8-2017

Factors Impacting Iron Transfer into the Rice Kernel During Parboiling

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Food Science

by

Katherine Wilkes
Purdue University
Bachelor of Science in Food Science, 2015

August 2017
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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Abstract

Efforts to deliver essential micronutrients through existing rice fortification methods are challenged by poor consumer acceptance of fortified rice, production costs, and equipment availability. Current research efforts seek to explore the potential of parboiling to improve nutrient delivery. This study investigates factors that affect iron transfer into the kernel endosperm via parboiling by evaluating the impact of varied milling durations, iron concentrations in the soaking water, and parboiling conditions upon iron penetration into the rice kernel. Long-grain rice grown in Arkansas was parboiled as rough, brown, and milled rice under various soaking and steaming temperature conditions. Visual quality of the milled parboiled rice was evaluated using a colorimeter utilizing the L* a* b* color scale. Compositional analyses quantified the impact of parboiling conditions upon the iron content of various rice kernel components.

This work demonstrates that hull and bran layers of the rice kernel act as a barrier to iron uptake via parboiling fortification. Hybrid CL XL745 and pureline Diamond rice cultivars exhibited differences in iron uptake and response to parboiling conditions. Parboiling brown and milled rice resulted in increased fortification efficiency compared to rice parboiled in the rough rice form. Parboiled conditions (soaking temperature and inclusion of a steaming step) were critical determinants of iron penetration into the rice kernel and of parboiled rice color. Milling studies indicated that iron fortificants primarily deposit in the external kernel layers.

Optimization of this fortification method may be particularly advantageous in regions of the world where rice is a staple crop and where it represents a major source of daily caloric intake. Understanding the impacts of parboiling soaking and steaming steps may allow rice processors to deliver increased nutrition via parboiled rice.

Table of Contents

List of Figures

Chapter 1:

Introduction and Literature Review

1.1 Iron Deficiency

Iron deficiency affects approximately one-fourth of the world's children and women of childbearing age, and is the leading cause of anemia worldwide (Stoltzfus 2014). It is particularly prevalent within communities that experience food insecurity, those that rely heavily on staple crops for primary nutrition, and those with low dietary diversity (Juliano 1993, WHO 2006). Iron deficiency causes growth stunting in children, fatigue, reduced cognitive capacity, and is a risk factor for increased mortality rates for infants, children, and pregnant women (Stoltzfus 2014).

1.2 Rice Fortification

Rice is the preferred or only domestically available staple crop for more than half of the world's population, with the majority of consumption occurring in Asia, but is emerging as a principal source of nutrition in Africa, Latin America, and the Caribbean (Ashong et al. 2012, Piccoli et al. 2012) as well. Approximately 750 million metric tons of paddy rice were harvested globally in 2014 alone (FAOSTAT 2014). The relatively low cost of fortification, year-round availability, potential for delivery of micronutrients with complementary macronutrients, and limited changes required to consumption behaviors make rice fortification a promising means of addressing micronutrient deficiencies (Piccoli et al. 2012). However, efforts to fortify rice via existing methods face inefficiencies and logistical challenges to adoption by both commercial processors and by households in affected regions (Alavi et al. 2008, Roks 2014).

1.3 Existing Approaches to Rice Fortification

The alleviation of iron deficiency and iron deficiency anemia via consumption of fortified rice has been demonstrated in supervised rice fortification programs for children in the Philippines and in India (Moretti et al. 2006, Angeles-Agdeppa et al. 2008). Currently, multiple methods for both bulk fortification and pre-mixing (blending highly-fortified kernels with conventional rice) are in use to address micronutrient deficiencies. Dusting rice with electrostatically-attracted mineral powders is largely considered ineffective, as rice washing methods commonly practiced in developing countries to remove adulterants also remove micronutrients (Alavi et al. 2008). Spray-coating rice kernels with highly-concentrated mineral solutions to form a "premix" prevents iron removal via preparation, but unpalatable fortified kernels are commonly identified and removed due to their appearance (Alavi et al. 2008). Emulsification, encapsulation, and nanoparticulation of fortifying agents may address bioavailability and stability issues, but are expensive to implement in developing regions and have met mixed success in rice fortification applications (Moretti et al. 2006). Cold extrusion methods blend highly-fortified, rice flour-based "pasta" that resembles rice grains with natural rice (Ashong et al. 2012). Hot, twin-screw extrusion produces harder, more transparent kernels that are more consistent with the appearance of natural rice (Alavi et al. 2008). These "kernels" require much greater capital equipment costs to produce—thus, this method is challenged by resources available in the regions most affected by micronutrient deficiencies. As such, further technological developments are needed to increase the rate at which deficiencies are alleviated at minimal cost to consumers and stakeholders.

1.4 Fortifying Agents

There are many complex, intertwined factors that must be considered when determining fortification levels; target population consumption behaviors, dietary diversity, age, gender, cultural practices, and food product characteristics. A customized degree of fortification may best serve the unique needs of different populations. WHO guidelines (WHO 2006) recommend limiting iron consumed via fortified food products to 5-10 mg iron/day.

Various compounds are currently in use to fortify food products with iron. Ferrous sulfate ($FeSO_4$) and ferrous pyrophosphate are commonly included in cereal applications, but their use is limited by organoleptic changes, solubility, and bioavailability (WHO 2006, Moretti et al. 2006). Increasing the solubility of fortifying agents increases solubility in human gastric juices, but washing and excess-water boiling practices can then more easily remove fortificant from kernels during food preparation (WHO 2006). Generally, decreases in particle size of fortifying compounds promote catalyzation of oxidative reactions and complexation with tannins, polyphenols, and sulfur compounds within the food matrix that may reduce bioavailability (Cook and Reusser 1983).

Phytin is the predominant storage form of phosphorous in cereals and a major inhibitor of mineral absorption. It chelates divalent molecules and prevents their absorption in the GI tract (Champagne et al. 2004, Delcour and Hoseney 2010). Hot soaking of rice has been shown to reduce the amount of phytin present in the kernel (Liang et al. 2008). Chelating agents, such as citrate and ethylenediaminetetraacetic acid (EDTA) are used to bind iron compounds to reduce their interaction with the rice matrix, thus increasing their bioavailability. While the high concentrations of NaFeEDTA in use in extruded, premixed kernels can cause some color change (Moretti et al. 2006), NaFeEDTA's high bioavailability in the presence of absorption inhibitors

makes it an ideal fortifying agent for cereals, grains, and flours (WHO 2006). Clinical trials support this assessment: Kenyan schoolchildren, who were originally diagnosed with iron deficiency and or/iron deficiency anemia, demonstrated greater improvement in hemoglobin concentrations and reduced prevalence of anemia after consuming whole-maize flour fortified with NaFeEDTA compared to those who consumed flour fortified with electrolytic iron (Andang'o et al. 2007). MacPhail et al. (1994) found the optimal ratio of iron to Na_2EDTA to be between 1:1 and 1:4 for maximum bioavailability. Prom-u-thai et al. (2011a), in the development of parboiling fortification, reported high rates of iron delivery and bioavailability in both whole and broken milled parboiled rice soaked in acidic solutions of FeEDTA. Cost is a critical challenge to implementation of fortification programs using NaFeEDTA. Bothwell and MacPhail (2004) estimated its cost to be 6-8 times greater than that of ferrous sulfate. However both Prom-u-thai et al. (2011b) and Bothwell and MacPhail (2004) point to the increased bioavailability as a potential cost savings. Parboiling has also been applied to fortify rice with combined iron and zinc (Prom-u-thai et al. 2011); folic acid (Kam et al. 2012); and folic acid, β-carotene, and iron (Thiruselvam et al. 2014). Further studies on parboiling fortification with more complex micronutrient systems are necessary to address a variety of deficiencies.

1.5 Parboiling

Parboiling is a hydrothermal rice processing method used to process approximately one-fifth of the world's supply of rice (Bhattaracharya 2004). Parboiling involves three steps: soaking, steaming, and drying. Rough rice is first soaked to a moisture content in excess of 28% MC[1], then steamed to gelatinize kernel starch (Appiani 2014). The rice is then dried to a MC of

[1] All MCs are expressed on a wet weight basis.

approximately 12%. Parboiled rice typically produces greater milling yields than non-parboiled rice, a difference that has been attributed to starch gelatinization and the "fusion" of fissured rice kernels (Miah et al. 2001, Appiani 2014). High temperatures achieved during soaking and steaming inactivate enzymes and reduce or eliminate insect and mold proliferation in parboiled rice, which enhances shelf life and final quality (Appiani 2014). Thus, there is an economic incentive to parboil rice in regions where the quality of the rice harvest is typically poor.

While the majority of parboiled rice is currently produced via small-scale production in South Asia, modern hot-soaking methods are replacing traditional techniques in large-scale production (Bhattaracharya 2004). Today, Thailand and India are the primary global exporters of parboiled rice, followed by Brazil and the United States (Bhattacharya 2004). Outside of South Asia, the countries that import parboiled rice are largely Middle Eastern and African, such as Saudi Arabia, Ghana, Nigeria, South Africa, and Zimbabwe (Bhattacharya 2004). Parboiled rice is golden in color, and when cooked, is texturally harder and fluffier than cooked fresh rice. In Western countries, parboiled rice is valued for its resistance to stickiness and tolerance of thermal abuse in both retail and food service markets (Appiani 2014).

Parboiled rice exhibits increased levels of thiamine, B vitamins, sugars, amino acids, calcium, phosphorus, and manganese when compared to non-parboiled rice (Champagne et al. 2004). These nutritional benefits provide another incentive to parboil rice, and have been shown to prevent *beriberi* (thiamine deficiency), whereas a diet based on non-parboiled rice cannot (Bhattacharya 2004). It is theorized that water soluble constituents in the bran layers diffuse into the rice endosperm during parboiling (Kam et al 2012) which allows them to be retained through milling. Further work suggests that vitamins and minerals may adhere to the endosperm via gelatinized starch (Bhattacharya 2004, Kam et al 2012).

1.6 Fortification via Parboiling

A potential answer to widespread iron deficiency may be a fortification method based on parboiling—iron would be introduced to the soaking water, allowing minerals to infuse into the kernel during the soaking and steaming steps. This approach may have lower operating and startup costs than current extrusion fortification methods and would utilize equipment not only available in large, commercial facilities, but also in homes and villages. Furthermore, if iron penetrates into the kernel during parboiling, as is indicated by initial work in this area (Prom-u-thai 2008, 2010) and pilot scale-up efforts **(**Thiruselvam et al. 2014), this approach potentially presents a fortification solution that may be effective despite traditional rice preparation methods, such as washing before cooking or boiling in excess water, that tend to remove nutrients from rice fortified using current methods.

1.7 Influence of Parboiling Conditions

The vast majority of parboiled rice is processed in the rough rice form, but brown rice parboiling has been studied for its reduced water and energy requirements (Bhattacharya 2004, Parnsakhorn and Noomhorm 2012). Parboiling brown rice may also reduce color change, prevent odor development, and yield cooked texture characteristics comparable to parboiled rough rice (Kar et al. 1998, Patindol et al. 2008, Parnsakhorn and Noomhorm 2012, Appiani 2014). Water diffusion rates into the rice kernel during soaking, and potentially the delivery rate of soluble fortifying agents, are hastened by increased water temperature and the removal of the rice hull (Bhattacharya 2004, Thakur and Gupta 2006, Kam et al. 2012). Iron uptake could be faster and fortification efficiency greater as soaking temperatures increase above the onset gelatinization temperature. However, increasing soaking temperatures have also been shown to cause

discoloration and nutrient loss when hulls break open (Appiani 2014). The rice steaming step in parboiling gelatinizes kernel starches, distributes water and kernel constituents throughout the endosperm, inactivates enzymes, and eliminates pests (Appiani 2014). Steaming also deepens the golden color of parboiled rice, which is generally considered undesirable, and inactivates antioxidants (which has a negative impact on shelf life) (Bhattacharya 2004, Appiani 2014). Appiani (2014) hypothesizes that the steaming step is critical to color and nutrient distribution because exposure to heat and pressure drives components to penetrate the endosperm—a concept that may have applications to iron uptake and distribution within the kernel.

1.8 Kernel Structure: Potential Barriers to Uptake

Rice (*orzya satvia* L.) is harvested as rough (paddy) rice. Rough rice kernels are protected by the hull, which makes up approximately 20% of the kernel weight and is composed of the lemma and palea, as seen in Figure I. The lemma and palea overlap to prevent pests from reaching the caryopsis, or brown rice kernel. The hull layers are rich in cellulose and notably, silica, which deters pests and fungal growth (Champagne et al 2004, Delcour and Hosney 2010). Hulls are removed to produce brown rice. Prom-u-thai et al. (2008) tracked rice hull iron content as iron fortificant concentrations in soaking water increased and demonstrated that rice hulls take up iron during the parboiling process. This indicates that dehulling rice before parboiling could improve the efficiency of iron uptake by the kernel endosperm during parboiling fortification.

Rice lipids are primarily located in the aleurone, subaleurone, and germ of rice bran (Champagne et. al 2004). These constituents are removed as the degree of milling increases. The mature endosperm houses the majority of rice's macronutrients, including both protein and starch (Delcour and Hosney 2010, Bhattacharya 2011). Pomeranz and Webb (1985) proposed

that high brown rice total lipid content (TLC) indicates increased bran layer thickness, while lower TLC values are associated with thinner bran layers. Rohrer and Siebenmorgen (2004) suggested that lipid, as a constituent of the bran, may differ in content among rice cultivars. Lanning and Siebenmorgen's work with cultivar characterization confirmed these ideas—pureline long-grain rice cultivars were shown to have significantly greater oil content than hybrid long-grain rice cultivars, and therefore more oil present in the rice bran layers (2011).

Prom-u-thai et al. (2008) observed ten-fold reductions in iron content in parboiled, fortified rice between unmilled (brown rice) samples and those milled for 60 s. These findings indicate that during parboiling, iron may deposit in regions of the rice kernel (bran, germ, pericarp) that are removed when brown rice is milled: this is and are supported by images of kernel iron distribution produced using Perls Prussian Blue staining as a component of the same study. Prom-u-thai et al. (2008) noted significant differences among the iron contents of fortified, parboiled end products from seven cultivars evaluated and suggested that cultivar characteristics may impact iron uptake during parboiling fortification and retention after milling. Differences between cultivars' bran layer thicknesses and oil contents may impact iron uptake and penetration into the kernel. Limiting removal of kernel layers after parboiling may be critical to maintenance of iron in the parboiled rice kernel.

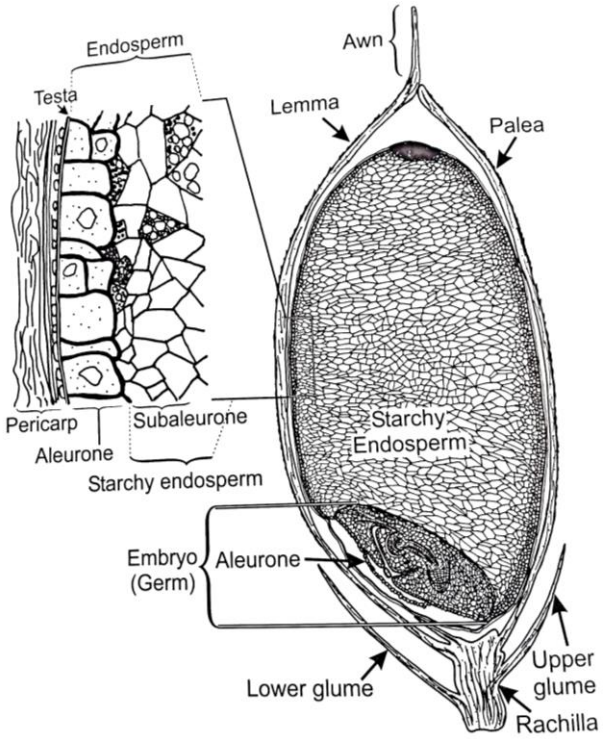

Fig. 5. Illustrations of a longitudinal section of a rice spikelet.

Figure 1.1 Structure of the Rough Rice Kernel (Champagne et al 2004)

1.9 Rationale

Collaborative efforts between scientists at Chiang Mai University and the University of Queensland have begun to investigate the viability of rice fortification with micronutrients using parboiling. The authors explored the impact of fortification using parboiling upon color, cooking quality, and sensory characteristics; consumer acceptability; and began process optimization for scale up by food processors (Prom-u-thai et al. 2009, 2010, 2011). Further work from Thiruselvam et al. (2014) confirms the success of parboiling fortification of rice at the pilot scale. This work suggests the viability of parboiling as a fortification method, but further research is needed to understand the process parameters that can be manipulated to maximize iron uptake into the kernel.

This study has two primary objectives regarding the mechanisms of iron transfer into rice via parboiling. The first objective was to determine the impact of rice feedstock, fortificant concentration, and parboiling conditions on the total iron content and color of rough, brown, and milled long-grain rice fortified using parboiling. The second objective was to assess the degree to which iron penetrates the rice kernel endosperm during parboiling by assessing the influence of milling duration on the iron content of brown and milled rice. By quantifying the impact of the above factors, this experiment may explain what factors drive and inhibit iron uptake into the rice kernel during parboiling.

Chapter 2

Materials and Methods

2.1 Initial Rice Preparation and Characterization

Long-grain hybrid rice cultivar, CL XL745, was harvested, dried to approximately 12% MC, and stored in a farm bin near Pocahontas, Arkansas in the fall of 2015. Rice was removed from the bin and transported to the University of Arkansas Department of Food Science in Fayetteville, Arkansas in June, 2016. Long-grain pureline rice cultivar, Diamond, was harvested from the Rice Research and Extension Center near Stuttgart, AR in September, 2016. Rice was stored in grain bags in a walk-in refrigerator at 5°C at the research station before transport to the University of Arkansas Food Science Department in September, 2016.

2.2 Rice Procurement

Upon arrival, both rough rice lots were cleaned using a dockage tester (Model XT4, Carter-Day, Minneapolis, MN). The Diamond lot was then dried in thin layers under ambient conditions to approximately 12.5% MC. The MCs of both lots were measured in duplicate using an oven drying method previously established by Jindal and Siebenmorgen (1986) in a laboratory oven (Oven F Air 6.3 CF, VWR, Radnor, PA). Rice was stored at 5°C in a walk-in refrigerator in sealed plastic containers before dehulling, milling, and sample preparation occurred.

2.3 Non-Parboiled Rice Milling Durations

In order to determine the necessary milling duration to achieve a degree of milling of 0.4% surface lipid content (SLC) for non-parboiled rice, 150-g rice samples of Diamond and CL XL745 long-grain rice cultivars were dehulled using a laboratory sheller (THU, Satake, Tokyo, Japan) with a clearance of 0.048 cm between the rollers. The resultant brown rice was then milled using a laboratory mill (McGill no. 2, RAPSCO, Brookshire, TX) equipped with a 1.5-kg weight on the lever arm, situated 15 cm from the milling chamber centerline. Samples were milled for durations between 10 and 50 s, in ten-second increments. After milling, the rice was aspirated to remove loose particles of bran and germ, then separated into head rice and broken rice fractions using a sizing device (Model 61, Grain Machinery Mfg. Corp., Miami, FL). Surface lipid content was quantified for head rice at each degree of milling using a Soxhlet method described in AACC method 30-20 (AACC Intl., 2000) with modifications to the petroleum ether washing duration as described by Matsler and Siebenmorgen (2005). Lipid extractions were performed using a Soxtec lipid extraction apparatus (Avanti 2055, Foss North America, Eden Prairie, MN). SLC values were plotted against milling duration and trendlines were produced. Milling durations to achieve approximately 0.4% SLC was determined for each cultivar using these trendlines.

2.4 Rice Thermal Property Measurement

To determine onset gelatinization temperatures for each cultivar, flour-water slurries were analyzed using differential scanning calorimetry (DSC, Pyris Diamond, Perkin-Elmer Co., Norwalk, CT). Head rice samples were ground into flour using a cyclone sample mill (3010-30, UDY, Fort Collins, CO) fitted with a 0.25-mm mesh sieve. Four mg of rice flour was weighed

into an aluminum pan and mixed with 8 μL deionized water. The aluminum pan was hermetically sealed and equilibrated at room temperature for 1 h prior to scanning from 25°C to 120°C at 10°C/min. Thermal properties, including onset, peak, and conclusion gelatinization temperatures, and enthalpy of each thermal transition were determined. Onset gelatinization temperatures were used to determine soaking temperatures used during parboiling. Onset gelatinization occurred at 73°C for CL XL745 and 73.5°C for Diamond rice.

2.5 Determination of Parboiled Milling Durations

In order to assess milling durations necessary to achieve 0.4% SLC for parboiled rice, 200-g samples of rough rice were first parboiled using a water bath (Thermo Fisher Scientific Model 2849, Marietta, OH) for soaking and an autoclave (Tuttnauer Brinkmann Model 2340E, Hauppauge, NY) for steaming. Each 200-g sample was soaked in 400 mL of ultra-purified water in an 800 mL beaker. Ultra-purified water was produced in the lab using a water purification system (Merck Millapore Direct Q3, Darmstadt, Germany). Samples were soaked for three hours at the DSC-determined onset gelatinization temperature less 4°C for each cultivar, drained, layered evenly on a stainless steel tray, then steamed for 10 minutes at 115°C. Samples were then dried to approximately 12.5% MC in a chamber with air conditions controlled at 27°C and 60% relative humidity by a temperature- and humidity-control unit (AA5582, Parameter Generation and Control, Inc., Black Mountain, NC). Dehulling was conducted using the same laboratory sheller specified previously, but parboiled rice samples were passed through the sheller two times. After dehulling, the parboiled brown rice samples were milled using the above procedure, but for durations between 30 and 70 s, in ten-second increments. After milling, the rice was aspirated to remove loose particles of bran and germ, then separated into head rice and broken

rice fractions using a sizing device (Model 61, Grain Machinery Mfg. Corp., Miami, FL). Surface lipid content was quantified for parboiled head rice at each degree of milling the method described above. SLC values were plotted against milling duration and trendlines were produced. Milling durations to achieve approximately 0.4% SLC was determined for each cultivar using these trendlines.

2.6 Rice Cultivar Characterization

Brown rice from each cultivar was evaluated for total lipid content (TLC) as an indicator of bran layer oil content. TLC values were quantified using brown rice flour per AACC method 30-20 (AACC Intl., 2000) with modifications to the petroleum ether washing duration, as described by Matsler and Siebenmorgen (2005) and using a Soxtec fat extraction system (Avanti 2055, Foss North America, Eden Prairie, MN). Brown and milled rice crude protein contents were assessed in accordance with to Approved Method 46-13.01 (AACC 2000) using a conversion factor of 5.95. Apparent amylose content of head rice was assessed by iodine colorimetry per the method described by Juliano (1971). Brown and milled rice dimensions (length, width, thickness, and surface area) were assessed using a SeedCount kernel imaging system (Seedcount, Graintec Scientific, Queensland, Australia).

2.7 Hydration Curves

In order to understand the hydration properties of rough, brown, and head rice, hydration curves were produced for all three parboiling feedstocks at all three soaking temperatures—onset temperature less 4°C, at onset temperature, and 4°C above onset temperature. Samples of approximately 5 g of rice were placed separately into 50 mL beakers containing 10 mL of ultra-

purified water which was preheated to the desired temperatures in a water bath. Two samples were removed from the water bath every 30 minutes for a total study duration of 3 hours. Any excess water was poured off and samples were quickly blotted with paper towels upon removal from the water bath to remove surface moisture. Moisture contents of each soaked sample were determined using approximately 5-g samples of the soaked rice and the oven method previously described. The MCs of the soaked rice samples were calculated based on the final dry weight and plotted against soaking time to obtain the hydration curve.

2.8 Sample Preparation

Once initial sample processing and moisture content verification was complete, rough rice was stored in Ziploc bags containing approximately 200 g of rice. Bulk brown rice was produced by a two-pass dehulling procedure using the laboratory sheller described previously followed by hand removal of remaining hulled kernels. Broken kernels were then removed using a test rice grader. Brown rice was graded for three minutes per 500 g with the holder at a 25° angle (TRG, Satake, Tokyo, Japan). To produce milled rice samples, rice was first dehulled using the two-pass procedure described previously, then milled to a target surface lipid content of approximately 0.4% as described above. Head rice was then separated out using the same test grading separation procedure described previously. Once brown and milled rice was produced, both forms of rice was stored in Ziploc bags of approximately 200 g of rice.

2.9 Parboiling: Soaking and Steaming

Rough, brown, and head rice samples were soaked in ultra-purified water containing NaFeEDTA at concentrations of 0, 250, 500, and 750 mg/L at onset gelatinization less 4°C, at onset, and 4°C above onset temperatures. Soaking took place in the laboratory water bath

specified above. Each 200-g sample was soaked in 400 mL of ultra-purified water in an 800 mL beaker. While pH was not controlled in this study, NaFeEDTA solution pH values were measured at ambient temperature using a handheld pH meter (Beckman Coulter, Inc., Fullerton, CA) Samples to be steamed were drained of any excess water, layered evenly on a stainless steel tray, then steamed for 10 minutes at 115°C in the benchtop autoclave specified previously.

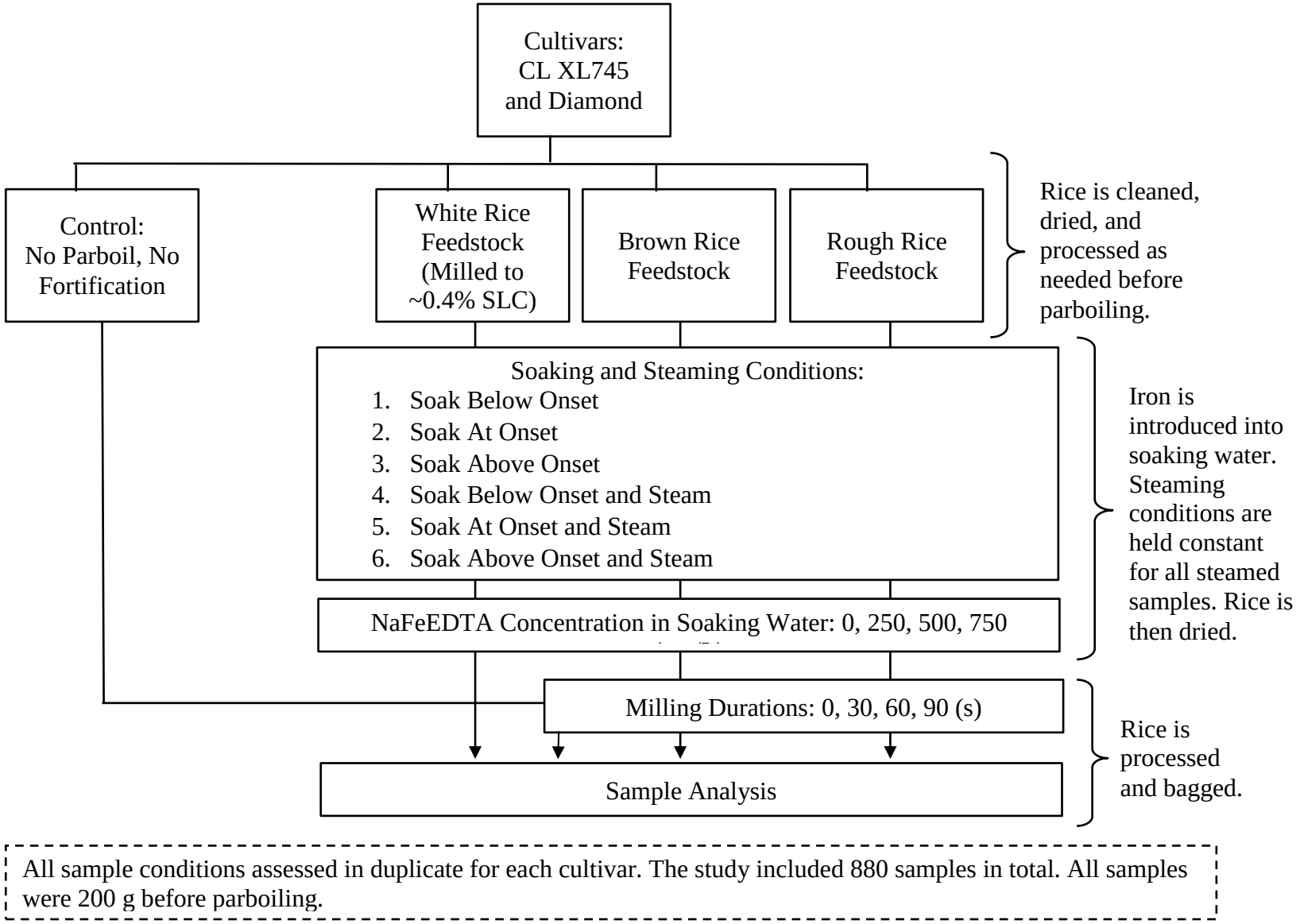

Figure 2.2 Visual Overview of Experimental Design

2.10 Post-Parboil Rice Processing

After soaking and steaming, rice was dried to approximately 12.5% MC for further processing. Samples were dried on wooden-framed screens at 27°C and 60% RH in a temperature- and humidity-controlled chamber as described previously. Once rice was dried, 150 g of each rough rice sample was then dehulled using the lab sheller and two-pass procedure described above. Brown rice was milled to a surface lipid content of approximately 0.4% using a laboratory mill and aspirated to remove any remaining germ or bran as described above. Broken rice was removed from milled rice samples using a sizing device a specified previously. Samples that were to remain in brown rice form (0 s milling duration) were separated into brown "head rice" and broken fractions using the same method.

2.11 Iron and Color Analyses

Once processed, head rice samples were evaluated for L* a* b* color ratings using a Hunter colorimeter (Colorflex, Hunter, Reston, VA). For each color analysis, a 60 x 15 mm transparent plastic Petri dish (Fisher Scientific, Waltham, MA) was filled with rice and leveled to maintain a bulk sample of consistent depth. Surface lipid content of a selection of milled parboiled samples and total lipid content (TLC) of a selection of brown rice samples were confirmed using the method described previously. Rice samples were sent to Altheimer Labs at the University of Arkansas (Fayetteville, Arkansas) for total iron content analysis. Rice was first digested by wet-ashing, a procedure which consisted of a pre-digestion in concentrated HNO_3 from room temperature to 60°C in 30 min followed by digestion at 90°C for 90 min with the addition of 30% H_2O_2. Aliquots of the digested samples were analyzed for Fe by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Spectro Arcos FHS16 (Spectro Analytical Instruments GmbH, Kleve, Germany).

2.12 Sampling and Data Analysis

Statistical analyses will be conducted using JMP Pro 13 (SAS Software Institute, Cary, NC Analysis of variance (ANOVA) with a significance level at 0.05 and variability assessments were used to determine the effects of cultivar, feedstock, parboiling conditions, fortification level, and the interactions between factors on the properties of fortified parboiled rice. Individual pairwise comparisons of means were conducted using Student's t tests.

Moisture content of hydration curve samples were modeled by feedstock. Rough, brown, and white rice feedstocks were evaluated using Gompertz models as given below.

$$y = a * Exp(-Exp(-b * (time(\min) - c)$$

Where *a* represents the asymptote, *b* represents the growth rate, and *c* represents the inflection point.

Chapter 3

Results and Discussion

3.1 Rice Physical and Chemical Properties

Total lipid content, gelatinization temperatures, kernel dimensions, amylose content, and protein content of CL XL745 and Diamond rice cultivars in brown and milled rice forms are shown in Table 3.1. Milled CL XL745 kernels were slightly longer (6.9 mm versus 6.6 mm), and thus had slightly greater surface area (10.3 mm^2 versus 9.8 mm^2), than Diamond kernels. Onset gelatinization was achieved at 73.0 °C for CL XL745 and 73.5 °F for Diamond. Protein content of Diamond rice was approximately 0.8% greater than CL XL745 in both brown and milled rice forms. Total lipid contents of CL XL745 and Diamond were 2.45% and 2.59%, respectively.

3.2 Hydration Curves

The hydration profiles of CL XL745 and Diamond in rough, brown, and milled rice form soaked below, at, and above onset gelatinization temperatures are given in Figure 3.1. For all three parboiling feedstocks, soaking duration was the primary driver for rice hydration. As layers of the kernel were removed (hull, bran and germ), temperature had a more significant impact on the rate and degree of rice hydration. The hydration profiles of rice soaked below, at, and above onset temperature varied within each feedstock. The impact of temperature is particularly profound when milled rice soaking temperature increased beyond the onset gelatinization temperature ("above onset" condition). Hydration of milled rice above onset was over six times greater than milled rice soaked under "below onset" or "at onset" conditions. This change was attributed to the impact of complete rice starch gelatinization and swelling upon rice cooking at the "above onset" condition (Fitzgerald 2004). Slight decreases in "above onset" milled rice MC at the end of the hydration study were attributed to losses of pasted rice solids after cooking.

Brown and rough rice hydration was restricted by hull, bran, and germ layers. Hybrid rice hydrated more rapidly and to a greater degree than the pureline, which may be attributed to the differences in kernel length and surface area between CL XL745 and Diamond rice. Brown and rough rice hydration profiles are consistent with paddy and brown rice hydration profiles soaked at temperatures between 30°C-60°C by Thakur and Gupta (2006). Bergman et al. (2004) hypothesize that both kernel surface area and protein content drive lower hydration rates. Increasing the size and strength of the protein network may prevent water binding and increase the structure of the kernel. Diamond rice had protein contents of 9.02% and 8.14% for rough and milled rice, respectively, and CL XL745 has 8.24% and 7.36% protein for brown and milled rice, respectively. Increased presence of protein in the Diamond rice may have reduced its hydration rate. Hydration curves for both cultivars parboiled in rough, brown, and white rice forms were assessed using Gompertz models. The Gompertz model is a sigmoid function characterized by slow growth at the start and end of the time period.

3.3 Relationship between Lipid Content and Iron Retention

Analyses of brown rice samples fortified at 750 mg/L NaFeEDTA indicated a significant impact of rice lipid content (encompassing TLC and SLC) upon total iron content of parboiled, fortified rice (Figure 5.2 and Table 5.7). This indicates that iron is removed with increased degrees of milling, thus, some iron is retained in exterior layers of the rice kernel. Brown rice parboiled with 750 mg/L NaFeEDTA took up 33.34 mg Fe per kg rice when in the brown rice form. 45.2% of the total Fe remained after 30 s of milling; 36.7% after 60 s; and 32.3% after 90 s of milling.

Retention was driven, to a lesser extent, by cultivar. Brown CL XL745 retained greater concentrations of iron than Diamond. This difference may be attributed to differences in oil

content or bran layer structure between the evaluated cultivars. Increased lipid content may prevent water uptake and thus prevent Fe uptake.

3.4 Assessment of Parboiling Factors Upon Total Iron Uptake

Factors that determine total iron content of parboiled fortified rice are depicted in Figure 5.2. Main effects are weighted by their impact on total iron content, while total effects include the impact of interacting factors. Feedstock was the primary driver for iron uptake. The concentration of NaFeEDTA in the soaking solution, soaking temperature, inclusion of a steaming step, and cultivar were secondary effects. Figure 5.3 depicts the conditions that maximized the total iron content of experimental parboiled, fortified rice. This analysis indicated that kernel layers present for parboiling and the Fe concentration of the soaking solution itself were the primary drivers for fortification.

3.5 Effect of Cultivar and Feedstock on Mineral Retention

Figures 3.2-3.4 present the comparative Fe contents of fortified-parboiled rough, brown, and white rice from lots parboiled in fortificant solutions from 0-750 mg/L. Hybrid cultivar CL XL745 exhibited greater degrees of iron uptake than Diamond, a pureline cultivar, for rough and white rice feedstocks, but were not significantly different for rice parboiled in the brown rice form. Increased length and surface area of CL XL745 kernels and greater protein content of Diamond rice (Table 3.1) may contribute to the reduced hydration and iron uptake of Diamond samples. Both dehulling and milling of rice before parboiling significantly improved the efficiency of iron fortification.

Rice hulls, which contain 9-20% lignin (Champagne et al. 2004), may absorb and attract iron during parboiling fortification. Lignin's aromatic structure, large surface area (Wu et al. 2008), and attractive functional groups (Krishnani et al. 2007) make it an ideal adsorption agent for metallic cations, including Fe (III). Steam activation, oxidation, and acid treatments can further improve cation adsorption (Marshall 2004). Rice hull ash, lignin, and biomatrices composed of rice hulls have been used to attract and collect metals such as Ni (II) (Lin and Wang 2011), Chromium (III), and Zn (Krishnani et al. 2007). Parboiling conditions would therefore potentiate husk attraction and adsorption of Fe (III) cations in solution. Prom-u-thai et al. (2008) confirmed this with husk iron retention values of fortified parboiled rice. Husk total Fe increased from 33.10 mg/kg in unfortified parboiled rice to 16140 mg/kg. In this way, parboiling in the rough rice form reduces iron uptake both physically, as a barrier, and chemically, when rice hulls bind iron cations present in the fortification system.

At equivalent degrees of milling and across all parboiling treatments, rice fortified with 750 mg/L of NaFeEDTA brown CL XL745 absorbed nearly five times as much iron as rough rice (3.74 mg/kg and 15.86 mg/kg, respectively). Diamond rice absorbed 3.00 mg/kg in the rough rice form and 15.07 mg/kg in the brown rice form. Milled CL XL745 rice absorbed 25 times the Fe (75.59 mg/kg) retained in rough rice. Diamond absorbed 51.01 mg/kg in the white rice form. The efficiency of iron delivery via parboiling with a brown feedstock may be impeded by both non-polar bran layer lipids and by complexation of exogenous minerals with phytin and bran layer proteins. Champagne et al. (1985) demonstrated that the addition of Fe (II) and Fe (III) formed insoluble complexes with albumin from rice bran. Phytin present in the bran layer can also bind divalent cations as they enter the rice kernel.

3.6 Effect of Soaking and Steaming on Mineral Retention

Total iron contents of rough, brown, and milled rice from soaking-only samples versus soaking-and-steaming samples at all three soaking temperatures are depicted in Figures 3.2-3.4. Endogenous total Fe contents (control samples, neither parboiled nor fortified) were 0-3.27 mg/kg and 0-4.82 mg/kg for CL XL745 and Diamond cultivars, respectively. Soaking treatments alone drove 2.8 times more iron into the kernel endosperm than the control. The addition of a steaming step significantly increased kernel iron uptake for a selection of conditions and feedstocks. Rough rice iron uptake was significantly greater for all soaking-and-steaming conditions compared to soaking alone. Parboiled fortified brown rice only saw a significant increase in Fe uptake for rice soaked under "above onset" conditions. White rice demonstrated significant differences between samples soaked "above onset" and steamed, and soaking-only samples soaked at "at onset" and "below onset" temperatures. The mean Fe content for both rice cultivars fortified at 500 mg/L and milled for 30 s increased from 0.13 mg/kg to 3.52 mg/kg for the rough rice feedstock; 11.22 mg/kg to 11.96 mg/kg for brown rice; and 38.92 mg/kg to 56.88 mg/kg for milled rice with the addition of a steaming step. This indicates the importance of the inclusion of a steaming step to maximize iron uptake.

3.7 Effect of Iron Fortificant Level and Milling Duration

For rough, brown, and white rice feedstocks, fortificant level and milling duration were the primary determinants of total iron content. For both cultivars and all treatments fortified to 500 mg/L and milled to 30 s, rough rice delivers a mean of 1.83 mg/kg Fe, brown rice delivers 11.59 mg/kg (6.1x that of rough rice), and white 48.29 mg/kg (25.6 times that of rough rice). These results indicate the critical nature of feedstock for optimizing fortification delivery. While Fe delivery was maximized with a white rice feedstock, rice quality and process considerations

prevent adoption of this method. However, parboiling brown rice with both soaking and steaming steps may deliver therapeutic levels of iron with economic fortificant concentrations while maintaining rice quality comparable to rice parboiled in the rough rice form.

As milling duration increased, total Fe content of all parboiled fortified rice decreases. In brown rice fortified to 500 mg/L, unmilled rice took up 25.13 mg/kg rice. With 30 s of milling total iron content decreased to 11.59 mg/kg; at 60 s of milling, 6.84 mg/kg; and 7.54 mg/kg at 90 s of milling. This suggests that iron deposits in external kernel layers, such as the bran and germ that are milled away. It is imperative, therefore, to optimize processing conditions such as feedstock, soaking temperature, and steaming conditions to drive as much fortificant as possible into the kernel endosperm. Examining the potential for reduced degrees of milling may also facilitate iron delivery via parboiling fortification. Iron uptake was 2.2 times greater in brown rice (milled 0 s) than in rice milled for 30 s. Brown and lightly-milled rice may deliver greater total Fe without increased input costs associated with high concentrations of the fortifying agent.

3.8 Rice Color

Fortified rice color is a primary determinant of consumer acceptance. Whiteness (L*) and yellowness (b*) values for both cultivars and all three feedstocks are given in Figures 3.5-3.7. Parboiling treatment and cultivar were the primary determinants of rice whiteness (L*) and yellowness (b*) values for each parboiling feedstock, with the exception of white rice. White rice b* values were also affected by fortificant concentration and interactions between the three evaluated variables, and cultivar was not a significant factor for L* values. Diamond exhibited significantly greater whiteness values than CL XL745 for rough and brown feedstocks, and significantly lower yellowness values for rough, brown, and white. This may be attributed to differences in cultivar composition, such as protein content, and their impact on Maillard

reactions that occur during the parboiling process. Rice parboiled in the rough form maintained significantly greater whiteness values than rice parboiled in the brown form. At equivalent milling durations, rough feedstock whiteness levels were significantly greater than those for brown and white rice. Hulls may act as a protective layer to prevent the formation of Maillard products. Bran layers may have a similar protective role, but can also contribute reactants to Maillard reactions. The work of Lamberts et al. (2006) confirmed that bran layer pigments diffuse into the kernel during parboiling and contribute to parboiled rice color change.

Parboiling soaking and steaming conditions were a critical determinant of rice color. Soaking-only treatments demonstrated significantly greater whiteness values than soaking-and-steaming treatments. These results were consistent with Parnsakhorn and Noomhorm's work with parboiled rice color (2012). Mean whiteness values across iron levels and feedstocks, at 30 s milling, were 60.44 and 65.93 for CL XL745 and Diamond parboiled in the rough form, respectively; 58.14 and 65.94 for the brown form; and 59.88 and 62.49 for the white. Yellowness values for the same conditions were 21.64 and 19.79 for CL XL745 and Diamond parboiled in the rough form, respectively; 20.05 and 18.17 for the brown form; and 16.74 and 16.72 for the white. Significant interactions between treatment and cultivar indicated that hybrid and pureline cultivars developed color differently as they were parboiled, which may be explained by differences in fortificant solution uptake rates caused by differences in kernel surface area, differences in protein content, or differences in bran layer oil contents. Increased lipid content is associated with darker, yellow-colored rice. Cultivar selection and screening may, therefore, be important for acceptability of the final fortified product.

Milling duration was a significant factor in both whiteness and yellowness values. As more of the bran and germ were milled away, samples became whiter and less yellow. For

samples parboiled in the rough form, whiteness values are significantly different both at 0 s and 90 s. For the brown form, whiteness values of and 90 s and 0 and 60 s are not significantly different. For samples parboiled in the rough rice form, the yellowness values all four milling durations are significantly different. For the brown form, yellowness values are significantly different for 30 s and 90 s. The degree of milling for parboiled fortified rice must be selected to optimize both total iron content of fortified kernels and rice color.

NaFeEDTA, and iron fortificants in general, can cause development of green or grey color in fortified rice. This color development is a critical impediment to consumer acceptance. Rough rice whiteness was not significantly different between 0 and 250 mg/L or between 250-750 mg/L of NaFeEDTA. Whiteness values were significantly different for the brown feedstock at 500 mg/L and 750 mg/L. Within the white feedstock, whiteness values for all fortificant concentrations were significantly different. For the brown feedstock, fortification level did not have an impact on yellowness values for the brown feedstock or white feedstock. Rough rice yellowness values were not significantly different between fortificant levels of 0-250, 250-750, and 750-500 mg/L NaFeEDTA. While the highest evaluated levels of iron fortificant showed differences in color, iron fortification level is secondary to treatment and cultivar in terms of the impact on rice color. Color development typical of parboiled rice may have helped mask the impact of NaFeEDTA upon rice color. Use of moderate levels of the fortifying agent will maximize total Fe content while minimizing the impact on visual quality of rice.

Table 3.1 Rice cultivar characterization

		CL XL745		Diamond	
		Mean	**St.Dev**	**Mean**	**St.Dev**
Protein Content (%)			**Brown**		
		8.24	0.07	9.02	0.07
			Milled		
		7.36	0.07	8.17	0.08
Amylose Content (%)		17.71	0.22	16.32	0.10
Total Lipid Content (%)		2.45	0.04	2.59	0.06
Dimensions			**Brown**		
	Length (mm)	7.20	0.40	7.14	0.47
	Width (mm)	2.25	0.12	2.23	0.15
	Thickness (mm)	1.99	0.09	2.02	0.08
	Surface area (mm^2)	11.2		10.9	
			Milled		
	Length (mm)	6.90	0.44	6.60	0.46
	Width (mm)	2.21	0.1	2.22	0.12
	Thickness (mm)	1.89	0.1	1.90	0.10
	Surface area (mm^2)	10.3		9.8	
Onset Gelatinization Temperature (°C)		73.0		73.5	

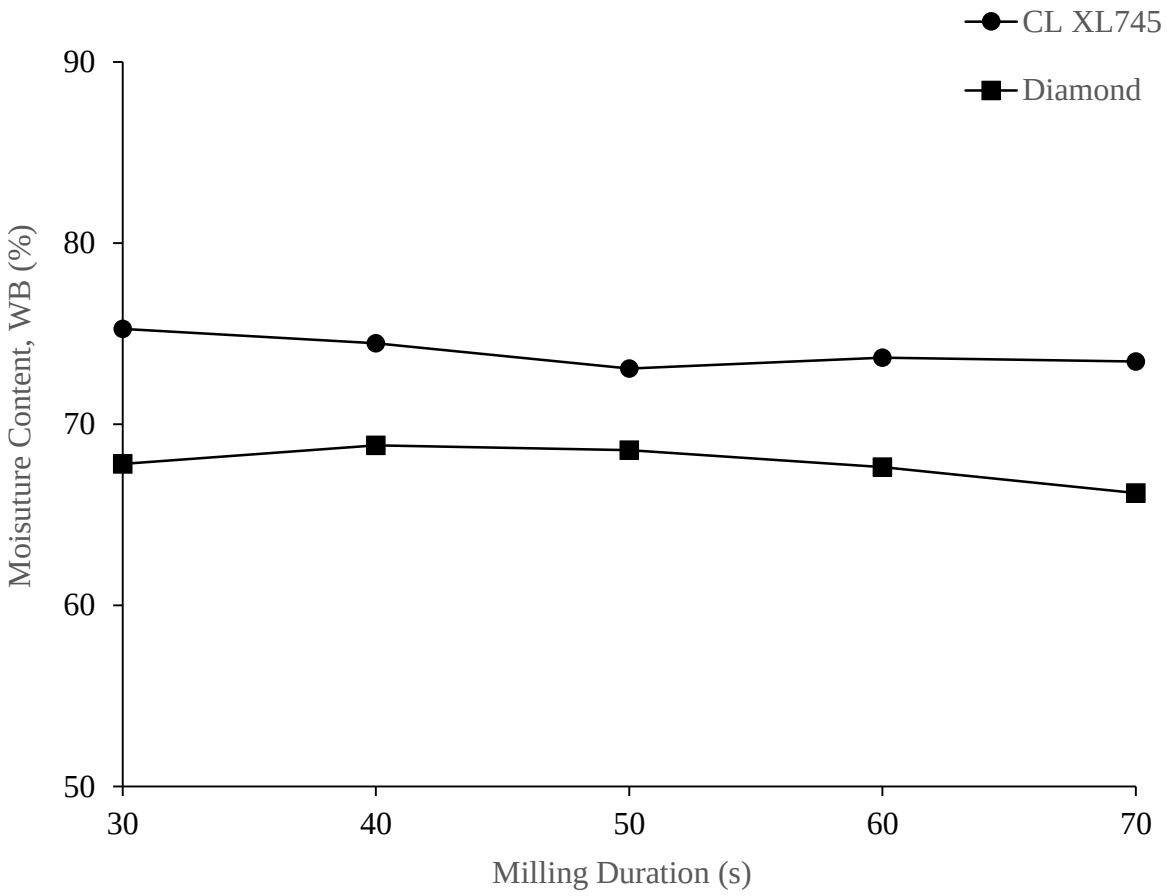

Figure 3.1 Head rice yields for parboiled CL XL745 and Diamond at milling durations from 30 to 70 seconds

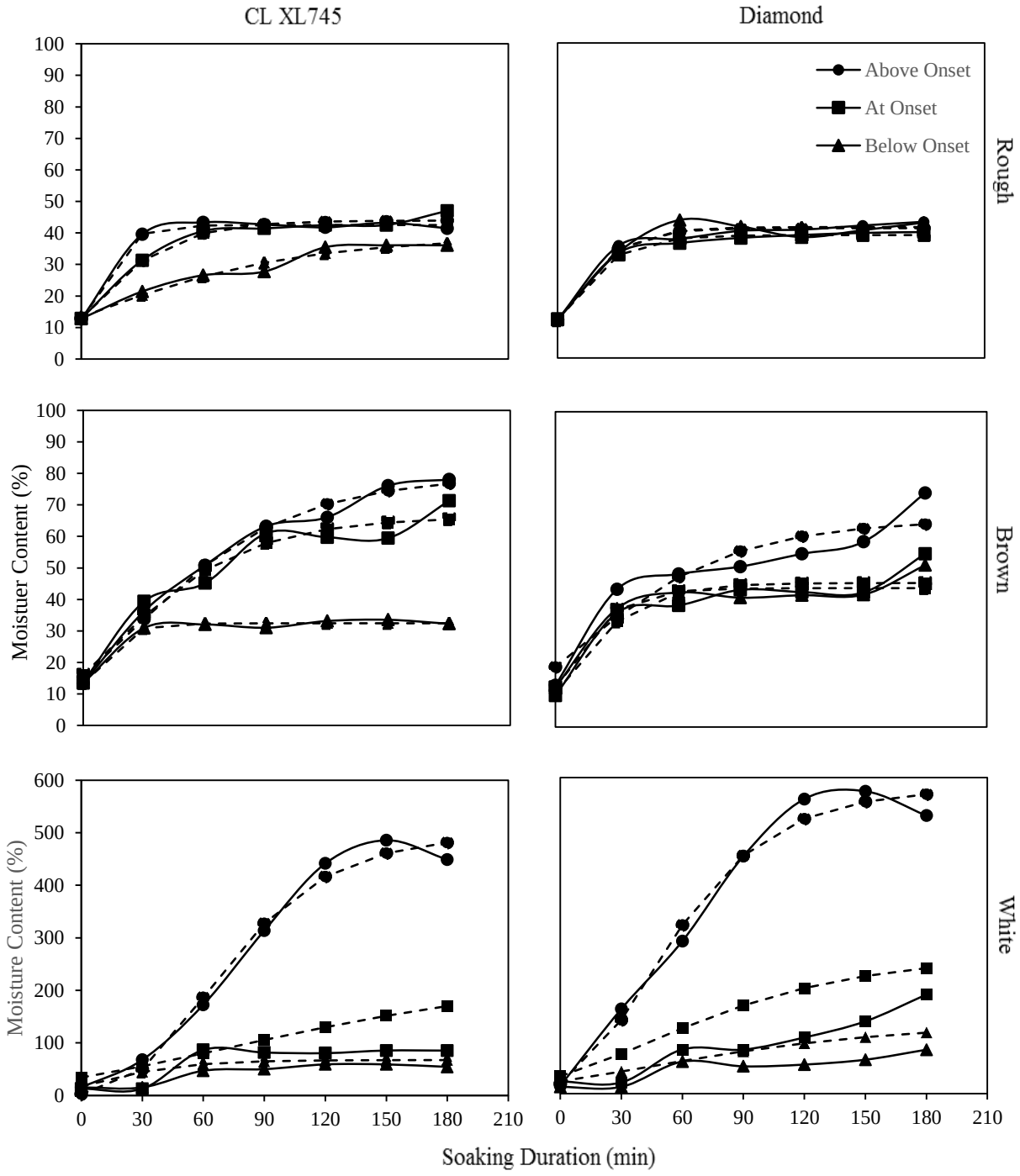

Figure 3.2 Hydration curves for rough, brown, and white rice feedstocks with Gompertz fit models

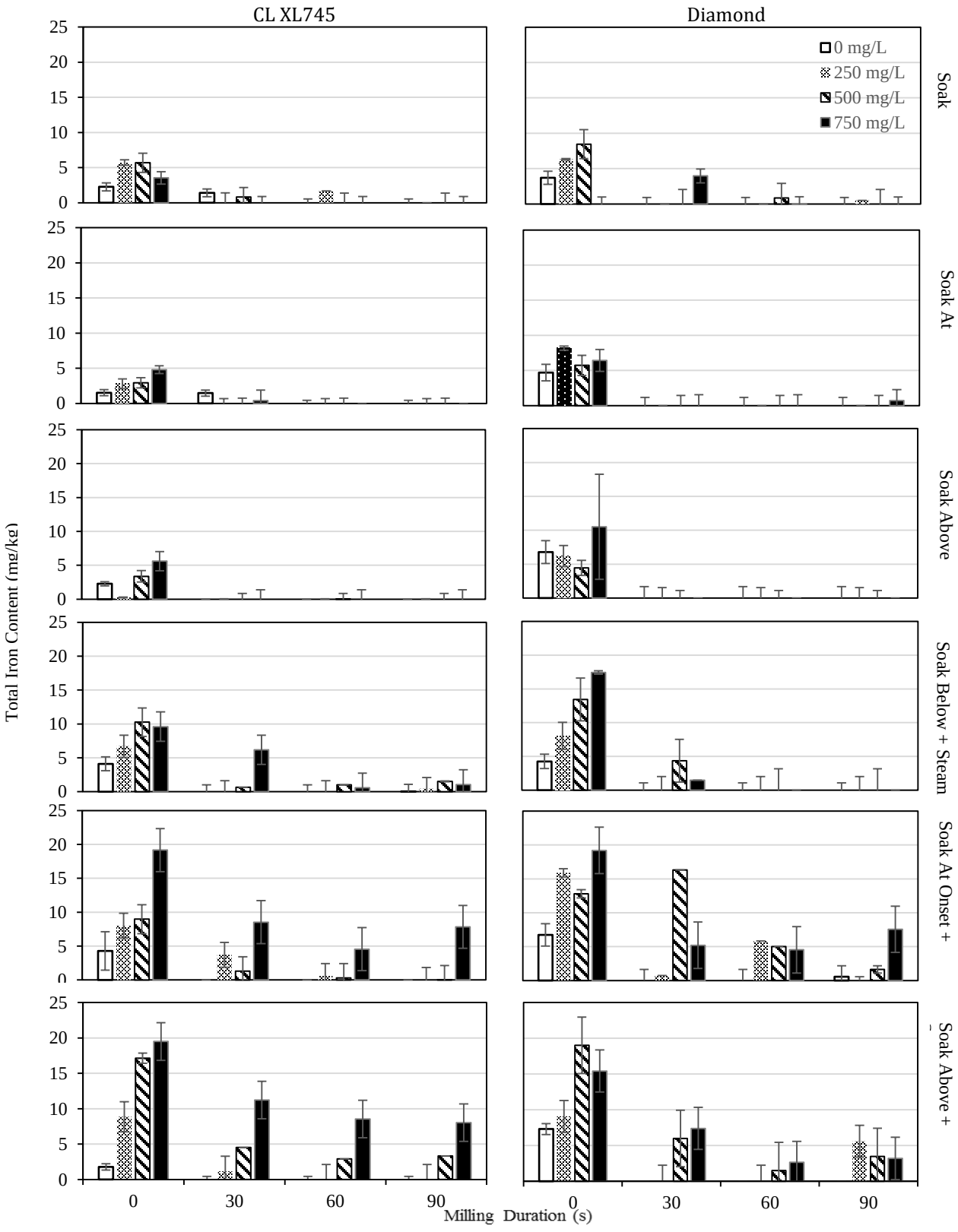

Figure 3.3 Total iron content of fortified rice parboiled in the rough rice form

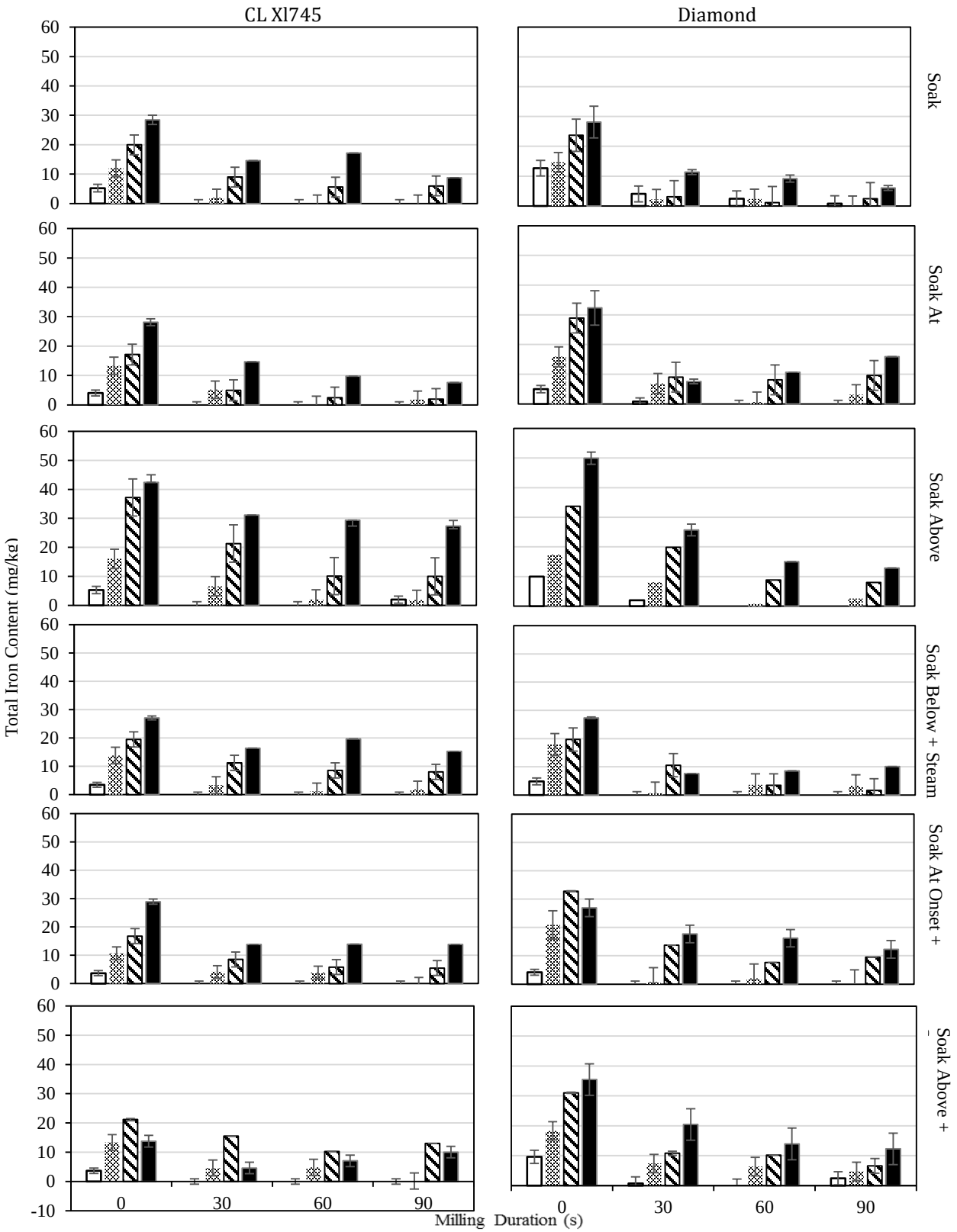

Figure 3.4 Total iron content of fortified rice parboiled in the brown rice form

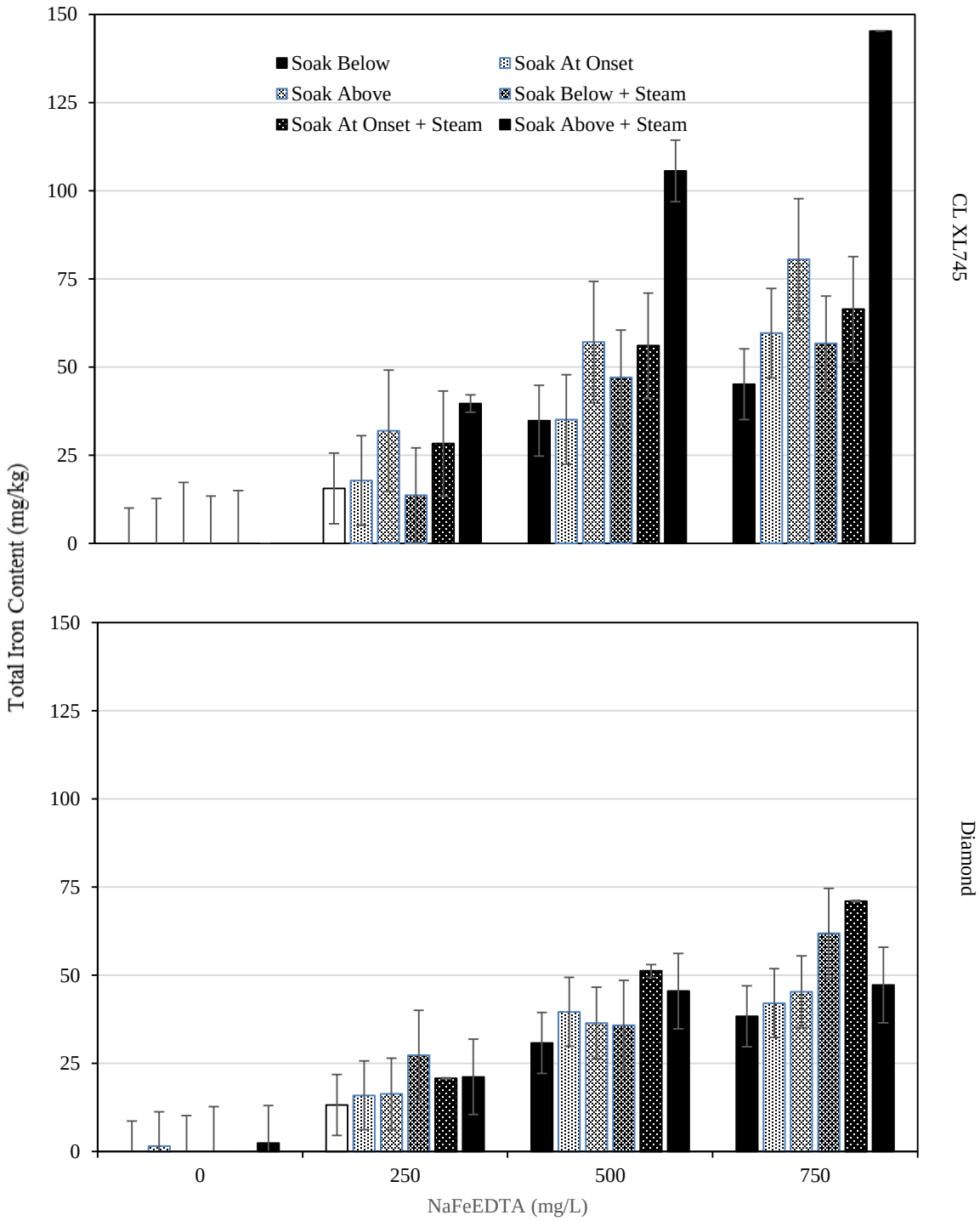

Figure 3.5 Total iron content of fortified rice parboiled in the white rice form

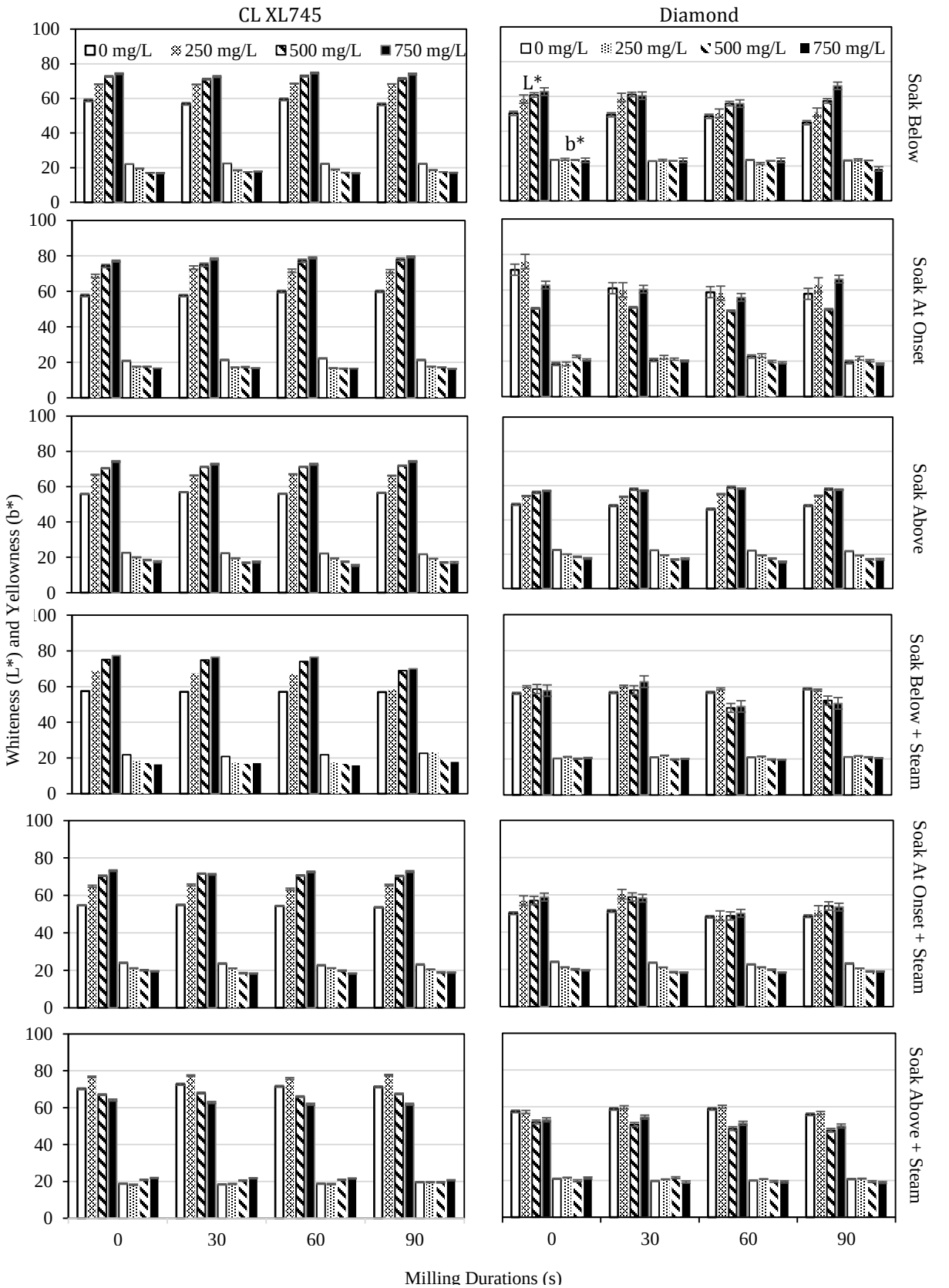

Figure 3.6 Whiteness (L*) and yellowness (b*) values of rice parboiled in rough rice form

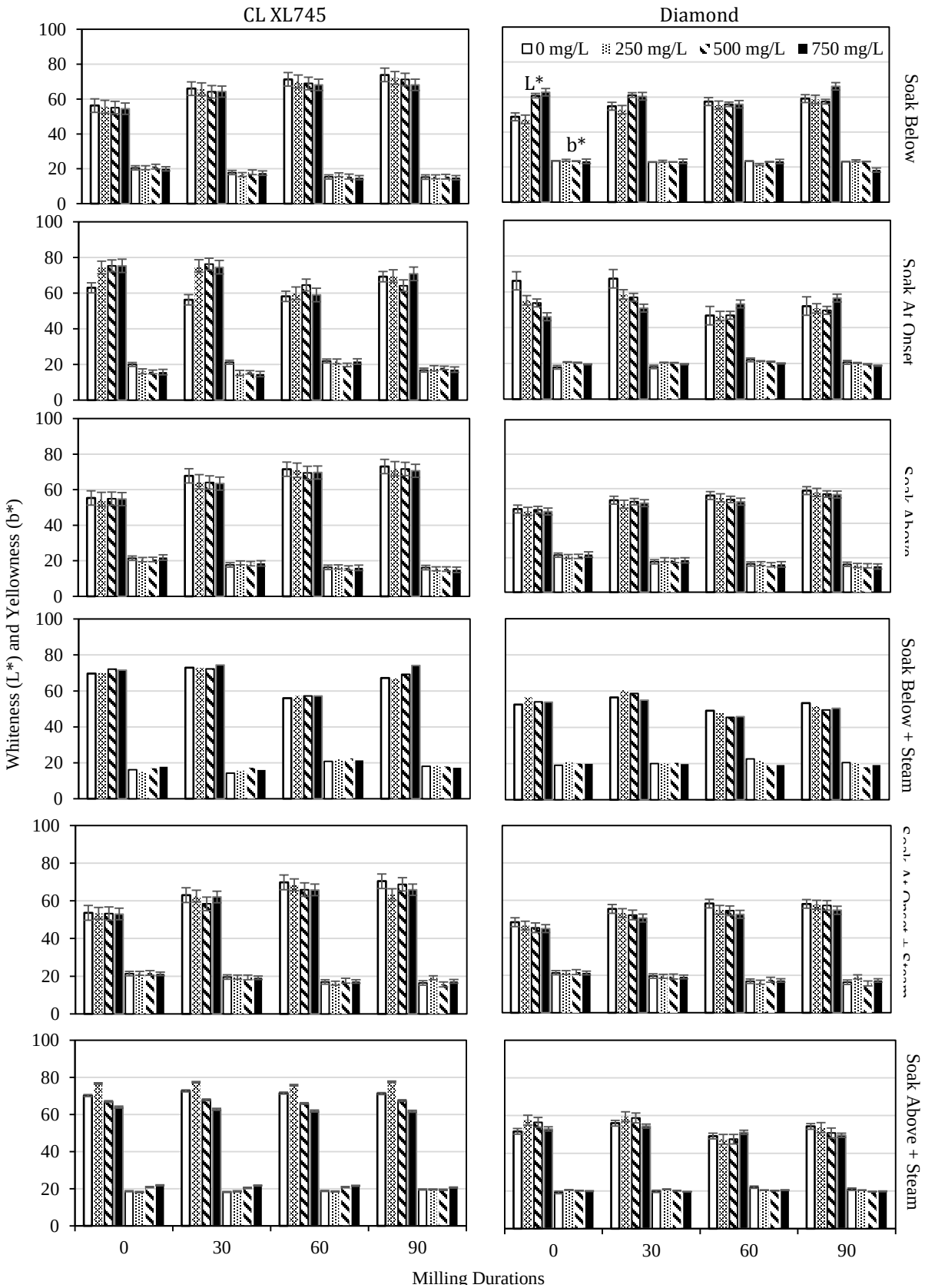

Figure 3.7 Whiteness (L*) and yellowness (b*) value of rice parboiled in the brown rice form

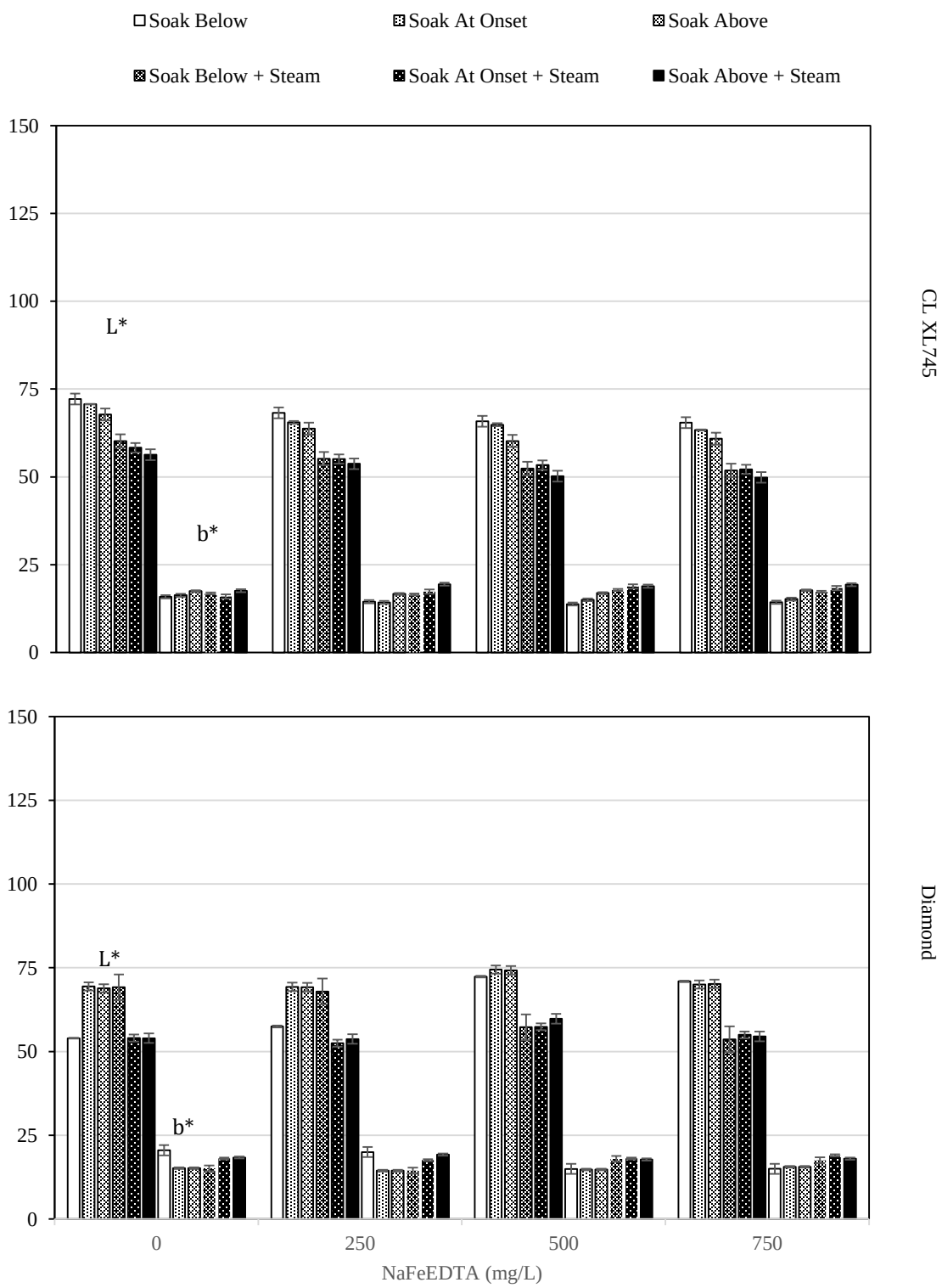

Figure 3.8 Whiteness (L*) and yellowness (b*) value of fortified rice parboiled in the white rice form

Chapter 4

Conclusion

This study suggests that kernel layers act as a significant barrier to iron uptake during parboiling. It demonstrated that modification of parboiling feedstock, parboiling conditions, milling duration, and fortification level will improve the efficiency of fortification levels. Increased soaking temperature and the inclusion of a steaming step significantly increased the concentration of iron driven into the starchy endosperm. Mean total iron content was 2.84 mg/kg for rice parboiled in the rough form, 9.92 mg/kg for the brown form, and 33.27 mg/kg for the milled rice form. Iron uptake was 3.4 times greater for brown rice than for rough rice, indicating that parboiling in the brown rice form may be a viable means to improve parboiling fortification efficiency. Cultivar was a significant driver of iron uptake for brown and white parboiling feedstocks, which may be determined by kernel characteristics such as surface area and protein content. Brown and milled rice color was driven primarily by cultivar and treatment, and interactions between those factors. NaFeEDTA fortificant only had a significant impact of rice whiteness and yellowness at very high concentrations. Further work with the modification of soaking solution pH, bioavailability assays, and pilot scaling may enhance the validity of current findings and further optimize parboiling fortification as a means of delivering iron fortification.

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Appendices

Table 5.1 Gompertz model of hydration in the rough rice form

CL XL745						
Model	**AICc**	**BIC**	**SSE**	**MSE**	**RMSE**	**R-Square**
Gompertz 3P	184.74	195.02	104.92	3.18	1.78	0.9786
Parameter	**Group**		**Estimate**	**Std Error**	**Lower 95%**	**Upper 95%**
Asymptote	Above		42.51	0.58	41.38	43.65
Growth Rate	Above		0.10	0.02	0.06	0.13
Inflection Point	Above		1.99	0.90	0.22	3.75
Asymptote	At Onset		43.93	0.70	42.56	45.31
Growth Rate	At Onset		0.04	0.00	0.03	0.05
Inflection Point	At Onset		4.71	1.68	1.42	7.99
Asymptote	Below		38.73	1.93	34.95	42.50
Growth Rate	Below		0.02	0.00	0.01	0.02
Inflection Point	Below		3.73	4.45	-4.99	12.45
Diamond						
Model	**AICc**	**BIC**	**SSE**	**MSE**	**RMSE**	**R-Square**
Gompertz 3P	194.46	204.74	132.26	4.01	2.00	0.9681
Parameter	**Group**		**Estimate**	**Std Error**	**Lower 95%**	**Upper 95%**
Asymptote	Above		41.21	0.68	39.87	42.55
Growth Rate	Above		0.07	0.01	0.05	0.08
Inflection Point	Above		2.84	1.40	0.10	5.58
Asymptote	At Onset		39.01	0.69	37.66	40.36
Growth Rate	At Onset		0.06	0.01	0.04	0.08
Inflection Point	At Onset		2.19	1.54	-0.82	5.21
Asymptote	Below		41.56	0.69	40.21	42.90
Growth Rate	Below		0.06	0.01	0.05	0.08
Inflection Point	Below		3.40	1.41	0.63	6.17

Table 5.2 Gompertz model of hydration in the brown rice form

CL XL745						
Model	**AICc**	**BIC**	**SSE**	**MSE**	**RMSE**	**R-Square**
Gompertz 3P	249.66	259.94	492.17	14.91	3.86	0.9707
Parameter	**Group**		**Estimate**	**Std Error**	**Lower 95%**	**Upper 95%**
Asymptote	Above		79.29	3.03	73.36	85.21
Growth Rate	Above		0.02	0.00	0.02	0.03
Inflection Point	Above		22.69	3.41	16.01	29.38
Asymptote	At Onset		66.24	2.27	61.79	70.69
Growth Rate	At Onset		0.03	0.00	0.02	0.03
Inflection Point	At Onset		13.29	3.42	6.60	19.99
Asymptote	Below		32.44	1.25	29.98	34.89
Growth Rate	Below		0.10	0.07	-0.03	0.23
Inflection Point	Below		-1.33	2.64	-6.50	3.84
Diamond						
Model	**AICc**	**BIC**	**SSE**	**MSE**	**RMSE**	**R-Square**
Gompertz 3P	282.04	292.32	1064.08	32.24	5.68	0.8870
Parameter	**Group**		**Estimate**	**Std Error**	**Lower 95%**	**Upper 95%**
Asymptote	Above		65.76	3.97	57.98	73.54
Growth Rate	Above		0.02	0.01	0.01	0.03
Inflection Point	Above		9.64	5.94	-2.00	21.28
Asymptote	At Onset		45.68	2.08	41.60	49.76
Growth Rate	At Onset		0.05	0.02	0.02	0.08
Inflection Point	At Onset		7.05	4.42	-1.60	15.71
Asymptote	Below		44.04	1.93	40.26	47.83
Growth Rate	Below		0.07	0.02	0.02	0.11
Inflection Point	Below		3.34	3.67	-3.85	10.52

Table 5.3 Gompertz model of hydration in the white rice form

CL XL745						
Model	**AICc**	**BIC**	**SSE**	**MSE**	**RMSE**	**R-Square**
Gompertz 3P	389.42	399.70	13718.29	415.71	20.39	0.9844
Parameter	**Group**		**Estimate**	**Std Error**	**Lower 95%**	**Upper 95%**
Asymptote	Above		497.08	16.40	464.94	529.22
Growth Rate	Above		0.03	0.00	0.02	0.03
Inflection Point	Above		59.30	2.45	54.51	64.09
Asymptote	At Onset		240.83	100.08	44.67	436.99
Growth Rate	At Onset		0.01	0.01	0.00	0.02
Inflection Point	At Onset		69.93	49.48	-27.05	166.91
Asymptote	Below		67.36	8.31	51.09	83.64
Growth Rate	Below		0.04	0.03	-0.02	0.10
Inflection Point	Below		7.79	12.83	-17.37	32.95
Diamond						
Model	**AICc**	**BIC**	**SSE**	**MSE**	**RMSE**	**R-Square**
Gompertz 3P	402.59	412.87	18773.05	568.88	23.85	0.9857
Parameter	**Group**		**Estimate**	**Std Error**	**Lower 95%**	**Upper 95%**
Asymptote	Above		578.30	15.54	547.84	608.76
Growth Rate	Above		0.03	0.00	0.02	0.03
Inflection Point	Above		41.99	2.26	37.55	46.43
Asymptote	At Onset		262.84	32.45	199.23	326.45
Growth Rate	At Onset		0.02	0.00	0.01	0.03
Inflection Point	At Onset		43.02	10.46	22.51	63.53
Asymptote	Below		134.91	47.62	41.58	228.24
Growth Rate	Below		0.01	0.01	-0.01	0.03
Inflection Point	Below		41.32	32.23	-21.85	104.49

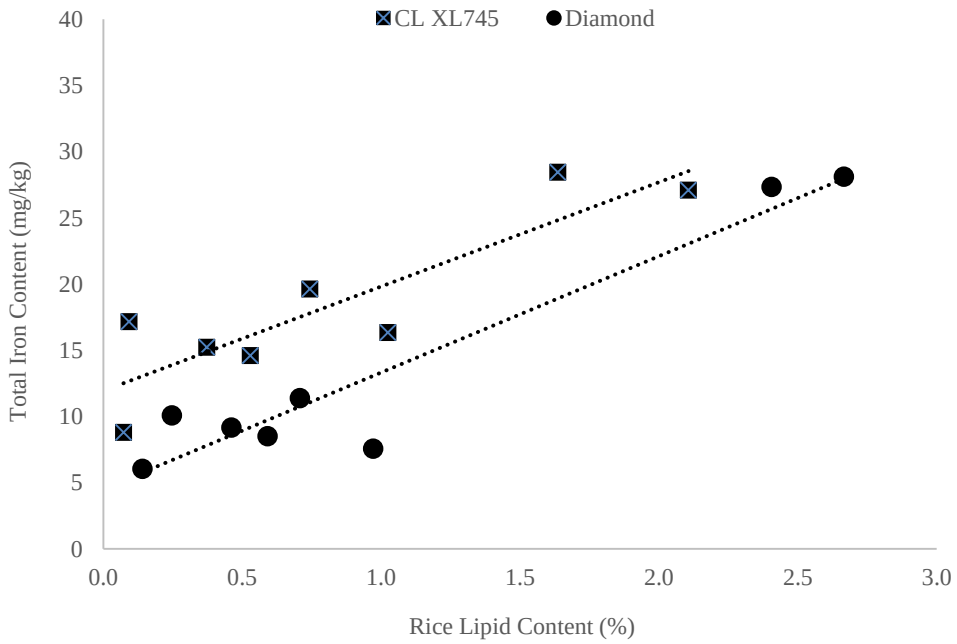


Figure 5.1 Relationships between rice lipid contents (SLCs and TLCs) and iron retention in the rice kernel (mg/kg) by thermal treatment[1]

[1]Samples included in this data set were brown rice parboiled using "Soak Below Onset" and "Soak Below Onset and Steam" conditions. Samples were all fortified in 750 mg NaFeEDTA/L solutions.

Table 5.4 Analysis of variance, effects upon total iron content (mg/kg), and Student's t-test for lipid and iron retention comparisons

Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	7	1687.28	241.04	15.4297
Error	24	374.92	15.62	
C. Total	31	2062.2074		<.0001

Effect Test				
Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
SLC	1.00	1253.87	80.26	<.0001
Cultivar	1.00	357.56	22.89	<.0001

Student's t-test			
Cultivar Level			**Least Square Mean**
CLXL745	A		19.6273
Diamond		B	12.6465

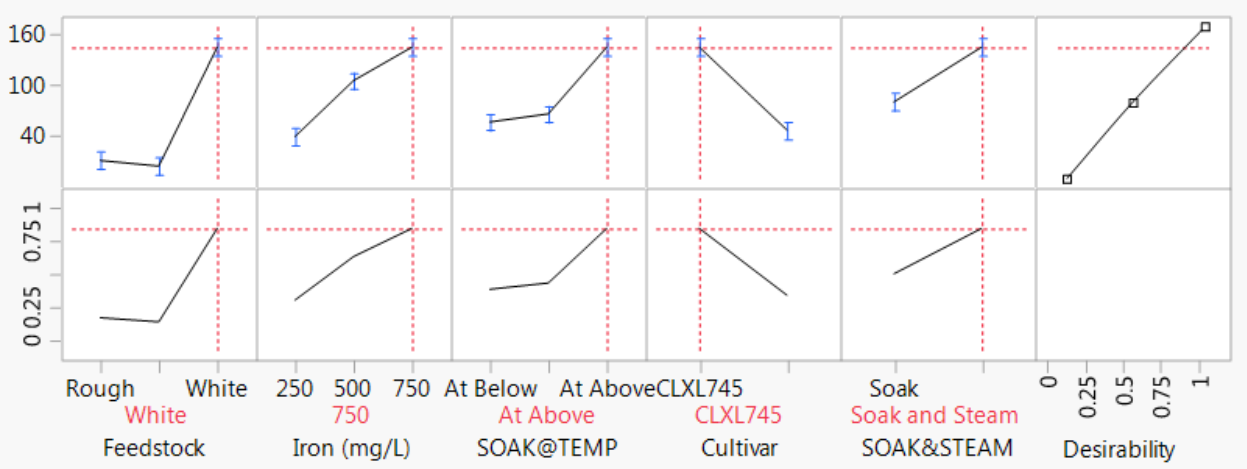
Figure 5.2 Fit least squares for parboiled fortified rice[1]

Summary Report

Column	Main Effect	Total Effect
Feedstock	0.63	0.877
Iron (mg/L)	0.074	0.223
SOAK@TEMP	0.027	0.145
SOAK&STEAM	0.015	0.085
Cultivar	0.013	0.098

[1]Rough, brown, and milled Diamond rice total iron content (mg/kg) were evaluated at 30 s of milling for samples parboiled at 250, 500, and 750 mg/L NaFeEDTA. White CL XL745 was milled for 43 s.

Figure 5.3 Parboiling conditions to maximize total iron content of fortified rice[1, 2]



[1]This analysis includes rough, brown, and white rice samples fortified with 250, 500, and 750 mg/L NaFeEDTA and milled for 30 s (white CL XL745 milled for 43 s).
[2]Total iron content was maximized (~145 mg/kg Fe) when rice was soaked above onset, then steamed in white rice form with 750 mg/L NaFeEDTA,

Table 5.5 Analysis of variance and effects tests for total iron content of rough rice feedstock

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	191	7350.3	38.5	9.5353
Error	192	774.9	4.0	
C. Total	383	8125.2		<.0001

Source	DF	Sum of Squares	F Ratio	Prob > F
Milling Duration	3	3528.31	291.41	<.0001
Iron (mg/L)	3	623.30	51.48	<.0001
Treatment	5	880.29	43.62	<.0001
Cultivar	1	77.40	19.18	<.0001
Cultivar*Milling Duration	3	196.80	16.25	<.0001
Treatment*Iron (mg/L)	15	640.17	10.57	<.0001
Iron (mg/L)*Milling Duration	9	304.52	8.38	<.0001
Treatment*Milling Duration	15	327.15	5.40	<.0001
Treatment*Cultivar	5	60.40	2.99	0.0126
Treatment*Cultivar*Iron (mg/L)	15	151.83	2.51	0.0021
Treatment*Iron (mg/L)*Milling Duration	45	294.63	1.62	0.0136

Table 5.6 Analysis of variance and effects tests for total iron content of brown rice feedstock

Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	191	41381.15	216.66	10.37
Error	192	4010.50	20.89	
C. Total	383	45391.65		<.0001
Effects test				
Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Iron (mg/L)	3	15245.53	243.29	<.0001
Milling Duration	3	12998.36	207.43	<.0001
Treatment	5	2175.05	20.83	<.0001
Cultivar*Milling Duration	3	860.87	13.74	<.0001
Treatment*Iron (mg/L)	15	2818.54	9.00	<.0001
Treatment*Cultivar	5	850.34	8.14	<.0001
Iron (mg/L)*Milling Duration	9	1010.99	5.38	<.0001
Treatment*Cultivar*Iron (mg/L)	15	1271.00	4.06	<.0001
Cultivar*Iron (mg/L)	3	234.49	3.74	0.0121
Treatment*Cultivar*Iron (mg/L)*Milling Duration	45	1726.05	1.84	0.0026
Treatment*Milling Duration	15	550.23	1.76	0.0436

Table 5.7 Analysis of variance and effects tests for total iron content of white rice feedstock

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	47	84660.74	1801.29	18.9658
Error	47	4463.87	94.98	
C. Total	94	89124.61		<.0001

Effects test

Source	DF	Sum of Squares	F Ratio	Prob > F
Iron (mg/L)	3	55590.75	195.10	<.0001
Cultivar	1	3011.32	31.71	<.0001
Treatment	5	7887.91	16.61	<.0001
Treatment*Cultivar	5	5865.59	12.35	<.0001
Cultivar*Iron (mg/L)	3	2251.74	7.90	0.0002
Treatment*Cultivar*Iron (mg/L)	15	5011.31	3.52	0.0005
Treatment*Iron (mg/L)	15	4413.37	3.10	0.0015

Table 5.8 Analysis of variance and effects tests for whiteness (L*) and yellowness (b*) color values for rough rice feedstock

Whiteness (L*) Value				
Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	47	17760.10	377.87	8.49
Error	336	14952.17	44.50	
C. Total	383	32712.27		<.0001
Effects test				
Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	5	16432.01	73.85	<.0001
Cultivar	1	200.60	4.51	0.0345
Yellowness (b*) Value				
Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	47	1333.21	28.37	9.12
Error	336	1045.60	3.11	
C. Total	383	2378.80		<.0001
Effects test				
Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Cultivar	1	286.47	92.06	<.0001
Treatment	5	803.63	51.65	<.0001
Treatment*Cultivar	5	135.81	8.73	<.0001

Table 5.9 Student's t-tests for whiteness (L*) and yellowness (b*) color values for rough rice feedstock

Whiteness (L*) Value						
Treatment Level						**Least Sq Mean**
Soak At Below	A					69.57
Soak At Above	A					67.55
Soak At Onset	A					67.30
Soak At Below + Steam		B				56.46
Soak At Above + Steam		B				54.73
Soak At Onset + Steam		B				54.42
Cultivar Level						**Least Sq Mean**
Diamond	A					62.39
CLXL745		B				60.95
Yellowness (b*) Value						
Treatment*Cultivar Level						**Least Sq Mean**
Soak At Onset + Steam,CLXL745	A					23.61
Soak At Above + Steam,CLXL745	A					23.48
Soak At Below + Steam,CLXL745	A					22.86
Soak At Onset + Steam,Diamond		B				20.59
Soak At Above,CLXL745		B				20.55
Soak At Below + Steam,Diamond		B				20.52
Soak At Above + Steam,Diamond		B				20.23
Soak At Above,Diamond		B	C			19.83
Soak At Onset,CLXL745			C	D		19.02
Soak At Below,CLXL745				D	E	18.88
Soak At Onset,Diamond				D	E	18.81
Soak At Below,Diamond					E	18.05

Table 5.10 Analysis of variance and effects tests for whiteness (L*) and yellowness (b*) color values for brown rice feedstock

Whiteness (L*) Value				
Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	47	17537.0	373.1	10.4
Error	336	12082.0	36.0	
C. Total	383	29618.9		<.0001
Effects test				
Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	5	15945.98	88.69	<.0001
Cultivar	1	219.21	6.10	0.0140
Yellowness (b*) Value				
Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	47	1002.18	21.32	6.4604
Error	336	1108.98	3.30	
C. Total	383	2111.16		<.0001
Effects test				
Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	5	790.12	47.88	<.0001
Cultivar	1	37.91	11.49	0.0008
Treatment*Cultivar	5	86.97	5.27	0.0001

Table 5.11 Student's t-tests for whiteness (L*) and yellowness (b*) color values for rough rice feedstock

Whiteness (L*) Value								
Cultivar Level								**Least Sq Mean**
Diamond	A							60.16
CLXL745		B						58.65
Treatment Level								**Least Sq Mean**
Soak At Below	A							66.59
Soak At Onset	A							66.54
Soak At Above		B						64.27
Soak At Below + Steam			C					53.24
Soak At Above + Steam			C					53.06
Soak At Onset + Steam			C					52.72
Yellowness (b*) Value								
Treatment*Cultivar Level								**Least Sq Mean**
Soak At Below + Steam,CLXL745	A							21.85
Soak At Above + Steam,CLXL745	A							21.79
Soak At Onset + Steam,CLXL745	A	B						21.17
Soak At Above + Steam,Diamond		B	C					20.27
Soak At Onset + Steam,Diamond			C					20.04
Soak At Below + Steam,Diamond			C					19.99
Soak At Above,CLXL745				D				18.75
Soak At Above,Diamond					D	E		18.55
Soak At Below,Diamond					D	E	F	18.09
Soak At Onset,Diamond					D	E	F	17.90
Soak At Onset,CLXL745						E	F	17.81
Soak At Below,CLXL745							F	17.24

Table 5.12 Analysis of variance and effects tests for whiteness (L*) and yellowness (b*) color values for white rice feedstock[1]

Whiteness (L*) Value				
Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	47	5342.21	113.66	194.21
Error	48	28.09	0.59	
C. Total	95	5370.30		<.0001
Effects test				
Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	5	3318.85	1134.11	<.0001
Cultivar	1	217.59	371.78	<.0001
Cultivar*Iron (mg/L)	3	391.02	222.70	<.0001
Treatment*Cultivar	5	403.43	137.86	<.0001
Iron (mg/L)	3	123.82	70.52	<.0001
Treatment*Iron (mg/L)	15	474.67	54.07	<.0001
Treatment*Cultivar*Iron (mg/L)	15	412.83	47.02	<.0001
Yellowness (b*) Value				
Analysis of Variance				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	47	304.67	6.48	15.84
Error	48	19.64	0.41	
C. Total	95	324.32		<.0001
Effects test				
Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	5	135.30	66.12	<.0001
Treatment*Cultivar	5	60.59	29.61	<.0001
Treatment*Iron (mg/L)	15	76.67	12.49	<.0001
Treatment*Cultivar*Iron (mg/L)	15	26.24	4.27	<.0001

[1]Student's t-tests for white rice are not included because three-way interactions were significant for this feedstock.