed (Muthayya et al., 2012; Steiger et al., 2014), and the method chosen depends on the local technology available, costs, and consumer preferences (WHO, 2018).

2.5.1 Dusting

Rice fortification by dusting, also known as powder enrichment, involves the addition of a pre-blended mixture of vitamins and minerals to the kernel surface that adheres to the grains by electrostatic forces (Dexter, 1998, Muthayya et al., 2012). However, dusting is considered inefficient due to high losses of micronutrients during washing and cooking in excess water (Muthayya et al., 2012, Steiger et al., 2014). Depending on the amount of water used and the cooking time, about 20-100% of the enrichment can be lost during washing and cooking (Hoffpauer, 1992). Powder enrichment is inexpensive compared to other rice enrichment methods, but is not recommended in developing countries, where intensive rice washing is practiced (Atungulu & Pan, 2014). Dusting of rice is commonly practiced in the U.S., and the label on the packaging warns consumers not to rinse the rice before cooking and not to cook in excess water (Steiger et al., 2014). To overcome limitations associated with dusting, three more sophisticated methods, including coating, hot and cold extrusion have been developed (Atungulu & Pan, 2014).

2.5.2 Coating

Enrichment of rice by coating is typically accomplished by the addition of a blend of highly concentrated vitamins and minerals to rice, followed by a water-insoluble food-grade coating substance (Dexter, 1998). Many different coating materials have been evaluated, including waxes, acids, gums, starches, and cellulosic polymers such as hydroxypropyl methylcellulose, ethyl cellulose, and methylcellulose. Ethyl cellulose or pectin-coated kernels have been shown to lower cooking and washing losses by 61% (Shrestha, Arcot, & Paterson, 2003). Coating is mainly practiced in the U.S., Costa Rica, and the Philippines (Steiger et al., 2014), but the major problems with this fortification technology are associated with color, odor, and taste of the fortified rice.

2.5.3 Hot extrusion

Extruded rice has the advantage that vitamins and minerals are incorporated into the rice matrix rather than adhering to the kernel surface (Atungulu & Pan, 2014). Hot extrusion is considered a highly sophisticated rice fortification technique to obtain high-quality fortified rice with high micronutrient retention. It involves the addition of nutrients and water to a dough of rice flour that is pressed through an extruder at 80-110°C, followed by slicing into grain-like structures similar to rice kernels (Steiger et al., 2014). However, high-shear and temperatures create a puffy and porous kernel structure that results in a rice texture that is significantly different from natural rice kernels, thus may not be acceptable in certain populations (Steiger et al., 2014). Hot extrusion is associated with high capital investment, but relatively low operating costs. In addition, high temperatures and pressure during the extrusion process can also lead to vitamin destruction (Atungulu & Pan, 2014).

2.5.4 Cold extrusion

In the cold extrusion process, a premix of rice flour, fortificant, binders, moisture barrier agents, and water are passed through a simple pasta press at normal temperatures (Steiger et al., 2014). Temperatures used in hot extrusion exceed melting temperatures of starch, whereas cold extrusion takes place at temperatures above glass transition but below starch melting temperatures (Mercier, Linko, & Harper, 1989; Steiger et al., 2014). Reconstituted fortified rice kernels produced by cold extrusion are similar to those obtained by the hot extrusion technology but are slightly laced with an opaque appearance. Consumer acceptance of cold extruded rice is also a concern because the products may be similar in texture to pasta and noodles rather than natural long-grain rice. Cold extrusion has lower start-up and capital costs, but has relatively high running costs compared to hot extrusion processing (Atungulu & Pan, 2014).

2.6 Parboiling

Vitamins and minerals, predominantly present in the outer germ and bran layers, are lost during rice milling (WHO, 2018). A process called rice parboiling has been practiced in many parts of the world to naturally improve rice nutrient content and milling yield of milled rice (Kumar, Singh, Chauhan, Chandra, Kumar, & Yadav, 2018). About 15-20% of the rice produced worldwide is in parboiled form (Buggenhout, Brijs, Celus, & Delcour, 2013a). Parboiling induces diffusion of nutrients from the bran to the inner endosperm layer by a three-step hydrothermal treatment of rough or brown rice that involves the soaking, steaming, and drying (Bhattacharya, 2004; Atungulu & Pan, 2014).

2.6.1 Soaking

Soaking, the first step in parboiling, facilitates hydration of the rice kernels. The rice kernel consists of pores and cracks which act as channels during water diffusion (Oli, Ward, Adhikari, & Torley, 2014). During hydration in the glassy phase, water diffuses into pores and cracks of the endosperm; and when the temperature exceeds the glass transition temperature, starch granules swell and increase in volume (Kashaninejad, Maghsoudlou, Rafiee, & Khomeiri, 2007). Hydration rate is affected by several factors including grain surface area, presence or absence of hair in the hulls and grain gelatinization temperature (Bhattacharya, Sowbhagya, & Swamy, 1972). During soaking, the rice absorbs up to 30% moisture that limits the undesirable "white core", a characteristic of rice kernels containing ungelatinized starch (Bhattacharya, 2004). Water distribution and penetration through the rice kernels can vary greatly depending on morphological structure of the grain, such as the packing of starch granules in the endosperm (Horigane, Takahashi, Maruyama, Ohtsubo, & Yoshida, 2006). Rice chemical composition also influences moisture absorption during soaking (Sittipiod & Shi, 2016). Proteins (Derycke et al., 2005; Buggenhout, Brijs, & Delcour, 2013b) and amylose-lipid complexes (Tester & Morrison, 1990) restrict swelling of starch granules and therefore cause uneven distribution of water and reduced level of moisture inside rice kernels (Maurice, Slade, Page, & Sirett, 1985). Traditionally, rice is soaked in water at ambient temperatures overnight (Atungulu & Pan, 2014). However, more sophisticated soaking techniques involve higher temperatures but shorter soaking durations to avoid fermentation and adverse effects on color, taste, and smell of the product (Luh & Mickus, 1991). The rate of hydration increases with increasing temperature, however if the soaking temperature is above the gelatinization temperature the rate of hydration increases exponentially and when moisture exceeds 30-32% splitting of husk occurs, which is accompanied by leaching and deformation of rice (Bhattacharya & Subba Rao, 1966). Hence, soaking at a high temperature should be strictly monitored such that the grains reach a moisture content of ~30% (Bhattacharya et al., 1972). Sufficient quantities of water absorbed during soaking ensure complete gelatinization of starch during the steaming step (Luh & Mickus, 1991).

2.6.2 Steaming

Steaming not only increases milling yield, improves storage characteristics and eating quality, but also achieves better vitamin retention in milled rice (Luh & Mickus, 1991). Steaming induces irreversible swelling and gelatinization of starch granules (Rao & Juliano, 1970; Ali & Bhattacharya, 1980), disruption of protein bodies (Rao & Juliano, 1970), formation of lipid-amylose complexes (Biliaderis, Tonogai, Perez, & Juliano, 1993), and inactivation of enzymes (Barber, De Barber, & Novo, 1983). Parameters including time exposed to steam, quantity of water absorbed, and steam temperature determine quality of the parboiled rice (Luh & Mickus, 1991). Steaming parameters also affect color of milled parboiled rice (Bhattacharya & Subba Rao, 1966). Heat induced color changes caused by chemical and physical transformations, including Maillard browning reactions, lead to darker color of parboiled rice compared to unparboiled rice (Luh & Mickus, 1991).

2.6.3 Drying

Drying of parboiled rice is essential to reduce moisture content to an optimal level for milling and subsequent storage. After parboiling, the rice contains around 35% moisture and must be dried to about 14% moisture for safe storage and milling (Luh & Mickus, 1991). Method of moisture removal during drying has a profound influence on milling quality (Bhattacharya, 2004). Breakage can be higher when dried with hot air or in the sun compared to air drying at room temperature. A steep moisture gradient and cooling causes the grain to break after drying, whereas tempering following drying can prevent the grain from damage (Cnossen & Siebenmorgen, 2000).

2.7 Fortification of Rice by Parboiling

Rice fortification by parboiling has been conducted on lab and pilot scale with minerals and vitamins including iron (Prom-u-thai et al., 2008; Prom-u-thai, Rerkasem, Fukai, & Huang, 2009; Thiruselvam, Cheong, Mohan, Paterson, & Arcot,, 2014; Patindol et al., 2017), calcium (Lee et al., 1995; Sirisoontaralak, Limboon, Jatuwong, & Chavanalikit, 2016), zinc (Prom-u-thai et al., 2011; Hotz, Kabir, Dipti, Arsenault, & Bipul, 2015; Patindol et al., 2017; Wahengbam, Green, & Hazarika, 2019), iodine (Tulyathan, Laokuldilok, & Jongkaewwattana, 2006), folic acid (Kam, Murray, Arcot, & Ward, 2012a, b; Kam, Arcot, & Adesina, 2012c; Thiruselvam et al., 2014) and β-carotene (Thiruselvam et al., 2014). Existing infrastructure, marketing networks, and great consumption of parboiled rice in most developing countries in Asia and Africa make parboiling a cost-effective method of rice fortification (Patindol et al., 2017). Minerals and vitamins added to the soaking water diffuse into the rice kernel during soaking and are pushed from the husk or bran layer into the endosperm in the steaming step (Patindol et al., 2017). Nutrient uptake of rice is affected by parboiling feedstock (brown or rough rice) (Patindol et al., 2017), fortificant concentration (Prom-u-thai et al., 2011; Kam et al., 2012a; Hotz et al., 2015; Patindol et al., 2017), and soaking duration (Patindol et al., 2017). Using brown rice as parboiling feedstock was shown to improve uptake of minerals during the parboiling process, whereas rice cultivar had little to no impact on the retention of minerals (Patindol et al., 2017). In fortification by parboiling with minerals and vitamins, a positive correlation was found between initial fortificant concentrations in the soaking solution and final nutrient content of milled fortified rice (Prom-u-thai et al., 2011; Kam et al., 2012a; Patindol et al., 2017). Sensory characteristics including kernel color and texture were not negatively affected by the fortification parboiling process (Prom-u-thai et al., 2009; Kam et al., 2012a; Patindol et al., 2017), and consumer acceptance of fortified parboiled rice was not different from non-fortified parboiled rice (Prom-u-thai et al., 2009; Kam et al., 2012b).

2.8 Fortified Rice Specifications

According to Code of Federal Regulations (CFR), the U.S. Food & Drug Administration (FDA) has established requirements for enriched rice. The standards provided by the FDA are based on final concentration in milled rice. Concentrations of fortificants permitted for each pound of fortified rice are listed in Table II-1. In the case of enriched parboiled rice, butylated hydroxytoluene (BHT) may be added as an optional ingredient in an amount not to exceed 0.0033% by weight of the finished food as an antioxidant (FDA, 2017).

2.9 References

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2.10 Tables and Figures

Table II-1 Concentrations of fortificants in fortified rice established by FDA.

Fortificant	Minimum Concentration	Maximum Concentration
Folic Acid	0.7 mg/lb	1.4 mg/lb
Iron	13 mg/lb	26 mg/lb
Vitamin D	250 United States Pharmacopeia (U.S.P.) units/lb	1000 U.S. P. units/lb
Calcium	500 mg/lb	1000 mg/lb

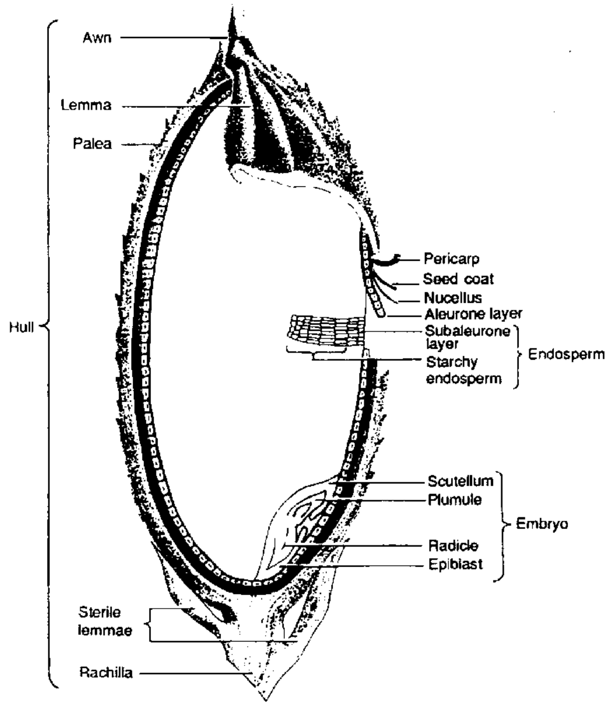

Figure II-1 The longitudinal section of a rice grain (Juliano & Bechtel, 1985).

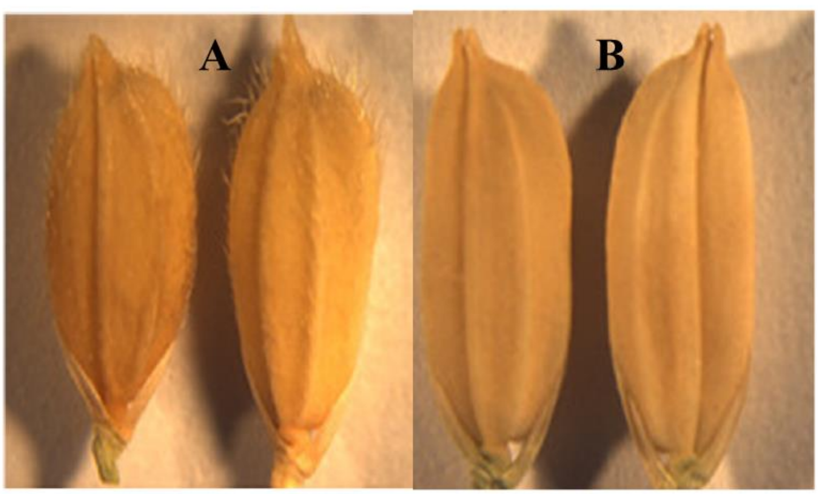

Figure II-2 Rough rice with hairy surface (A) and rough rice with non-hairy surface (B) (Bhattacharya, 2004).

III. Development of a Limited-Water Soaking Method on the Fortification of Rice with Calcium and Iron by Parboiling

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3.1 Abstract

Fortifying rice using parboiling has been shown to have a higher retention rate of nutrients and acceptable sensory characteristics. However, the conventional soaking step employs excess water and therefore generates large amounts of wastewater containing nutrients that, when discarded without treatment, can lead to nutrient overload in soil. A limited-water soaking method to fortify rice with calcium and iron by parboiling was developed and evaluated. Fortified rice quality attributes, mineral contents, the amount of wastewater, total solids in wastewater, washing-retention and bioavailability of calcium and iron were determined and compared with properties of rice that was fortified in excess-water soaking. The limited-water soaking method on average reduced the amount of effluent by 89% and the amount of total solids in wastewater by up to 85%. Rice fortified by the limited-water soaking method showed similar color and head rice yield, and slightly lower mineral uptakes compared to conventional soaking. Rice fortified simultaneously with calcium and iron in limited-water soaking contained about 999 mg Ca and 33 mg Fe per kg milled rice, and approximately 66% of calcium and 71% of iron remained in the rice after simulated washing. Calcium and iron dilute HCl-solubility was not affected by the soaking condition. Therefore, the limited-water soaking method can reduce the cost of fortification and wastewater treatment without affecting rice quality attributes.

3.2 Introduction

Milled rice, although a staple food for over half of the world's population, does not contribute significantly to dietary intake of micronutrients due to the removal of micronutrient-rich germ and bran layers during milling. Populations consuming milled rice as the staple food may, therefore, suffer from multiple micronutrient deficiencies. The most frequent nutritional disorder in both developing and developed countries is iron deficiency (Diaz, de las Cagigas, & Rodriguez, 2003), which can result in microcytic anemia, decreased cognitive capacity, as well as impaired immune and endocrine functions (McLean, Cogswell, Egli, Wojdyla, & de Benoist, 2008). Furthermore, populations relying on a cereal-based diet are more likely to encounter calcium deficiency because cereals are not a good source of calcium (Sirisoontaralak, Limboon, Jatuwong, & Chavanalikit, 2016). Recommended dietary allowances (RDAs) support a daily dietary intake of 1,000 mg of calcium (Dietary Reference Intakes, 2010), whereas predominantly rice-consuming populations in Africa and South America average a dietary calcium intake of ~400 to 700 mg/day and many in Asian countries consume less than 500 mg/day (Balk et al., 2017).

Rice is considered a highly effective tool for food fortification and has great potential to complement other interventions, such as supplementation and dietary diversification, whereas changes in a population's eating habits are not necessary (Muthayya et al., 2012). Recently, rice fortification by parboiling has been evaluated as an alternative to the currently available fortification technologies including dusting, coating, and extrusion (Lee, Hammer, & Eitenmiller 2000; Patindol, Fragallo, Wang, & Durand-Morat, 2017; Steiger, Müller-Fischer, Cori, & Conde-Petit, 2014). Dusting and coating technologies involve adhesion of nutrients to the surface of the rice, which leads to low retention rate due to washing or cooking of rice with excess water (Steiger et al., 2014). Extruded fortified rice has a higher micronutrient retention but is more expensive and reconstructed rice kernels have a different appearance than normal parboiled rice (Muthayya et al., 2012). Parboiled fortified rice was shown to have higher nutrient retention rate and acceptable sensory characteristics (Hotz et al., 2014; Prom-u-thai et al., 2008; Patindol et al., 2017; Sirisoontaralak et al., 2015). Wahengbam, Green, & Hazarika (2019) demonstrated that fortification of low-amylose rice with zinc by parboiling is a cost-effective method to produce parboiled rice that can rapidly and economically enhance the amount of Zn intake in a rice-based diet.

Parboiling is a traditional way to improve the nutritional value of rice via a three-step hydrothermal treatment that involves the soaking, steaming, and drying of rough rice (Bhattacharya, 2004). By adding fortificants such as minerals and vitamins to the soaking water, rice can be fortified using the parboiling method. Thiruselvam, Cheong, Mohan, Paterson, & Arcot (2014) showed that simultaneous fortification of rice with folic acid, β-carotene and iron by parboiling was feasible. About 12% of the added folic acid and β-carotene, and 46% of iron diffused into the endosperm during the fortification process. According to the Food and Drug Administration (FDA), enriched rice may contain 1,111-2,222 mg Ca/kg and 29-59 mg Fe/kg of milled rice (FDA, 2019). Higher soaking temperatures and fortificant concentration lead to higher calcium uptake by the rice (Sirisoontaralak et al., 2016). Calcium-fortified rice containing about 1,340 mg Ca/kg of milled rice was shown to have a firmer texture than non-fortified parboiled rice (Lee, Hettiarachchy, McNew, & Gnanasambandam, 1995). Prom-u-thai, Fukai, Godwin, Rerkasem, & Huang (2008) fortified rice using a mix of iron sulfate ($FeSO_4$) and ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) in a 2:1 molar ratio, yielding fortified rice containing 144 mg Fe/kg. Panelists were not able to detect significant differences in the fortified rice soaked in 250 mg Fe/kg paddy rice when compared to non-fortified parboiled rice;

however, a higher Fe level (450 mg Fe/kg) resulted in more yellow rice kernels and the development of off-flavors (Prom-u-thai, Rerkasem, Fukai, & Huang, 2009). Patindol et al. (2017) investigated the effects of feedstock, parboiling conditions, and fortificant concentrations on rice fortified by parboiling with iron and zinc simultaneously. Brown rice was shown to be a better feedstock than rough rice and soaking brown rice in 200 mg Fe/kg paddy rice increased Fe content to about 75.0 mg Fe/kg of milled rice. In these studies, the iron content of fortified rice was higher than the FDA requirements.

The conventional parboiling process employs excess water in the soaking step and generates about 1-1.2 L of effluent per kg of paddy (Rajesh, Bandyopadhyay, & Das, 1999). In rice fortification by parboiling, if micronutrients are added to the soaking water, most of the nutrients would enter the water effluent. When discharged into the environment without treatments, the wastewater would lead to nutrient overload in soil and therefore present an environmental hazard (Kim, Im, Park, Lee, Benham, & Jang, 2008; Alderson, Santos, & Filho, 2015). The establishment and maintenance of an effluent treatment plant in rice mills to ensure that effluent meets disposal standards are expensive and often disregarded (Business Standard, 2015). Thus, there is a need to reduce the soaking water used to decrease the cost of effluent treatment and to make the parboiling process viable for fortifying rice. The objective of the study was to develop and evaluate a limited-water soaking condition for the fortification of rice with calcium and iron by parboiling. The milling quality, mineral content, mineral retention and solubility, and kernel color of fortified rice by parboiling in excess water and limited-water were analyzed and compared.

3.3 Materials and Methods

3.3.1 Rice cultivar

Patindol et al. (2017) demonstrated that brown rice is a better feedstock than rough rice in mineral fortification by parboiling. Therefore, brown rice was used in this study. Rough rice (2017 crop; ~12% moisture content) of a long-grain pureline cultivar, RoyJ, was provided by the University of Arkansas Rice Research and Extension Center in Stuttgart, AR. The sample was cleaned using a dockage tester (Carter-Day Company, Minneapolis, MN), and then sorted using a thickness grader with a screen size of 1.74 mm to remove thin kernels and maintain kernels of uniform size. Rough rice was dehulled using a Satake THU-35 dehusker (Satake Corporation, Hiroshima, Japan) to obtain brown rice. The onset gelatinization temperature of the brown rice flour was 70.9°C, as determined by a differential scanning calorimeter (model Diamond, Perkin-Elmer Co., Norwalk, CT, USA).

3.3.2 Fortification-parboiling process

Food grade calcium lactate ($C_6H_{10}CaO_6$) from Spectrum Chemical MFG Corp (Gardena, CA), and ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$) from Fisher Scientific (Fair Lawn, NJ) were used as the source of calcium (Ca) and iron (Fe), respectively. The fortification solutions of 50 g/L calcium lactate (~9.2 g/L pure Ca), and 995 mg/L ferrous sulfate (~200 mg/L pure Fe) were selected according to results by Lee et al. (1995), Patindol et al. (2017), and Sirisoontaralak et al. (2016).

Brown rice was parboiled in the two individual solutions and their combination using both excess and limited-water soaking conditions. The equilibrium moisture content was 40.5% when brown rice was soaked at 65°C (Newton et al., 2011); thus, 50% of water was added to brown rice for limited-water soaking in order to ensure full hydration of the rice. The conventional excess water soaking method was achieved by soaking 1 part of brown rice (100 g) in 2 parts of water (200 g) in a 1000-mL beaker. Limited-water soaking was achieved by adding 0.5 parts of water (50 g) to 1 part of brown rice (100 g) in a CLARITY^{TM} vacuum pouch (10”×12”, 3 mm thick, Riverside, MO). Prior to vacuum sealing at 95% vacuum, rice was spread evenly in a thin layer inside the pouch. Soaking and steaming conditions were the same for both excess- and limited-water parboiling. Samples were placed in a preheated water bath (Boekel/Grant ORS-200, Boekel Scientific, Feasterville, PA) at 67°C for 3 hr. The soaking temperature was about 3°C below onset gelatinization temperature to prevent starch gelatinization during soaking. After soaking, the excess solution was carefully collected and weighed, and the soaked rice was steamed in an autoclave (Brinkmann 2340E, Tuttnauer USA Co. Ltd., Hauppauge, NY) at 120°C for 10 min. Steamed rice was dried in an EMC chamber at 65% relative humidity and 26°C to ~12% moisture content. All trials were conducted in duplicate.

3.3.3 Head rice yield

The parboiled brown rice sample was milled using a laboratory mill (McGill Miller #2, Rapsco, Brookshire, TX) for 60 s and separated (Grainman, Grain Machinery Mfg., Miami, FL) into broken kernels, head rice, and bran. Head rice was aspirated for 2 min to remove residual bran using an aspirator (Seedburo Equipment Company, Chicago, IL) (Patindol et al., 2017). Head rice yield (HRY) was calculated by dividing head rice mass over brown rice mass and expressed as percentage.

3.3.4 *Kernel color and white belly*

Head milled rice color was measured using a colorimeter (ColorFlex, Hunter Associates Laboratory, Reston, VA). The whiteness (*L**), redness *(a*)*, and yellowness *(b*)* values were taken as averages of the two readings for each sample, (Patindol et al., 2017). The percentage of white belly was measured using image analysis (WinSEEDLE Pro 2005a; Regent Instruments, Sainte-Foy, Quebec, Canada). The software calibration was done according to Shad, Steen, Devlieghere, Mauromoustakos, & Atungulu (2019). Approximately 100 head rice kernels were spread on an acrylic tray holder (152 × 100 × 20 mm), and prior to scanning, a blue plastic tray was placed on top of the tray holder to provide background. The kernels were digitally scanned to determine the amount of white belly within each kernel, which was expressed as the ratio of the white belly area to the entire scanned area of 100 kernels.

3.3.5 *Determination of Ca and Fe contents*

Head rice was ground into flour using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO) fitted with a 0.5-mm screen. Flour and bran were analyzed for Ca and Fe with inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Spectro Arcos FHS16 (Spectro Analytical Instruments GmbH, Kleve, Germany) according to Patindol et al. (2017).

3.3.6 *Mineral retention and solubility*

The retention and solubility of minerals in parboiled rice after washing were determined by following the methods described in Patindol et al. (2017). Head rice (~2 g) was added to a 50-mL plastic centrifuge tube with 20 mL of ultrapure water, and vortexed for 10 s at a high speed. The wash-water was carefully discarded prior to repeating the procedure two more times. Washed head rice was transferred into a 50-mL polystyrene weighing boat lined with Whatman filter paper #1. After 2 min, the filter paper was removed, and the washed head rice was dried at 40°C for 50 min to approximately 10% moisture content. For mineral solubility, head rice flour (2 g) was added into a 50-mL plastic centrifuge tube, added with 35 mL of 0.1 N HCl (pH = 1.6), vortexed, and incubated in a water-bath shaker at 37°C and 100 rpm oscillation for 30 min. After centrifuging at 1,000 × g for 10 min, the supernatant was discarded, and the residue was dried in an oven at 40°C overnight. The dried samples were analyzed for Ca and Fe by ICP-OES. Mineral retention and mineral solubility were calculated as follows:

$$Retention\ (\%) = \frac{\text{Mineral Content of Washed Rice}}{\text{Original Mineral Content (Unwashed Rice)}} \times 100$$

$$Solubility\ (\%) = \frac{(\text{Original Mineral Content} - \text{Residual Mineral Content})}{\text{Original Mineral Content}} \times 100$$

3.3.7 Statistical analysis

All parboiling conditions were conducted in duplicates for each fortificant solution and analyzed with JMP Pro Version 14 (SAS Software Institute, Cary, NC, USA) using analysis of variance (Tukey's HSD test and Student's t-test) with significance level at $P<0.05$. Two-way analysis of variance (ANOVA) was used to establish the effect of fortificant, soaking condition, and their interaction on parboiled rice quality attributes at $P<0.05$.

3.4 Results and Discussion

3.4.1 Soaking conditions and fortificant on parboiled rice properties

Head rice yield (HRY) is an economically important trait of commercial rice because the price of rice for whole kernels is typically twice that of broken kernels (Patindol et al., 2017). The HRY of parboiled rice ranged from 84.2 to 89.1% and was not affected by the limited-water soaking condition (Table III-1). Thus, using the limited-water soaking method in the fortification process did not have a negative impact on the breakage susceptibility of fortified parboiled rice when compared with conventional excess-water soaking.

Color of parboiled rice determines consumer acceptability and market value and is affected by parboiling conditions, enzyme activity, and nonenzymatic browning (Maillard reaction) (Bhattacharya, 2004). The color of fortified parboiled rice was significantly affected by the addition of iron to the soaking solution under both soaking conditions, but there were minimal to no color changes when calcium was used as a fortificant (Table III-1). In general, $L*$ and $b*$ values were lower in the iron-fortified rice. Previous studies also observed a decrease in rice whiteness when rice was fortified at high $FeSO_4$ concentrations as a result of Fe accumulation at the outer layer of cells of the endosperm (Prom-u-thai et al., 2009). Iron may promote enzymatic and nonenzymatic reactions during parboiling, leading to more discoloration. It has been shown that the presence of Fe (II) metals increased the polyphenol oxidase (PPO) activity, the key enzyme in enzymatic browning, thus generating more brown or black pigments (Arabaci, 2015). The whiteness of calcium-fortified rice was not different from non-fortified rice, agreeing with Lee et al. (1995). The combination of calcium and iron in the soaking solution resulted in a parboiled rice color more similar to the control, suggesting that the presence of calcium reduced the influence of iron on rice color. Loypimai, Sittisuanjik, Moongngarm, & Pimthong (2017) observed less discoloration in rice parboiled at 4% NaCl concentration and suggested that high mineral concentrations may exert an inhibiting effect on enzymatic and nonenzymatic browning reactions. When compared with the excess-water soaking, the limited-water soaking method resulted in slightly higher $L*$, $a*$, and $b*$ values in the iron-fortified rice, and higher $a*$ and $b*$ values but similar L^* in the nonfortified rice. Tian et al. (2014) found that parboiled rice whiteness (L^*) was increased and color intensity (B value) was decreased by both vacuum and high-pressure soaking compared with atmospheric-pressure soaking. Presumably, enzymatic reactions in rice were reduced due to lower oxygen concentrations, thus generating less color pigments. The effect of vacuum soaking on rice whiteness was only noted in the iron-fortified rice in the present study. Because the limited-water soaking method used only 95% vacuum and some oxygen still present in the system, the rate and extent to which enzymatic and nonenzymatic browning occurred may be similar to those under the excess-water soaking. Therefore, similar whiteness of nonfortified, calcium-, and calcium + iron fortified rice was observed.

White belly, occurring as a dull white spot in the center of a kernel, is another important quality attribute of parboiled rice, indicating incomplete starch gelatinization as a result of insufficient or uneven moisture distribution during parboiling (Srisang and Chungcharoen, 2019). The iron-fortified rice had a significantly lower white belly content (~4.2%), whereas the control and calcium-fortified rice had similar white belly contents ranging from 5.4 to 9.1%. These results suggest that Fe ions present in the soaking water may promote a more homogenous diffusion of moisture into the endosperm and therefore increased starch gelatinization inside the rice kernels, thus decreased white belly content (Srisang and Chungcharoen, 2019). Previous works found that soaking kernels under pressure or vacuum improved hydration rate of corn and rice compared to soaking under atmospheric pressure (Velupillai and Verna, 1982). Nevertheless, the moisture content of nonfortified parboiled rice was similar, ~33%, between the excess- and limited-water soaking methods (data not shown). Therefore, limited-water soaking might not have a major impact on hydration rate and therefore did not affect the extent of starch gelatinization, and thus, white belly content was mainly affected by the fortificant.

Pasting properties obtained with an RVA can be used to predict cooked rice texture. In general, a lower paste viscosity is associated with a softer cooked rice texture (Patindol et al., 2010). Cold paste viscosity was significantly lower in flour from rice fortified with iron and/or calcium regardless of soaking condition with the exception of calcium-fortified rice by the limited-water soaking method (Table III-1). A recent study concluded that the presence of iron and zinc in rice flour decreased paste viscosity by interacting with proteins, thus decreasing starch swelling (Patindol et al., 2017). Similarly, Lee et al. (1995) found that calcium-fortified rice flour displayed decreases in gelatinization temperature, peak viscosity, and breakdown viscosity. Calcium can inhibit starch gelatinization by forming cross-bonds with linear amylose molecules (Sirisoontaralak et al., 2016). Thus, the presence of calcium and other salts can inhibit swelling, and subsequently reduce viscosity and breakdown of starch granules (Oosten, 1982). RVA profiles (Figure III-1) show that the control rice had higher overall pasting viscosities than the fortified rices, suggesting that the fortified rices may develop a softer texture than the non-fortified rice after cooking. Compared with the excess-water soaking method, the limited-water soaking method resulted in significantly higher cold paste viscosities for calcium- and calcium + iron-fortified rice. However, nonfortified and iron-fortified rice by the limited-water soaking method, showed cold paste viscosities similar to the excess-water soaking method. Tian, Zhao, Xie, Wang, Xu, & Jin, (2014) found that cooked rice soaked under vacuum and high hydrostatic pressure had smaller empty spaces inside the kernels compared to rice soaked under atmospheric pressure as revealed by scanning electron microscopy, presumably due to enhanced moisture absorption and distribution. The enhanced moisture absorption and distribution could improve starch swelling and explain the higher cold paste viscosities of rice soaked under vacuum in the limited-water soaking method.

3.4.2 Wastewater and total solids in wastewater

The limited-water soaking method utilized one fourth of the amount of fresh water used in the conventional excess-water soaking method. Thus, limited-water soaking not only reduced the amount of effluent, but also the amount of total solids in the wastewater (Table III-2). The excess-water soaking method generated between 1.5 kg (no minerals in soaking solution) and 1.9 kg (calcium + iron in soaking solution) of wastewater per kg of brown rice. In contrast, the limited-water soaking method generated only 0.2 kg of wastewater per kg of brown rice, thereby, on average, reducing the amount of wastewater by up to 89%. The greater the amount of fortificants added to the soaking solution, the greater the amount of total solids was present in wastewater. Therefore, the combination of calcium and iron in the excess-water soaking method yielded the highest amount of total solids in wastewater with about 72.3 g of solids per kg of brown rice, which included 16 g and 0.3 g of calcium and iron, respectively. In contrast, the limited-water soaking method significantly reduced the amount of total solids in wastewater by approximately 85% due to less calcium and iron were added to the soaking solution. The amount of total solids in wastewater was about 11.1 g per kg of brown rice, which included approximately 2.3 g of calcium and 0.01 g of iron.

3.4.3 Mineral content

The inward diffusion of micronutrients into the endosperm is facilitated by the moisture gradient between the rice kernels and the surrounding environment during the soaking stage (Lund, 1984). The standards provided by the Food & Drug Administration (FDA) permit 1100-2200 mg of calcium and 28-56 mg of iron per kg of fortified milled rice (FDA, 2019). Rice fortified with calcium (50 g/L calcium lactate) by excess- and limited-water soaking contained about 1101 mg/kg and 983 mg/kg of calcium, respectively, when brown rice was used as the feedstock and parboiled brown rice was milled for 60 seconds (Table III-3). Soaking milled rice in 30 g/L calcium lactate at ambient temperature and 50 g/L calcium lactate at 60°C at a milled rice:soaking water ratio of 1:0.75 (w/v) at ambient temperature for 3 hr resulted in a calcium uptake of about 1344 mg/kg (Lee et al., 1995) and 1892 mg/kg (Sirisoontaralak et al., 2016), respectively. The differences in calcium uptake are attributed to the use of different feedstocks, i.e. brown rice vs. milled rice. Iron content was increased to approximately 88 mg/kg by the excess-water soaking method and to 26 mg/kg by the limited-water soaking method when rice was fortified in a solution containing 200 mg/L pure iron. The iron content in the excess-water soaking method was similar to a previous study of 75 mg/kg (Patindol et al., 2017). Rice fortified simultaneously with calcium and iron by the limited-water soaking method contained significant amounts of minerals. The consumption of about 400 g of cooked calcium + iron rice, fortified via the limited-water soaking method, can make up for about 15% of the recommended dietary allowances (RDAs) of calcium, and ~72% (male) and ~32% (female) of the RDAs of iron.

The excess-water soaking method resulted in significantly higher mineral uptakes when rice was fortified with either calcium or iron separately, whereas the soaking method did not affect mineral uptake in rice fortified with calcium and iron simultaneously (Table III-3). The effect of lower mineral uptake in limited-water soaking was more pronounced for iron than for calcium may be due to the significantly lower $FeSO_4$ concentration (995 mg/L) than the calcium lactate concentration (50 g/L) in the soaking solution, which consequently lowered diffusion gradients. In the limited-water soaking method, although rice and soaking solution were spread evenly inside the vacuum pouch, a large amount of kernels were in contact with the inner side of the pouch or other adjacent kernels, and therefore the total surface available for mineral diffusion into the kernel may have been reduced. Nevertheless, the fortified rice by the limited-water soaking method in this study almost met the requirements for calcium and iron content of fortified milled rice by the FDA. To increase mineral content and meet the FDA requirements of fortified rice, the fortification parboiling process by the limited-water soaking method could be improved by increasing mineral concentration in the soaking solution (Lee et al., 1995; Prom-u-thai et al., 2008; Prom-u-thai et al., 2009; Thiruselvam et al., 2014; Patindol et al., 2017), by increasing soaking temperature and/or duration (Sirisoontaralak et al., 2016), or by reducing milling time (Prom-u-thai et al., 2008). With the limited-water soaking method, a slight increase in fortificant concentration in the soaking water would still generate less total solids in wastewater than excess-water soaking while increasing mineral uptake of fortified rice.

3.4.4 Washing retention and extractability with dilute HCl

Washing rice is a common practice prior to cooking in order to remove dust and dirt and to obtain a more desirable cooked rice texture. However, washing removes a significant amount of rice components including protein, ash, and water-soluble vitamins and minerals (Steiger et al., 2014). Fortification by parboiling has been shown to produce fortified rice with relatively high washing stability compared to alternative fortification methods such as dusting or coating (Steiger et al., 2014). The limited-water soaking method increased calcium and iron retention when they were added individually (Table III-3). In the limited-water soaking method, the vacuum may create small pores in rice to improve mineral retention (Tian et al., 2014). Additionally, minerals may form complexes with amylose and become entrapped in the rice kernel (Sirisoontaralak et al., 2016). However, when rice was fortified with calcium and iron simultaneously, washing retention was unaffected by the soaking method, and was greater than the 46.2% Ca retention reported by Sirisoontaralak et al. (2016) in fortified rice after washing.

In one study, to evaluate mineral bioavailability, fortified rice flour was exposed to 0.1 N HCl to simulate human digestion (Prom-u-thai et al., 2008). A greater amount of dissolved minerals in the 0.1 N HCl implies that more minerals may be absorbed in the human digestive system (Sirisoontaralak et al., 2016). The dilute-HCl solubility of calcium and iron was neither affected by fortificant combinations, nor by soaking method. In this study, mineral solubilities were lower than the ~72% reported by Sirisoontaralak et al. (2016) and the ~66% reported by Patindol et al. (2017). The differences in the mineral retention and solubility may be due to different cultivars and/or feedstocks. For example, Fe retention has been shown to vary between cultivars with the highest retention rate in cultivar YRF 2 (97.9%) and the lowest in Opus (20.1%) (Prom-u-thai et al., 2008). Calcium solubility was greater when milled rice was used as the feedstock for fortification by parboiling according to Sirisoontaralak et al. (2016) compared with the brown rice used in this study.

3.4.5 Mineral distribution

Figure III-2 presents the distribution of the individual mineral and their combination in milled rice, bran, and wastewater by both soaking methods. In calcium-fortification, the amounts of pure calcium in the excess-water and limited-water soaking solutions were 1837 mg and 459 mg, respectively. After parboiling in the excess-water soaking method, approximately 6% of the added calcium was retained in the milled rice, whereas the bran and wastewater contained about 8% and 86% of the added calcium, respectively (Figure III-2A). The limited-water soaking greatly increased the percentage of calcium uptake in the milled rice and lowered the amount of calcium in the wastewater. In the limited-water soaking method, about 21% of the added calcium in the soaking solution was retained in the milled rice and 53% was present in the wastewater. In iron-fortification, the excess-water and limited-water soaking solutions contained about 40 mg and 10 mg of pure iron, respectively. Of the added iron, approximately 23% and 27% diffused into the endosperm in the excess-water and limited-water soaking solutions, respectively (Figure III-2B). Tian et al. (2014) observed a more effective penetration of water into the peripheral portion of starch granules in rice kernels when soaked under a vacuum. Thus, the inward diffusion of calcium and iron was positively affected by the application of a vacuum in the limited-water soaking method even though the amount of minerals added to the soaking solution was less than that in the excess-water soaking method. The components of rice bran, including albumin, phytic acid, cellulose, lignin, and hemicellulose, have a high affinity for cations (Champagne, Rao, Liuzzo, Robinson, Gale, & Miller, 1985). Therefore, a significant amount of minerals was retained in the bran, which was also observed by Thiruselvam et al. (2014).

3.4.6 Statistical analysis

The effects of fortificant, soaking condition, and their interaction on fortified rice quality attributes were analyzed by two-way ANOVA, and the results are shown in Table III-4. HRY was neither affected by soaking condition nor by the interaction between fortificant and soaking condition; however, the fortificant had a minor impact on HRY ($p=0.0498$). The whiteness (L^*), redness (a^*), and yellowness (b^*) values were significantly affected by the fortificant at $p<0.0001$, $p=0.0004$, and $p<0.0001$, respectively. Limited-water soaking had no impact on the whiteness (L^*) ($p=0.748$) of the fortified rice, whereas the redness (a^*) and yellowness (b^*) values were affected by the soaking condition at $p<0.0001$. The interaction of fortificant and soaking condition on rice kernel redness (a^*) was minimal ($p=0.0552$), but it was significant for whiteness (L^*) and yellowness (b^*). Calcium and iron contents of fortified rice, as well as the amount of wastewater and total solids in wastewater were all significantly affected by fortificant, soaking condition, and their interaction at $p<0.0001$.

3.5 Conclusions

This study demonstrated the effectiveness of the limited-water soaking method developed to fortify rice with calcium, iron, and their combination. Fortification by parboiling under the limited-water soaking condition is an economically feasible process to produce fortified rice because it reduces cost for fortificants, water, and wastewater treatment. Water usage in the soaking step was reduced by 75%, resulting in significant decreases in the amount of wastewater and solids in wastewater. The limited-water soaking method had little impact on rice quality attributes, including head rice yield, color, and pasting properties. The simultaneous fortification of rice with calcium and iron significantly increased the calcium and iron contents in parboiled rice to a level close to FDA regulations on fortified rice. Further improvements in the limited-water soaking method, such as an increase in fortificant concentrations in the soaking solution or soaking and/or steaming time and temperature could increase mineral uptake to the required level by the FDA without affecting mineral contents in wastewater significantly. In conclusion, the limited-water soaking method has great potential to produce fortified parboiled rice in a more sustainable and environmentally beneficial way while also delivering vital nutrients to a large number of people.

3.6 References

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3.7 Tables and Figures

Table III-1 Effect of fortificant and soaking condition on head rice yield (HRY), color parameters, white belly content, and cold paste viscosity of milled fortified rice[a].

			Color				
Fortificant	**Soaking Condition**	**HRY (%)**	**Whiteness (*L**)**	**Redness (*a**)**	**Yellowness (*b**)**	**White Belly (%)**	**Cold Paste Viscosity (cP)**
Control	Excess	88.8 ± 1.2^{a}	39.4 ± 0.4^{bc}	4.2 ± 0.1^{bc}	24.3 ± 0.1^{bc}	9.1 ± 1.0^{a}	437.5 ± 3.5^{a}
	Limited	87.7 ± 1.3^{a}	39.7 ± 1.0^{a-c}	5.4 ± 0.2^{a}	26.1 ± 0.3^{a}	6.7 ± 0.7^{a-c}	447.0 ± 4.2^{a}
Calcium	Excess	88.9 ± 2.9^{a}	40.9 ± 0.4^{a}	4.6 ± 0.2^{ab}	25.2 ± 0.6^{ab}	8.3 ± 0.4^{ab}	407.5 ± 2.1^{b}
	Limited	84.2 ± 3.2^{a}	40.7 ± 0.9^{ab}	5.0 ± 0.1^{a}	25.3 ± 0.5^{ab}	8.8 ± 0.3^{a}	452.5 ± 0.7^{a}
Iron	Excess	89.8 ± 1.4^{a}	35.3 ± 0.6^{f}	4.0 ± 0.3^{bc}	20.7 ± 0.7^{e}	4.2 ± 1.3^{c}	375.0 ± 15.6^{d}
	Limited	89.1 ± 1.5^{a}	36.8 ± 0.7^{ef}	5.1 ± 0.5^{a}	23.9 ± 0.3^{c}	4.1 ± 1.2^{c}	377.5 ± 9.2^{cd}
Calcium + Iron	Excess	85.5 ± 1.6^{a}	38.8 ± 0.2^{cd}	3.7 ± 0.1^{c}	22.5 ± 0.2^{d}	6.0 ± 0.9^{bc}	337.5 ± 4.9^{e}
	Limited	84.8 ± 1.0^{a}	37.6 ± 0.8^{de}	4.6 ± 0.4^{ab}	23.3 ± 0.6^{cd}	5.4 ± 1.3^{c}	403.0 ± 0.0^{bc}

[a]Means ± standard deviation of two replications with the same letter in the same column are not significantly different (P<0.05) based on Tukey's HSD test.

Table III-2 Amount of wastewater, total solids, and the amount of calcium and iron in wastewater per kg of brown rice in excess- and limited-water parboiling[a].

Fortificant	Soaking Condition	Wastewater (kg/kg brown rice)	Total solids in wastewater (g/kg brown rice)	Minerals in wastewater (g/kg brown rice)	
				Ca	Fe
Control	Excess	1.5±0.1^{b}	12.4±0.2^{c}	0.0	0.0
	Limited	0.2±0.0^{c}	6.0±0.0^{e}	0.0	0.0
Calcium	Excess	1.6±0.0^{b}	69.8±1.0^{b}	15.8±0.0^{b}	0.0
	Limited	0.2±0.0^{c}	12.1±0.1^{c}	2.4±0.1^{c}	0.0
Iron	Excess	1.6±0.0^{b}	10.4±0.3^{d}	0.0	0.22±0.0^{b}
	Limited	0.2±0.0^{c}	5.4±0.3^{e}	0.0	0.01±0.0^{c}
Calcium + Iron	Excess	1.9±0.1^{a}	72.3±0.1^{a}	16.0±0.0^{a}	0.28±0.0^{a}
	Limited	0.2±0.0^{c}	11.1±0.4^{cd}	2.3±0.0^{c}	0.01±0.0^{c}

[a]Means ± standard deviation of two replications with the same letter in the same column are not significantly different ($P<0.05$) based on Tukey's HSD test.

Table III-3 Effect of soaking condition on calcium and iron contents of fortified milled rice, washing retention, and dilute-HCl solubility[a].

		Mineral content (db, mg/kg milled rice)		Washing Retention (%)[b]		Dilute- HCl Solubility (%)[c]	
Fortificant	**Soaking Condition**	**Ca**	**Fe**	**Ca**	**Fe**	**Ca**	**Fe**
Control	Excess	42.9±2.0^{d}	6.2±0.6^{d}	0.0	0.0	0.0	0.0
	Limited	49.8±0.8^{d}	9.1±0.4^{d}	0.0	0.0	0.0	0.0
Calcium	Excess	1100.7±6.8^{a}	9.7±1.4^{d}	86.4±7.4^{ab}	0.0	59.7±8.0^{a}	0.0
	Limited	983.3±9.5^{b}	6.9±0.2^{d}	96.3±0.4^{a}	0.0	67.0±2.1^{a}	0.0
Iron	Excess	32.9±5.5^{d}	88.1±2.9^{a}	0.0	52.7±9.1^{b}	0.0	32.6±8.6^{a}
	Limited	36.5±6.1^{d}	26.0±2.0^{c}	0.0	79.4±3.1^{a}	0.0	35.4±7.3^{a}
Calcium + Iron	Excess	823.9±20.8^{c}	36.2±0.1^{b}	75.7±0.6^{bc}	72.6±4.0^{a}	62.4±0.2^{a}	28.0±1.2^{a}
	Limited	998.5±6.4^{b}	33.2±0.8^{b}	65.7±1.6^{c}	71.4±0.8^{a}	67.7±0.6^{a}	31.1±3.3^{a}

[a]Means ± standard deviation of two replications with the same letter in the same column are not significantly different ($P<0.05$) based on Tukey's HSD test.
[b]Values are residual calcium and iron content after rinsing with water, expressed as percentage retention.
[c]Values are residual calcium and iron content after extraction with 0.1 N HCl, expressed as percentage solubility.

Table III-4 Two way analysis of variance (ANOVA) of head rice yield (HRY), whiteness (*L**), redness (*a**), yellowness (*b**), calcium content, iron content, wastewater, and solids in wastewater as affected by fortificant, soaking condition, and their interaction at a significant level P<0.05.

	Sum of Squares	F Ratio	Prob>F
Head Rice Yield			
Fortificant	47	3	0.0498*
Soaking Condition	17	4	0.799
Fortificant*Soaking Condition	19	1	0.295
Whiteness (*L)**			
Fortificant	98	84	<0.0001*
Soaking Condition	0	0	0.748
Fortificant*Soaking Condition	7	6	0.0031*
Redness (*a)**			
Fortificant	2	9	0.0004*
Soaking Condition	6	74	<0.0001*
Fortificant*Soaking Condition	1	3	0.0552
Yellowness (*b)**			
Fortificant	52	76	<0.0001*
Soaking Condition	15	67	<0.0001*
Fortificant*Soaking Condition	12	17	<0.0001*
Calcium content			
Fortificant	34192	225	0.0001*
Soaking Condition	1639	11	0.0304*
Fortificant*Soaking Condition	42631	280	<0.0001*
Iron content			
Fortificant	1000	298	<0.0001*
Soaking Condition	2114	631	<0.0001*
Fortificant*Soaking Condition	1742	520	<0.0001*
Wastewater			
Fortificant	0	21	0.0002*
Soaking Condition	9	4135	<0.0001*
Fortificant*Soaking Condition	0	16	0.0006*
Solids in Wastewater			
Fortificant	4490	9855	<0.0001*
Soaking Condition	4429	29154	<0.0001*
Fortificant*Soaking Condition	3026	6639	<0.0001*

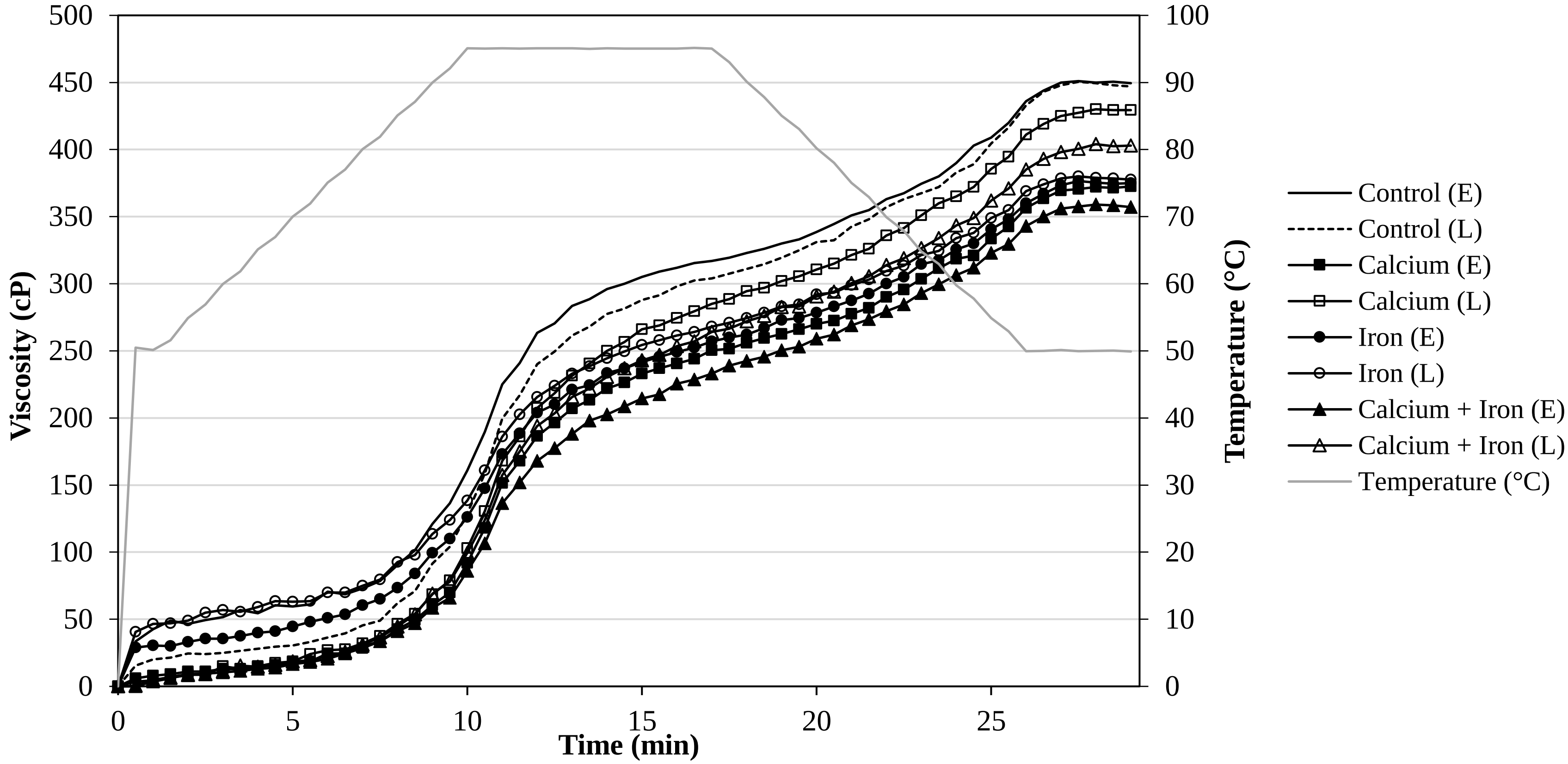

Figure III-1 Pasting profiles of non-fortified and fortified rice flours as affected by fortificant and soaking condition (E=excess-water and L=limited-water).

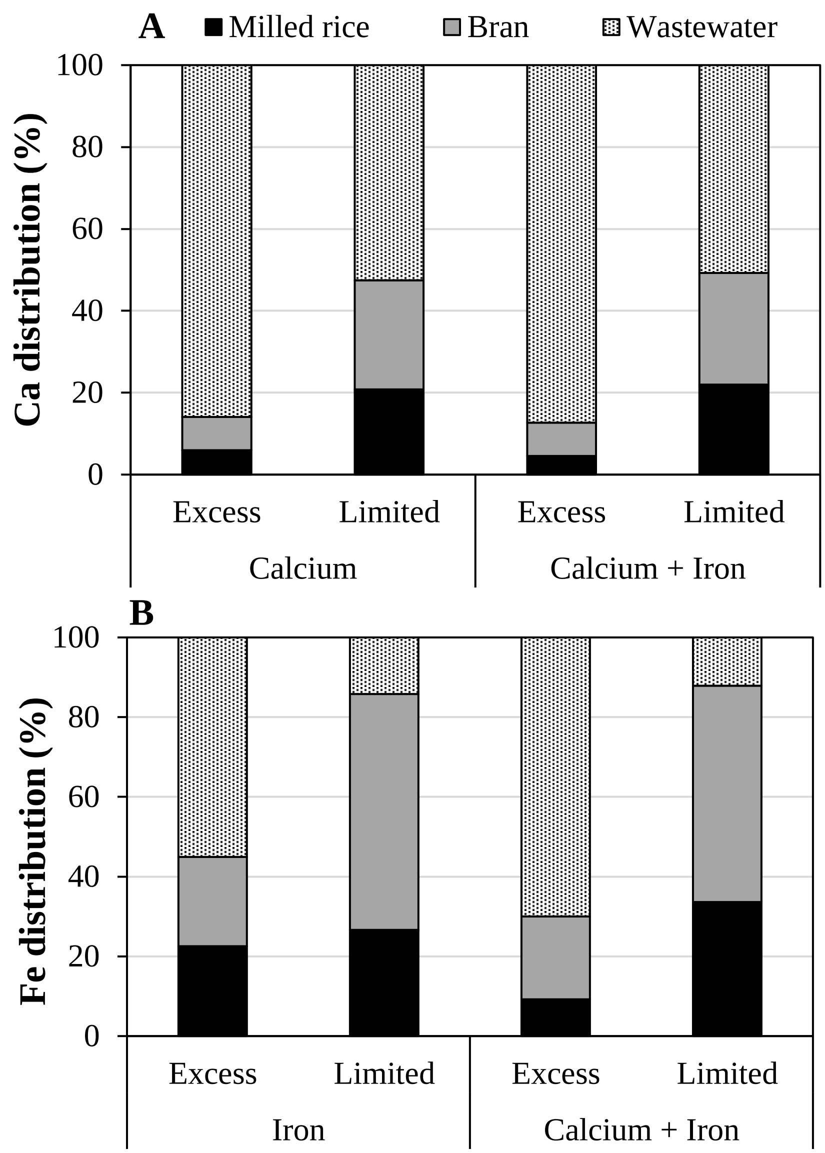

Figure III-2 Percentage distribution of calcium after fortification with calcium and calcium + iron (A) and iron after fortification with iron and calcium + iron (B) in milled rice, bran, and water. Calculations were based on the amount of each mineral added to the soaking solution.

IV. Simultaneous Fortification of Rice with Folic Acid and β-Carotene or Vitamin A by Limited-Water Parboiling

4.1 Abstract

Rice fortification by parboiling has been used to fortify rice with a single or a combination of multiple nutrients. While several studies have demonstrated the success of rice fortification with minerals by the parboiling technique, the number of studies that used vitamins as fortificants is limited with varying results. A limited-water soaking method has been introduced as a feasible and more sustainable alternative to the conventional excess-water soaking in parboiling. This study investigated the application of limited-water soaking in the simultaneous fortification of rice with water- and fat-soluble vitamins. Pure and water-soluble forms of β-carotene and vitamin A were first compared to identify a more effective form to be combined with folic acid. Water-soluble forms of β-carotene and vitamin A were easy to incorporate into the soaking water and presented effective fortification without affecting rice milling quality, the amount of wastewater and solids in wastewater. Milled rice fortified with folic acid combined with water-soluble forms of β-carotene or vitamin A in the limited-water soaking method contributed to about 75% of the recommended dietary allowances (RDAs) for folic acid, about 173% (female) and 134% (male) of the RDAs for β-carotene, and to about 58% (female) and 45% (male) of the RDAs for vitamin A. Rice fortified with β-carotene showed a higher uptake and a more orange color compared to the others. Thus, fortification of rice with vitamins in limited-water parboiling is an efficient process obtaining fortified rice that can significantly increase vitamin intake with limited environmental impacts.

4.2 Introduction

Worldwide, more than 2 billion people suffer from micronutrient deficiencies, affecting at least one-third of Africa's population (World Food Programme (WFP), 2018), while folic acid and vitamin A are the most common vitamin deficiencies in developing countries (World Health Organization (WHO), 2018). Inadequate folic acid intake is primarily associated with high risk of giving birth to infants with neural tube defects along with other birth defects (Flores, Vellozzi, Valencia, & Sniezek, 2014; Rogers et al., 2018). Vitamin A deficiency can lead to a weakened immune system, growth retardation in children, xerophthalmia, and an increased risk of infectious diseases (WFP, 2018).

Fortification of foods is a common strategy to combat micronutrient deficiencies and has been implemented in many countries to increase folic acid and vitamin A intake (Rogers et al., 2018; WFP, 2018). Rice (*Oryza sativa*) is the most consumed staple food in the world, and therefore an effective vehicle for fortification purposes at a population-level intervention. Recently, fortification of rice by parboiling was introduced as an efficient technology to produce enriched rice as it displayed better retention of nutrients after washing and cooking (Prom-u-thai, Fukai, Godwin, Rerkasem, & Huang, 2008; Kam, Murray, Arcot, & Ward, 2012a; Hotz, Dipti, Arsenault, & Bipul, 2015; Sirisoontaralak, Limboon, Jatuwong, & Chavanalikit, 2016; Patindol, Fragallo, Wang, & Durand-Morat, 2017) compared to rice fortified by available fortification methods such as dusting, coating, and extrusion (Steiger, Müller-Fischer, Cori, & Conde-Petit, 2014)

Parboiling is a three-step hydrothermal treatment of rough or brown rice that involves soaking, steaming and drying (Bhattacharya, 2004). By adding minerals or vitamins to the soaking water, rice has been fortified by parboiling with zinc (Prom-u-thai, Huang, Cakmak, & Rerkasem, 2011; Hotz et al., 2015; Patindol et al., 2017; Wahengbam, Green, & Hazarika, 2019), calcium (Lee, Hettiarachchy, McNew, & Gnanasambandam, 1995; Sirisoontaralak et al., 2016), iron (Prom-u-thai et al., 2008; Prom-u-thai, Rerkasem, Fukai, & Huang, 2009; Thiruselvam, Cheong, Mohan, Paterson, & Arcot, 2014; Patindol et al., 2017), folic acid (Kam, Murray, Arcot, & Ward, 2012a, b; Kam, Arcot, & Adesina 2012c; Thiruselvam, 2014) and β-carotene (Thiruselvam et al., 2014). Rice fortified with minerals was shown to have similar sensory characteristics and firmer texture compared to non-fortified rice (Prom-u-thai et al., 2009; Lee et al., 1995). Soaking time did not have a significant impact on folic acid uptake (Kam et al., 2012a), and consumer acceptance of folic acid and β-carotene fortified rice was not different from that of commercial white rice (Kam et al., 2012b; Thiruselvam et al., 2014). Retinyl acetate and retinyl palmitate, along with β-carotene, a provitamin A, are the most common forms of vitamin A fortificants used in fat-based foods, such as margarine. On the other hand, water-soluble matrices have been used to improve their solubility and bioavailability in fortified cereal products (WHO, 2006).

The conventional fortification parboiling process generates large amounts of wastewater that represents a serious environmental concern. A recently developed limited-water soaking method for the fortification of rice with minerals reduced the amount of effluent by about 89% and the amount of total solids in wastewater by up to 85% (Jannasch & Wang, 2020). The present study evaluated the limited-water soaking method for the fortification of rice with folic acid combined with vitamin A or β-carotene. Two forms of β-carotene and vitamin A, including pure forms dissolved in Tween® 80 and water-soluble forms, were compared for fortification efficiency in the limited-water parboiling. The more effective forms of β-carotene and vitamin A, determined by rice vitamin uptake and feasibility during the parboiling process, were then combined with folic acid in the fortification-parboiling process. The amount of wastewater and total solids in wastewater, rice milling quality, vitamin content, and kernel color were determined.

4.3 Materials and Methods

4.3.1 Materials

Long-grain rough rice of RoyJ (2017 crop; ~12% moisture content) was provided by the University of Arkansas Rice Research and Extension Center in Stuttgart, AR. Rice was cleaned using a dockage tester (Carter-Day Company, Minneapolis, MN) and sorted using a thickness grader with a screen size of 1.74 mm to remove kernels of less than 1.74 mm in thickness, which can have adverse effects on parboiled rice quality (Jannasch, Carvalho, Patindol, & Wang, 2020). Brown rice, which has been shown to be a better feedstock for fortification by parboiling (Patindol et al., 2017), was obtained by dehulling rough rice using a Satake THU-35 dehusker (Satake Corporation, Hiroshima, Japan). The onset gelatinization temperature of the brown rice, as determined by a differential scanning calorimeter (model Diamond, Perkin-Elmer Co., Norwalk, CT), was 70.9°C.

Food grade folic acid was provided by Glanbia Nutritionals, Inc. (Carlsbad, CA). Tween® 80 (Polysorbate 80; Polyoxyethylene (20) Sorbitan Monooleate) from Spectrum Chemical Mfg. Corp. (Gardena, CA) was used to dissolve pure β-carotene (Thermo Fisher Scientific, Ward Hill, MA) and vitamin A palmitate, 1.70 MIU/g, USP (Spectrum Chemical Mfg. Corp., Gardena, CA). Water-soluble (WS) forms of β-carotene (β-carotene 10% CWS/S) and vitamin A (dry vitamin A palmitate, type 250 CWS/F) were provided by DSM Nutritional Products Ltd. (Basel, Switzerland). One gram of water-soluble β-carotene and vitamin A contained 100 mg and 75 mg of pure β-carotene and vitamin A, respectively.

4.3.2 *Soaking methods and fortification-parboiling process*

Both excess-water and limited-water soaking methods were conducted according to Jannasch & Wang (2020). 2 parts (200 mL) of soaking solution were added to 1 part (100 g) of brown rice in a beaker in the excess-water soaking, whereas 0.5 part (50 mL) of soaking solution and 1 part (100 g) of brown rice were vacuum sealed in a CLARITY™ vacuum pouch (10”×12”, 3 mm thick, Riverside, MO) in the limited-water soaking method. Rice was soaked in a water bath (Boekel/Grant ORS-200, Boekel Scientific, Feasterville, PA) at 67°C for 3 hr. Excess soaking solution was carefully drained and weighed to determine the amount of wastewater. The mass of total solids in wastewater was obtained by evaporating the wastewater in an oven at 135°C for 24 hr. Samples were steamed in an autoclave (Brinkmann 2340E, Tuttnauer USA Co. Ltd., Hauppauge, NY) at 120°C for 10 min. Rice was dried at 26°C and 65% relative humidity in an EMC chamber to ~12% moisture content. For vitamin analyses, head rice was ground into flour using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO) fitted with a 0.5-mm screen. All trials were conducted in duplicate.

4.3.3 *Comparison of pure versus water-soluble forms of β-carotene and vitamin A as fortificants*

The amount of fortificant added to the soaking solutions was calculated based on vitamin uptakes found in previous studies (Kam et al., 2012a; Kam et al., 2012b; Thiruselvam et al. 2014). Pure β-carotene (175 mg) and vitamin A (66.7 mg; 34 mg pure retinol) were suspended in 4.5 g and 0.3 g of Tween® 80, respectively, using a glass stirring rod. The suspension was then added with 1000 mL deionized water while mixing until completely dissolved. Water-soluble β-carotene (1.75 g) and vitamin A (0.45 g) were dissolved in 1 L of deionized water. All fortification-parboiling trials were conducted in duplicate using the limited-water soaking method.

4.3.4 Fortification with folic acid, β-carotene, vitamin A, and their combinations

Fortification of brown rice with folic acid, β-carotene, and vitamin A individually, as well as the simultaneous fortification of folic acid with β-carotene or vitamin A, were conducted via excess- and limited-water parboiling. Based on the outcomes of section *2.3*, water-soluble forms of β-carotene and vitamin A were used as fortificants in the simultaneous fortification, and β-carotene concentration was lowered because utilizing a soaking solution of water-soluble β-carotene at 1.75 g/L resulted in β-carotene levels in fortified rice exceeding the RDAs. Soaking solutions containing 35 mg folic acid and 0.5 g water-soluble β-carotene (50 mg pure β-carotene) or 0.45 g water-soluble vitamin A (34 mg pure retinol) per liter were used.

4.3.5 Head rice yield

Dried parboiled brown rice was milled using a laboratory mill (McGill Miller #2, Rapsco, Brookshire, TX) for 60 s, separated into head rice and broken kernels (Grainman, Grain Machinery Mfg., Miami, FL), and aspirated for 2 min to remove residual bran (Seedburo Equipment Company, Chicago, IL). Head rice yield (HRY) was expressed as percentage of mass of head rice in the brown rice sample after milling.

4.3.6 Kernel color

A colorimeter (ColorFlex, Hunter Associates Laboratory, Reston, VA) was used to measure whiteness (*L**), redness (*a**), and yellowness (*b**) of milled fortified rice for two readings for each sample.

4.3.7 Folic acid analysis

The extraction and purification of folic acid from rice flour and bran was performed in duplicate according to Kam et al. (2012c) with modifications. Approximately 2 g of rice flour or 0.3 g of rice bran was added to 50-mL centrifuge tubes with 20 mL phosphate buffer (0.2 M sodium phosphate, monobasic, monohydrate containing 1% w/v sodium ascorbate, pH 7.0). As internal standards, 0.1 mL of folic acid dissolved in phosphate buffer (10, 20, 30, and 40 mg/L) was added to unfortified parboiled rice flour and bran. Tubes were placed in a boiling water bath for 8 min and subsequently cooled for 5 min at room temperature. Samples were incubated with α-amylase (3,000 U/mL, 0.17 mL) at 37°C for 2 hr and then boiled for 3 min to inactivate the enzyme. Samples were cooled at room temperature and then centrifuged at 2,100 ×g for 15 min. Strong anion exchange (SAX) cartridges (500 mg, 6 mL, Phenomenex, Torrance, CA) were used to purify the supernatants by solid-phase extraction (SPE). Cartridges were preconditioned with methanol (2× 2.5 mL) and deionized water (2× 2.5 mL) prior to loading with 5 mL of the supernatants. After washing the cartridges with deionized water (3× 2.5 mL), the folic acid was eluted from the solid phase with 6 mL of 0.1 M sodium acetate containing 10% (w/v) sodium chloride and 1% (w/v) ascorbic acid, and filtered through a 0.45-µm PTFE filter (VWR International, Radnor, PA) prior to HPLC analysis. Folic acid was analyzed following an adapted method of Ningsih & Megia (2019) using an HPLC system (Waters Corp, Milford, MA) equipped with a model 600 pump, model 717 plus autosampler and model 996 photodiode array detector. Separation was carried out at ambient temperature in a 4.6 mm × 250 mm Waters Symmetry C18 column (Waters Corp., Milford, MA) with a 50-µL sample loop at 1.5 mL/min. The mobile phase was 2% acetic acid in water (A) and 0.05% acetic acid in water/acetonitrile (50:50 v/v) (B). The linear gradient began at

5% B and increased to 25% B over 5 min. Folic acid was detected at 283 nm and expressed as mg folic acid per kilogram of sample. All samples were prepared in duplicate and injected two times.

4.3.8 β-carotene and vitamin A analysis

The extraction of β-carotene and vitamin A was performed in duplicate according to Lamberts & Delcour (2008) with modifications. Rice flour (3.0 g) or rice bran (0.1 g) was mixed with ascorbic acid (0.3 g), ethanol (95%, 15.0 mL), and sodium chloride solution (0.01 g/mL, 3.0 mL) in a 50-mL centrifuge tube in a water bath at 85°C for 5 min under stirring with a magnetic stirring bar at 300 rpm. As internal standard, 0.1 mL of pure β-carotene and vitamin A dissolved in methanol (200 mg/L) was added to unfortified parboiled rice flour and bran. The heated mixture was saponified with potassium hydroxide solution (0.6 g/mL, 3.0 mL) at 85°C for 20 min with additional vortex mixing after 8 and 15 min. The mixture was cooled in an ice bath and then added with cold sodium chloride solution (10 g/L, 15.0 mL). Beta-carotene and vitamin A were extracted with three 10 mL portions of hexane/ethyl acetate (9:1 v/v). Samples were vortex mixed for 15 sec and centrifuged at 2,100 ×g for 5 min after each solvent addition, and the organic layers were collected in a separate centrifuge tube. The combined organic layers were washed with 10 mL of deionized water, mixed, and centrifuged at 2,100 ×g for 5 min, and the aqueous layer was discarded. The organic phase was dried under nitrogen gas at room temperature, and the residue was dissolved in methanol-acetonitrile (4:6 v/v). The amount of methanol-acetonitrile mixture varied between 2 and 5 mL depending on the amount of β-carotene in the sample and for all of the vitamin A samples was 2.4 mL. The extracts were filtered through a 0.45-µm PTFE filter prior to analysis by the HPLC system as described previously at a column temperature of 9°C. Beta-carotene was separated using a mobile phase of methanol:acetonitrile:2-propanol (40:58:2) at 0.45 mL/min and detected at 450 nm (Hentschel et al., 2002). Vitamin A was separated using a mobile phase of acetonitrile: methanol: methylene chloride (70:15:15) at 1.8 mL/min, and detected at 325 nm (Kim & Quadro, 2010).

4.3.9 Statistical analysis

All parboiling conditions were conducted in duplicate for each fortificant solution and analyzed with JMP Pro Version 14 (SAS Software Institute, Cary, NC, USA) using analysis of variance (Tukey's HSD test) with a significance level at $P<0.05$. Two-way analysis of variance (ANOVA) was used to establish the effect of fortificant, soaking condition, and their interactions on parboiled rice attributes at $P<0.05$.

4.4 Results and Discussion

4.4.1 Comparison of pure versus water-soluble forms of β-carotene and vitamin A as fortificants

4.4.1.1 Type of fortificant on parboiled rice properties

Parboiled rice quality and consumer acceptability are strongly associated with head rice yield (HRY) and kernel color, respectively (Luh & Mickus, 1991; Bhattacharya, 2004). The HRY of β-carotene and vitamin A fortified parboiled rice ranged 88.3-90.3% and was not significantly different from that of nonfortified parboiled rice, regardless of fortificant type (Table IV-1). The whiteness (L^*) of rice fortified with both, pure and water-soluble forms of β-carotene and vitamin A, was higher than that of the control of nonfortified rice, but the trends for redness (a^*) and yellowness (b^*) were not clear (Table IV-1). Rice fortified with water-soluble β-carotene exhibited higher a^* and b^* values compared to non-fortified rice and rice fortified with pure β-carotene in Tween® 80, indicating that water-soluble β-carotene imparted a more orange appearance to parboiled rice. Both, L^* and a^* were not affected by the type of vitamin A used, whereas b^* was slightly lower in rice fortified with water-soluble vitamin A. Because vitamin A has minimal to no effects on kernel color, the use of the vitamin A as fortificant instead of β-carotene could be implemented in populations where rice with a less orange color is preferred.

4.4.1.2 Wastewater and total solids in wastewater

The wastewater from rice fortification via excess-water parboiling contains large amounts of nutrients and organic materials, and thus presents an environmental hazard when discharged without proper treatment (Kumar, Priyadarshinee, Roy, Dasgupta, & Mandal, 2016). Regardless of fortificant type, fortification with β-carotene and vitamin A generated the same amount of wastewater (0.2 kg per kg of brown rice) as the control (Table IV-1). Fortification with pure β-carotene required larger amounts of Tween® 80 compared to fortification with pure vitamin A, and thus, produced more solids in wastewater. Water-soluble forms of β-carotene and vitamin A resulted in a similar amount of solids in wastewater, which was only slightly higher than the control without fortificants. Therefore, water-soluble fortificant forms are not only easier to disperse in the soaking solution, but also can reduce cost of wastewater treatment.

4.4.1.3 Type of fortificant on β-carotene and vitamin A uptake

The level of vitamin fortification depends on consumption levels of the food in the target population. Although fortified rice can contribute significantly to vitamin intake for most consumers, the fortificants must not exceed RDAs and take into account countries with high levels of rice consumption (WFP, 2018). Thus, a portion of 400 g of fortified cooked rice (150 g uncooked rice) per day must provide less than or close to 700 µg (female) and 900 µg (male) of vitamin A, or 1,400 µg (female) and 1,800 µg (male) for β-carotene (Institute of Medicine, 1998, 2001). The results show that soaking of brown rice in water-soluble β-carotene was more efficient than in pure β-carotene with Tween® 80 for β-carotene uptake (Table IV-2). Higher β-carotene uptake was also found in rice bran when water-soluble β-carotene was used as fortificant. The vitamin A content of rice fortified with the water-soluble form was slightly lower compared to that of rice fortified with pure vitamin A in Tween® 80, whereas the vitamin A levels in bran were similar for both forms of vitamin A. Milled rice vitamin uptake, expressed as percentage of the amount of vitamin retained in the milled rice over the amount of fortificant added in the soaking solution, was significantly greater for water-soluble β-carotene, but was lower for water-soluble vitamin A. The color of fortified rice was shown to correlate with its vitamin uptake. Rice fortified with water-soluble β-carotene contained greater amounts of β-carotene and exhibited higher redness (*a**) and yellowness (*b**), resulting in a more orange color as shown in Table IV-1. Fortification with vitamin A in Tween® 80 showed higher vitamin A uptake with rice kernels appearing more yellow (higher *b** value) than rice fortified with water-soluble vitamin A.

The daily consumption of 400 g of cooked fortified rice (150 g uncooked rice) contributes to about 31% (female) and 24% (male) of the RDAs for β-carotene when using pure β-carotene in Tween® as fortificant, compared to 491% (female) and 382% (male) of the RDAs for β-carotene when water-soluble β-carotene is used. The same amount of fortified cooked rice provides a daily vitamin A intake of approximately 81% (female) and 63% (male), and 45% (female) and 35% (male) when using vitamin A in Tween® and water-soluble vitamin A, respectively. Water-soluble forms of β-carotene and vitamin A were selected for the simultaneous fortification of rice with folic acid considering the ease of soaking solution preparation in addition to fortification efficiency. Due to the high fortification efficiency, the concentration of water-soluble β-carotene was lowered to 50 mg/L in the following fortification processes.

4.4.2 *Fortification with folic acid, β-carotene, and vitamin A*

4.4.2.1 Head rice yield and color of fortified parboiled rice

The HRY and kernel color of fortified parboiled rice under both excess-water and limited-water soaking by different fortificants are presented in Table IV-3. The HRY, ranging 88.3-90.3%, was neither affected by fortificant nor by soaking condition, and the whiteness ($L*$) was higher in fortified rice than in non-fortified rice, agreeing with the previous results presented in Table IV-1. The redness ($a*$) and yellowness ($b*$) of folic acid and vitamin A fortified rice was similar to those of non-fortified rice due to low concentrations of folic acid and vitamin A in the soaking solution. Fortifying rice with β-carotene individually or in combination with folic acid resulted in slightly higher $b*$ values but similar $a*$ values relative to the control, indicating that fortified rice appeared less orange when a lower β-carotene concentration was used in the soaking solution compared to the concentration used in section *4.1.1*. The limited-water soaking method did not affect $L*$ and $b*$ values, regardless of fortificant, but resulted in higher $a*$ values with either β-carotene or vitamin A. These results agree with recent findings in mineral fortification by parboiling, where soaking condition had minimal impacts on the HRY and color of calcium and iron fortified rice (Jannasch & Wang, 2020).

4.4.2.2 Soaking condition on wastewater and total solids in wastewater

The limited-water soaking method aimed at reducing wastewater and solids in wastewater by using 25% of the amount of soaking solution as in the conventional excess-water soaking method. The present results showed that the limited-water soaking method produced about 87% and 45% less wastewater and solids in wastewater, respectively, compared to the excess-water soaking method (Table IV-3). Both fortificant type and fortificant combinations had no significant effect on the amount of wastewater under each soaking condition. The amount of solids in wastewater was similar for all fortificants under limited-water soaking and varied slightly in excess-water soaking. Simultaneous fortification with folic acid and vitamin A produced a similar amount of solids in wastewater as fortification with folic acid and β-carotene individually.

4.4.2.3 Soaking condition on vitamin content of milled rice and bran

Fortification of rice by parboiling with a combination of multiple nutrients is considered a feasible and efficient method to combat and simultaneously prevent several micronutrient deficiencies (Steiger et al., 2014; Thiruselvam et al., 2014; Patindol et al., 2017). The vitamin content in fortified milled rice was significantly increased by fortification with folic acid, β-carotene, and vitamin A individually and in their combination (Table IV-4). Compared to the excess-water soaking method, the limited-water soaking method slightly decreased the folic acid content and improved β-carotene retention in milled rice. The vitamin A content of rice fortified with only vitamin A was lower in the limited-water soaking method but was similar in both soaking conditions when vitamin A was combined with folic acid. Significant amounts of fortificants were retained in the bran with vitamin A showing the most difference. Milled rice vitamin uptake was significantly improved by the limited-water soaking method. Similarly, Jannasch & Wang (2020) found that the percentage of mineral uptake was higher in limited-water parboiling. This might be due to a more effective penetration of water into the rice kernels when soaked under vacuum (Tian et al., 2014). Milled rice fortified with folic acid (35 mg/L) and either β-carotene (50 mg/L) or vitamin A (34 mg/L) in the limited-water soaking contained ~2 mg/kg folic acid, ~16 mg/kg β-carotene, and ~3 mg/kg vitamin A. Thus, a daily consumption of 400 g of cooked fortified rice can contribute to about 75% of the RDAs for folic acid, to 173% (female) and 134% (male) of the RDAs for β-carotene, and to about 58% (female) and 45% (male) of the RDAs for vitamin A. Recent works using higher folic acid and β-carotene concentrations under the excess-water soaking method produced fortified rice with folic acid and β-carotene content that exceeded the RDAs with large variations (Kam et al., 2012a; Thiruselvam et al., 2014).

4.4.2.4 Statistical analysis

Effects of fortificant, soaking conditions, and their interaction on HRY, amount of wastewater, solids in wastewater, kernel color, folic acid, β-carotene, and vitamin A content of fortified rice were analyzed by two-way ANOVA (Table IV-5). HRY was neither affected by fortificant nor by the soaking condition, but the amount of wastewater and solids in wastewater were significantly affected by soaking conditions ($p<0.0001$). The color, including whiteness (L^*), redness (a^*), and yellowness (b^*), of fortified rice was primarily affected by fortificant ($p<0.0001$). In addition, L^* ($p=0.0179$) and a^* ($p<0.0001$) were affected by soaking condition, whereas b^* was affected by the interaction of fortificant and soaking condition ($p=0.0084$). The contents of β-carotene, and vitamin A in fortified rice were not affected by fortification with a single vitamin or the combination of two vitamins, whereas folic acid content was slightly affected by the presence of β-carotene and vitamin A ($p=0.0388$). Soaking condition had a significant impact on the contents of folic acid ($p=0.0043$) and β-carotene ($p=0.0019$), whereas only the interaction between fortificant combination and soaking condition was associated with vitamin A content of fortified rice.

4.5 Conclusions

This study demonstrated the effectiveness of the limited-water soaking method in rice fortification by parboiling with folic acid, β-carotene, vitamin A, and their combinations. Water-soluble forms of β-carotene and vitamin A resulted in high vitamin uptakes in milled rice while generating a similar amount of solids in wastewater as the parboiling process without fortificants.

Compared to the conventional excess-water soaking method, the limited-water soaking method had no impact on milling quality of rice and consumed about 75% less water and fortificant, thus reducing cost for the fortification process and wastewater treatment. Simultaneous fortification with folic acid and β-carotene or vitamin A using the limited-water soaking condition produced fortified rice that significantly contributes to folate and vitamin A intake in populations that consume large quantities of rice.

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4.7 Tables and Figures

Table IV-1 Effect of pure and water-soluble forms of β-carotene on head rice yield (HRY), milled rice color, amount of wastewater, and solids in wastewater in the limited-water soaking method[a].

Fortificant	Vitamin concentration (mg/L)	HRY (%)	Color			Wastewater (kg/kg brown rice)	Solids in wastewater (g/kg brown rice)
			Whiteness (L^*)	Redness (a^*)	Yellowness (b^*)		
Control	0	88.3±1.1a	54.4±0.0c	3.6±0.1b	22.1±0.3c	0.2±0.0a	5.1±0.6c
Pure β-carotene in Tween® 80	175	89.7±0.7a	58.2±0.5a	2.4±0.3c	24.9±0.1b	0.2±0.0a	8.9±0.1a
Water-soluble β-carotene 10% CWS/S	175	90.3±0.0a	56.4±0.2b	6.0±0.1a	31.0±1.0a	0.2±0.0a	6.7±0.5b
Vitamin A in Tween® 80	34	90.0±0.9a	57.4±0.0ab	2.0±0.2c	24.1±0.4b	0.2±0.0a	6.6±0.1b
Water-soluble vitamin A, type 250 CWS/F	34	90.3±1.7a	57.7±0.8ab	2.5±0.2c	22.2±0.1c	0.2±0.0a	6.2±0.1bc

[a] Means ± standard deviation of two replications with the same letter in the same column are not significantly different ($P<0.05$) based on Tukey’s HSD test.

Table IV-2 Effect of pure and water-soluble forms of β-carotene on vitamin content of milled rice and bran, and percentage of vitamin uptake of milled rice based on amount of vitamin added to soaking solution[a].

Fortificant	Vitamin concentration (mg/L)	Vitamin content (db[b], mg/kg)				Milled rice vitamin uptake (%)	
		Milled rice		Bran			
		β-carotene	Vitamin A	β-carotene	Vitamin A	β-carotene	Vitamin A
Control	0	0.0±0.0	0.0±0.0	2.3±1.0	74.3±10.6	-	-
Pure β-carotene in Tween® 80	175	2.9±0.6	-	136.2±1.1	-	2.8±0.6	-
Water-soluble β-carotene 10% CWS/S	175	45.8±15.7	-	3105.5±51.5	-	45.7±15.7	-
Vitamin A in Tween® 80	34	-	3.8±0.0	-	629.2±33.5	-	19.0±0.1
Water-soluble vitamin A, type 250 CWS/F	34	-	2.1±0.4	-	679.8±25.8	-	10.9±2.1

[a] Means ± standard deviation of two replications.
[b]Dry basis.

Table IV-3 Head rice yield (HRY) and color of rice fortified with folic acid, β-carotene, and vitamin A in excess- and limited-water soaking methods along with amount of wastewater and solids in wastewater generated in the parboiling process[a].

Fortificant	Soaking condition	HRY (%)	Color			Wastewater (kg/kg brown rice)	Solids in wastewater (g/kg brown rice)
			Whiteness (*L**)	Redness (*a**)	Yellowness (*b**)		
Control	Excess	88.3±0.7a	54.7±0.4d	3.0±0.3bc	22.0±0.7cd	1.5±0.0a	12.3±0.1a
	Limited	88.3±1.1a	54.4±0.0d	3.6±0.1ab	22.1±0.3cd	0.2±0.0b	5.1±0.6c
Folic acid	Excess	89.7±0.2a	59.1±0.2ab	1.6±0.1f	21.2±0.2cd	1.5±0.1a	10.0±0.1b
	Limited	90.2±0.1a	58.5±0.4abc	2.2±0.4def	22.3±0.6cd	0.2±0.0b	6.6±0.1c
β-carotene	Excess	89.1±0.9a	58.9±0.3abc	3.0±0.4bcd	24.9±0.6b	1.6±0.1a	10.5±0.7b
	Limited	89.1±0.0a	57.4±0.6bc	4.1±0.1a	25.8±0.4b	0.2±0.0b	5.9±0.3c
Vitamin A	Excess	90.3±1.1a	57.8±0.3abc	1.5±0.1f	22.9±0.2c	1.6±0.1a	11.5±0.3ab
	Limited	90.3±1.7a	57.6±0.8abc	2.5±0.2cde	22.2±0.1cd	0.2±0.0b	6.2±0.1c
Folic acid + β-carotene	Excess	88.7±0.8a	57.2±0.2c	3.2±0.1bc	27.6±0.4a	1.6±0.1a	11.4±0.7ab
	Limited	89.7±0.0a	57.6±1.0abc	3.6±0.0ab	26.2±0.6ab	0.2±0.0b	6.6±0.2c
Folic acid + vitamin A	Excess	89.6±0.5a	59.3±0.2a	1.5±0.2f	20.7±0.4d	1.5±0.0a	10.6±0.6b
	Limited	88.8±1.9a	58.4±0.0abc	2.2±0.1ef	21.5±0.3cd	0.2±0.0b	5.9±0.3c

[a] Means ± standard deviation of two replications with the same letter in the same column are not significantly different (P<0.05) based on Tukey's HSD test.

Table IV-4 Vitamin content of milled rice and bran in excess- and limited-water soaking methods, and percentage vitamin uptake of milled rice based on amount of vitamin added to soaking solution[a].

Fortificant	Soaking condition	Vitamin content (db[b], mg/kg)						Milled rice vitamin uptake (%)		
		Milled rice			Bran					
		Folic acid	β-carotene	Vitamin A	Folic acid	β-carotene	Vitamin A	Folic acid	β-carotene	Vitamin A
Control	Excess	0.2±0.0	-	-	0.2±0.0	2.3±1.0	14.5±0.8	-	-	-
	Limited	0.2±0.0	-	-	0.2±0.0	2.3±1.0	14.5±0.8	-	-	-
Folic acid	Excess	2.2±0.0	-	-	10.5±2.6	-	-	2.8±0.0	-	-
	Limited	1.8±0.3	-	-	7.1±1.2	-	-	9.3±1.2	-	-
β-carotene	Excess	-	6.8±0.6	-	-	333.7±54.5	-	-	6.0±0.5	-
	Limited	-	15.5±1.8	-	-	481.6±24.1	-	-	55.4±6.4	-
Vitamin A	Excess	-	-	4.4±0.9	-	-	674.9±23.3	-	-	5.6±1.1
	Limited	-	-	2.1±0.4	-	-	679.8±25.8	-	-	10.9±2.1
Folic acid + β-carotene	Excess	3.6±0.8	6.2±0.8	-	10.5±2.7	424.6±26.4	-	4.5±0.9	5.3±0.7	-
	Limited	2.0±0.1	16.1±1.1	-	16.6±1.0	418.8±22.1	-	10.3±0.5	57.9±3.9	-
Folic acid + vitamin A	Excess	3.5±0.2	-	2.3±0.2	16.7±2.7	-	611.3±6.7	4.5±0.2	-	3.0±0.3
	Limited	2.4±0.6	-	2.7±0.4	14.8±2.0	-	592.5±24.5	12.1±3.1	-	14.3±2.0

[a] Means ± standard deviation of two replications.
[b]Dry basis.

Table IV-5 Two-way analysis of variance (ANOVA) head rice yield, amount of wastewater, solids in wastewater, and vitamin content as affected by fortificant, soaking condition, and their interaction at a significant level $P<0.05$.

	P-Value		
	Fortificant	**Soaking condition**	**Fortificant* Soaking condition**
Head rice yield	0.1362	0.7972	0.8360
Wastewater	0.4582	<.0001*	0.4582
Solids in wastewater	0.0732	<.0001*	0.0010*
Whiteness (*L**)	<.0001*	0.0179*	0.1655
Redness (*a**)	<.0001*	<.0001*	0.2386
Yellowness (*b**)	<.0001*	0.5980	0.0084*
	Fortificant combination	**Soaking condition**	**Fortificant combination* soaking condition**
Folic acid content	0.0388*	0.0043*	0.1939
β-carotene content	0.7025	0.0019*	0.3476
Vitamin A content	0.1325	0.0633	0.0213*

V. Overall summary

This study demonstrated the effectiveness and feasibility of fortification by limited-water parboiling with multiple nutrients including calcium, iron, folic acid, β-carotene, and vitamin A. Reduction of water usage in the soaking step significantly reduced amount of wastewater and solids in wastewater, thus can reduce cost for fresh water, fortificants, and wastewater treatment. The fortification parboiling process greatly improved mineral and vitamin content of parboiled rice, whereas rice quality attributes were only minimally affected. In the fortification with lipophilic β-carotene and vitamin A, the use of their respective water-soluble forms was shown to be more time- and resource efficient compared to the use of pure fortificant forms in combination with an emulsifier. In conclusion, the implementation of fortification by parboiling using the limited-water soaking method in predominantly rice consuming countries is a cost-effective and sustainable way to improve overall nutrient intake without affecting consumer's eating habits. Studies elucidating validity for process upscaling of limited-water parboiling, consumer acceptance of fortified parboiled rice, and nutrient bioavailability may further enhance this study.