# **Incorporating iron fortificant in ultrasonicated waxy rice led to its stickier and firmer characteristics**

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#### **Abstract**

Incorporating iron in milled rice is an excellent recommendation for regaining mineral loss during milling and addressing micronutrient deficiency issues. This paper investigated the waxy rice variety iron fortification on sonicated milled grains and the effects of iron on the textural attributes of cooked fortified rice measured by a texture profile analyzer (TPA). Through enhanced absorption, modified rice grain induced by ultrasonic treatment has successfully increased iron uptake by 13.2% compared to non-sonicated waxy rice, with excellent retention of 99.33% after washing and cooking. The textural hardness of fortified sonicated rice significantly decreased  $(p < 0.01)$  by 19%, attributed to the microporous formation after the ultrasonic treatment. The rice stickiness increased by 39.5% after ultrasonication, and iron fortification was associated with the enhancement of the leached amylopectin-iron network resulting in a stronger attraction between the fortified rice and the TPA probe. The improved network formation in fortified rice was confirmed in the increased elastic (G') and viscous (G") moduli during temperature ramp and frequency sweep experiments. The observed impacts of micronutrient fortificants on the textural and rheological attributes may be helpful in the development of rice and rice products with enhanced eating quality.

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# **Introduction**

Micronutrient deficiency is a problem affecting people worldwide, especially children in developing countries lack essential nutrients resulting in stunted growth, poor health, and cognitive development issues. Human diet supplementation with essential micronutrients is a tactical solution to address this issue. Fortification or nutritional enrichment of commonly consumed foods is an effective mass-level intervention in preventing malnutrition across a broad population spectrum. Numerous international and national public health programs have been implemented to combat malnutrition by fortifying dairy products, cooking oil, and cereal flour<sup>[\[1](#page-6-0)]</sup>. Among these food fortifications, rice continues to serve as a top priority vehicle for fortification since it is consumed by most of the world's population. The Food and Agriculture Organization (FAO) promotes rice fortification programs to increase [th](#page-6-1)e daily intake of essential nutrients in addressing malnutrition<sup>[[2](#page-6-1)]</sup>.

Asia, the rice-eating region of the world, has a massive iron (Fe) deficiency problem affecting 70%−95% of the population. Ideally,F[e](#page-6-1) dietary intake should range between 13.7 and 20.5 mg daily<sup>[[2\]](#page-6-1)</sup>. However, t[h](#page-6-2)[e](#page-6-3) availability of Fe in milled rice is only at around 2 mg·kg<sup>-1[[3](#page-6-2)[,4\]](#page-6-3)</sup>. Most well-polished white rice has almost nil Fe content due to nutrient loss upon milling and processing. Brown rice contains slightly higher Fe contents, but most rice eaters, especially young children, preferred it less due to its hard and chewy texture compared to white rice. This calls for a critical intervention that involves multiple approaches to address the problem. Iron deficiency among adults has several serious consequences, including anemia, impaired work efficiency, learning disabilities, and restrained activities. In children, iron deficiency slows down growth, weakens immunity, and retards cognitive development<sup>[\[2](#page-6-1)]</sup>. Numerous methods for iron fortification in rice have been investigated. Postharvest iron enrichment techniques include the coating process<sup>[[5\]](#page-6-4)</sup>, extrusion technology<sup>[\[6](#page-6-5)]</sup>, parboiling techniques<sup>[\[7](#page-6-6)]</sup>, and cold plasma treatment<sup>[[8](#page-6-7)]</sup>. The fortified rice grains, dubbed 'pre-mix grains', are then combined with regular milled rice (e.g., at a ratio of 1:200) to achieve the desired level of fortification following the daily recommended nutrient intake. However, these iron fortification strategies entail time-consuming processes and lower retention rates upon cooking. Additionally, the appearance and sensory properties of nutrient-coated and fortified extruded kernels are undesirable to most consumers compared to raw rice. A simple, efficient, and economically viable technique for iron fortification with improved eating qualities is highly desirable when applied to milled rice samples.

Ultrasonication is a technique that agitates particles in a sample using sound waves. Acoustic cavitation modifies the sample through microbubble formation in the liquid medium, which then grows and collides implosively, impacting the sample's surface. The technique is beneficial in removing dirt, fragmenting samples, accelerating dissolution, synthesizing nanomaterials, and disrupting biological processes. In food processing, ultrasonication has been helpful in mixing, degassing, dissolution, discoloration, extraction, crystalliz[at](#page-6-8)ion, sterilization, and emulsification, among other processes<sup>[\[9\]](#page-6-8)</sup>. In

rice samples, ultrasonic treatment increases the capacity of rice to absorb water due to the formation of pores, microcavities, fissures, and cracks in rice grain morphology<sup>[[10](#page-6-9)]</sup>. The modification on the grain surface is attributed to the ultrasonic cavitation process, in which bubbles are created in a liquid medium and then collapse, forming tiny jets that interact directly with the surface of a rice grain or starch granules suspended in a liquid. Mineral fortification on sonicated milled rice can be achieved *via* soaking due to the increased water uptake, which also absorbs the minerals dissolved in it<sup>[\[10](#page-6-9)]</sup>. A cursory survey of the literature showed the absence of studies on waxy rice varieties investigating the textural and rheological impacts of ironfortified rice, hence this study. Waxy rice is ubiquitous in Asian culture as it is used in rice-based snacks, such as the different cultural versions of rice cakes. Therefore, iron fortification of waxy rice is highly desired to increase the variety of food vehicles for daily iron intake. This work aimed to investigate the iron fortification of waxy rice using the sonic-treatment method of fortification and its effects on the grain texture upon cooking. The iron uptake in sonicated milled waxy rice and its textural attributes can be helpful in the development of rice and ricebased products with improved eating qualities.

# **Materials and methods**

# **Rice sample**

IR65, a waxy rice variety, was used in this study. Rice was grown during the 2017 dry season in the International Rice Research Institute's (IRRI) irrigated field plots in Los Baños, Laguna, Philippines, under optimal field management conditions. Seeds were harvested and dried to 12%−14% moisture content. Paddy rice samples were dehulled and polished (Rice sheller THU-35 A, Satake Corporation) (Kett Mill). Through sizeexclusion chromatographic analysis, the molecular weight distributions of debranched starch of IR65 were as follows: DP > 1,000 (0.23% ± 0.10%), DP 121−1,000 (3.63% ± 0.14%), DP 37−120 (34.39% ± 0.32%), and DP 6−36 (61.45% ± 0.09%).

# **Production of iron-fortified milled IR65**

<span id="page-1-0"></span>The ultrasound treatment and iron fortification of milled IR65 grains([Fig. 1](#page-1-0)) were carried out according to the method described in our previous work<sup>[\[11\]](#page-6-10)</sup>. In a 50 mL test tube, milled rice was soaked in deionized water (1:1) and sonicated for 5 min in an ultrasonic bath (Fisher Scientific FS30), operating at

a working frequency of 40 kHz and a power output of 130 W. The water temperature was maintained constant throughout the treatment by adding ice to the ultrasonic bath, which prevented starch gelatinization during the sonication process. The treated rice was drained and air-dried overnight at room temperature. Fortification with iron was accomplished by soaking 10 g of sonicated rice in 1,000 mg·L−1 ferrous sulfate (Dr. Paul Lohmann GmbH KG, Emmerthal, Germany) for 60 min at a rice-to-water ratio of 1:2. Following soaking, the treated rice was drained and air-dried overnight at room temperature to obtain the fortified rice pre-mix samples. As a control, the same procedure was performed on non-sonicated rice.

# **Bulk analysis of iron using inductively coupled plasma-optical emission spectroscopy**

The iron content of rice samples was determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES) to determine the bulk uptake concentration. After placing an oven-dried rice sample (0.600 g) in a culture tube (25 mm  $\times$ 200 mm), 12 mL of a perchloric acid: nitric acid mixed acid solution (1:10) was added to facilitate the rice sample digestion. The sample was pre-digested in the digestor equipped with the flexi glass baffle system from room temperature to 60 °C for 25 min. The digestion was then continued at a temperature range of 60 to 225 °C. The tube was left alone, and the contents were digested completely until 1 mL of colorless to slightly yellow clear digest remained. The digested sample was cooled, and 45 mL of 1% HNO<sub>3</sub> was added to the tube. A vortex mixer was used to mix the tube, and the sample was immediately transferred to a labeled polypropylene tube. The digest was then analyzed for Fe and P using ICP-OES with WinLab32 soft-ware (PerkinElmer, Model Optima 5300DV, USA)<sup>[\[12\]](#page-6-11)</sup>.

To determine the amount of iron retained after cooking, 1.0 g of rice was placed in a 50 mL test tube and washed three times with 10 mL deionized water. After that, water was added to achieve a rice-to-water ratio of 1:1.6. Aluminum foil was wrapped around the test tube, and it was immersed in boiling water for 15 min. Then, the cooked sample was lyophilized. The freeze-dried cooked rice sample was ground, and the same procedure for iron analysis, as described above, was followed.

# **Texture profile analysis of cooked rice**

In a 50-mL test tube, each sample was prepared by rinsing 25 unbroken rice kernels twice and immersing them in a specific rice-to-water ratio of 1:1.4 for waxy milled rice. The test



**Fig. 1** Postharvest iron fortification of waxy rice using ultrasonic treatment.

#### Incorporating iron fortificant in ultrasonicated waxy rice

tubes were then sealed with aluminum foil and tied with a rubber band to prevent the evaporation of water. The test tube was heated in a boiling water bath for 15 min and then placed in a water bath (50 °C) until the texture profile analysis was performed. For each cooking replicate, three cooked rice grains of similar heights (from the same tube kept at 50 °C) were selected and placed parallel at the center of the base plate so that the probe entirely compressed the grains. Compression testing of cooked, unbroken rice grains was performed twice using a TA XT-Plus Texture Analyzer equipped with a cylindrical probe (35 mm, Stable Micro Systems Ltd., Surrey, UK). The texture analyzer was set up with the following parameters: a pretest speed of 1 mm·s<sup>-1</sup>, test speed and post-test speed of 5 mm·s−1 , the target mode was set to 90% strain, time was set for 5 s, trigger type was set to autoforce, trigger force was set to 3 g, and tare mode was set to auto. Two compression tests were performed using the Exponent Lite software to control the instrument, showing the divided regions representing downstrokes (increasing values) and upstrokes (decreasing values). The peak of the first positive curve is characterized by hardness. Adhesiveness was defined as the area beneath the negative curve, representing the effort required to remove the plunger from the sample on the base plate. The ratio of the second positive curve area to the area of the first positive curve defined cohesiveness. Springiness is the ratio of the time elapsed between the upstroke and the peak of the second curve (T2) to the time elapsed between the starting point and the peak of the first curve (T1), representing sample height recovery following initial compression. Adhesion was calculated by multiplying the hardness by the cohesiveness. Chewability was determined as a result of the interaction of gumminess and springiness. When the first depression is applied, resilience is defined as the ratio of the area before the deformation target to the area after the deformation target. Three compression replicates were conducted for each cooking repli-cate<sup>[[13](#page-6-12)]</sup>.

#### **Rheological properties**

the power model  $\eta^* = K^* \dot{\gamma}^{n^*-1}$  was applied to parameterize the mechanical spectra- consistency coefficient further  $(\eta^*)$  and flow behavior index (n<sup>\*</sup>)<sup>[[15](#page-6-14)]</sup>. Measurements were performed in The effects of iron incorporation on rice rheological properties were measured with an AR2000 (TA Instruments, New Castle, DE) using a parallel metal plate probe with a 40 mm diameter and a 1 mm gap from the Peltier plate. The Peltier plate was filled with 1.3 mL rice dispersion at a concentration of 25% (db). During the test, the sample was covered with a solvent trap filled with water to reduce evaporation. A controlled heating ramp (35–95 °C) was followed by a steady cooling ramp (95–35 °C) at 4 °C min<sup>-1</sup> ramp rate<sup>[[14](#page-6-13)]</sup>. The oscillation frequency was set to 1 cycle per s (1 Hz). The gel was then subjected to an angular frequency sweep test by first equilibrating at 35 °C for 1 min, then applying 0.1 to 100 rad·s−1 at 0.1 Pa oscillatory stress. The storage modulus (G'), loss modulus (G"), and loss tangent (tan =  $G''/G'$ ) rheological parameters were reported. Moreover, for the frequency sweep data, duplicate.

#### **Statistical analysis**

Statistical analysis was performed using one-way ANOVA with Tukey's posthoc test at a significance level of  $= 0.01\%$ . ASTATSA [\(https://astatsa.com/OneWay\\_Anova\\_with\\_Tukey](https://astatsa.com/OneWay_Anova_with_TukeyHSD/) [HSD](https://astatsa.com/OneWay_Anova_with_TukeyHSD/)) was used for all statistical analyses.

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# **Results and discussion**

### **Effect of iron fortification on waxy rice**

Among iron fortificants, ferrous sulfate is the cheapest and most widely used in food fortification because of its excellent bioavailability<sup>[\[16\]](#page-6-15)</sup>. [Figure 2](#page-2-0) shows the photographs of rice samples where an evident color change was observed in both ironsoaked non-sonicated and sonicated rice. This qualitative observation indicates ferrous sulfate incorporation in the rice grain.

The endogenous iron content of raw IR65 rice is  $7.55 \pm 0.071$ mg·kg<sup>-1</sup> ([Fig. 3](#page-2-1)). The hygroscopic nature of rice allows water entry into the grain during soaking, allowing iron to come along with the hydration process. When the rice was treated with ultrasonication, iron incorporation was higher. After one hr of soaking, the iron content of sonicated rice increased to 368.00 ± 12.73 mg·kg−1 , which is 13.2% higher than the nonsonicated (319.50  $\pm$  10.61 mg·kg<sup>-1</sup>) sample. This higher iron content is due to the rice grain's morphological changes induced by ultrasonication. The cavitation forces by exploding microbubbles generate shear forces and microjetting, resulting in rice kernel cracks and fissures. The retention of iron in

<span id="page-2-0"></span>

**Fig. 2** Photographs of raw, sonicated, and iron-soaked (nonsonicated and sonicated) IR65 rice.

<span id="page-2-1"></span>

Fig. 3 Iron content of uncooked and cooked fortified rice. Values with different letters are significantly different (\* *p* < 0.01).

fortified sonicated IR65 rice after washing and cooking is 99.33% attributed to the efficient penetration of the mineral into the rice kernel's core, as previously reported<sup>[[11](#page-6-10)]</sup>. Significant loss of absorbed iron was noted in the non-sonicated sample, confirming that soaking alone without sonic treatment absorbs iron only at the surface, which is prone to leach out upon washing. Ultrasonic-treated food materials have shown improve-ment in mass transfer properties<sup>[[17](#page-6-16)]</sup>, enhancing the hydration process and micronutrient uptake. Similarly, higher iron uptake was reported in sonicated kidney beans<sup>[\[18\]](#page-7-0)</sup>. In our previous work on non-waxy rice samples, a 28-fold increase in iron concentration (321  $\pm$  13.43 mg of iron per kg) over the endogenous iron content of non-waxy milled rice was achieved after sonication and soaking in an aqueous iron solution with 82.9% retention after washing and cooking<sup>[\[19\]](#page-7-1)</sup>.

#### **Effects of sonication and fortification on rice textural properties**

The textural attributes of rice after fortification measured by sensory evaluation of human panels and instrumental evaluation by TPA are essential factors in assessing consumer acceptability and palatability. However, for practicality and simplicity, TPA was utilized in this work<sup>[\[20\]](#page-7-2)</sup> to determine the effect of iron soaking on rice's textural properties. TPA involves subjecting the cooked rice grains to two compression cycles mimicking the first and second bites on the food sample. It provides critical information on the mechanical responses, such as the hardness, adhes[iveness,](#page-3-0) springiness, and cohesiveness of the cooked rice. [Figure 4](#page-3-0) presents cooked raw and iron-soaked rice samples' instrumental textural attributes (hardness, stickiness,

<span id="page-3-0"></span>

Fig. 4 Textural properties of cooked raw and iron-fortified IR65 rice. (a) Hardness, (b) stickiness, (c) cohesiveness, (d) springiness, (e) gumminess, (f) chewiness. Values with different letters are significantly different (\* *p* < 0.01, \*\* *p* < 0.05).

cohesiveness, springiness, gumminess, and chewiness). The results demonstrate that sonicating and incorporating iron through soaking significantly affects rice hardness, stickiness, and gumminess, while other properties show no significant difference.

In Asia, hardness or firmness is an essential characteristic of cooked rice<sup>[[21](#page-7-3)]</sup>. Using TPA, hardness (g) is the force at the peak of the first curve in the two-cycle compression test<sup>[[22](#page-7-4)]</sup>. Because of the absence of amylose, IR65 is classified as a waxy variety with a soft mouthfeel texture having a lower hardness value than more rigid amylose-containing rice. The firmness or hardness of cooked iron-soaked non-sonicated and sonicated rice were 1,082.71  $\pm$  52.78 and 1,282.27  $\pm$  204.14 g, respectively, compared to cooked raw rice  $(1,584.80 \pm 165.55 \text{ g})$ . Incorporating iron through soaking significantly decreases the hardness of both rice samples. Our prior work showed that sonication alone didn't induce any changes in the hardness of IR65<sup>[[19](#page-7-1)]</sup>. This finding indicated the role of the fortificant in changing the firmness or hardness of waxy rice. The decrease in hardness in non-sonicated rice, markedly lower by 31.7%, may still be associated with fissures' formation during soaking. Rice being hydrophilic, absorbs moisture during soaking, resulting in swelling and internal stress development<sup>[[23](#page-7-5)]</sup> and leading to cracks<sup>[[24](#page-7-6)]</sup>.

Similarly, the decrease in hardness (significantly lower by 19.0%) for sonicated fortified rice is also attributed to the fissure induced by ultrasonic treatment and iron soaking. Rice grain develops cracks and crevices produced by cavitation during ultrasonication. While sonicated fortified rice has a higher hardness value than the non-sonicated sample, the difference didn't show any significant effect. However, comparing both samples, the drop in the hardness value is attributed to the incorporation of iron into the grain. Longer amylopectin chains have a higher recrystallization rate during the short-te[rm](#page-7-7) retrogradation process, resulting in harder-cooked rice<sup>[\[25\]](#page-7-7)</sup>. Ferrous sulfate may act as a filler that may inhibit gelatinized starch's realignment, causing a lesser hardness value. The softer rice characteristics may be caused by the molecular interaction of FeSO $_4$  and the amylopectin chains during the cooked grain's cooling process or retrogradation. Likewise, gumminess also

shows a similar trend as a derived product of hardness. This textural effect was observed in our previous works on fortified sonicated non-waxy rice samples<sup>[[26](#page-7-8)]</sup>.

Stickiness is a vital physical and sensory property of food that influences consumers' preferences<sup>[\[27\]](#page-7-9)</sup>. This paper reported the absolute value of the adhesiveness or stickiness of rice samples. In contrast to hardness, sonication alone increased the stickiness of IR65 from 102.15  $\pm$  20.53 to 174.99  $\pm$  42.15 g·s, as previ-ously reported<sup>[\[19\]](#page-7-1)</sup>. The cracks and fissures in treated grain may facilitate the leaching of additional amylopectin onto the rice surface, resulting in a stronger attraction between the probe and cooked rice samples $^{\lceil 11 \rceil}$  $^{\lceil 11 \rceil}$  $^{\lceil 11 \rceil}$ . The addition of FeSO $_4$  didn't significantly increase the value of the non-sonicated grain (135.59  $\pm$ 18.40 g·s), while a significant increment of 39.51% was observed in sonicated IR65 (168.86  $\pm$  29.17 g·s). Like sonicated IR65, the FeSO<sub>4</sub> acts as a physical cross-linker to the leached amylopectin, causing a higher attracting force between the TPA probe and cooked fortified rice. Compared to the parboiling technique, iron-fortified rice is stickier than unfortified rice<sup>[\[28\]](#page-7-10)</sup>.

#### **Effects of sonication and fortification on rice rheological attributes**

The effect of ultrasonication and iron fortification on rice rheological properties was further investigated by subjecting the ground samples to dynamic oscillatory temperature ramp and frequency sweep experiments. Dynamic oscillatory shearing provides structural information by distinguishing between elastic and viscous contributions to measured stress as a func-tion of frequency or temperatures in a non-destructive way<sup>[\[29\]](#page-7-11)</sup>. Compared to other techniques, such as thermal and viscoanalyzer, rheometry provides the food material's behavior under conditions (e.g., changing temperature and frequency) and may simulate real-world conditions experienced by the food samples during processing. The mechanical spectra ([Fig. 5](#page-4-0)), which presents the elastic (G') and viscous moduli (G") ([Fig. 5a](#page-4-0)) as well as the tan *δ* [\(Fig. 5b](#page-4-0)) of the rice samples during controlled gelatinization (35 to 95 °C) and retrogradation (95 to 35 °C), provides information on the swelling and staling be haviors of waxy rice flour components as the temperature changes. Results showed that the raw and treated rice samples

<span id="page-4-0"></span>

Fig. 5 Dynamic shear curves of IR65 rice samples during temperature ramp experiment showing (a) elastic (G') and viscous moduli (G"); and (b) tan *δ*. For the color version of the figure, the reader is referred to the online version of this article.

exhibited viscoelastic solid behaviors during thermal processes as observed in higher G' than G" values in agreement with the previous reports<sup>[\[29\]](#page-7-11)</sup>. The changes in rice rheological behaviors during controlled heat-induced gelatinization have been proposed to undergo four stages, namely: (1) suspension into sol (increments in modulus), (2) sol transition to gel (at  $G'_{max}$ ), (3) network destruction (G<sub>max</sub> to 95 °C), and (4) network strengthening (95 to 35 °C). These stages were observed in all samples. In general, ultrasonic treatment resulted in a greater G'max than non-sonicated rice. The increase in G' of sonicated milled rice accelerates the degradation of starch granules by cavitational forces, making the granules more permeable to water as the temperature rises<sup>[\[30\]](#page-7-12)</sup>.

Compared with the G'<sub>max</sub> of raw rice ([Table 1](#page-5-0); 333.73  $\pm$  79.11 Pa), ultrasonically treated waxy rice increased its  $G'_{max}$  by 592.67  $\pm$  56.74 Pa<sup>[\[19\]](#page-7-1)</sup>, suggesting that ultrasonication altered the natural network formation during gelatinization of rice starch components. Further, an increase in G'max was observed upon the incorporation of iron in sonicated rice. Compared to the raw and sonicated IR65, the iron-fortified sonicated IR65 and ironsoaked IR65 have a higher G'<sub>max</sub> value of 833.23  $\pm$  199.95 Pa and 760.50  $\pm$  145.24 Pa, respectively. Moreover, the G" of ironfortified sonicated IR65 exhibited similar behavior. Increased

<span id="page-5-0"></span>**Table 1.** Dynamic rheological properties of waxy rice in the presence of  $FeSO<sub>4</sub>$ .

	Samples		
	Raw IR65	Iron soaked IR65	Sonicated iron-fortified IR65
Heating			
$G'_{max}$	$333.73 \pm 79.11^a$	760.50 ± 145.24 <sup>b**</sup>	$833.23 \pm 199.95^{b*}$
$G''_{max}$	$107.84 \pm 14.34$ <sup>a</sup>	$175.57 \pm 30.09^a$	$194.43 \pm 39.46^{b**}$
tan $\delta_{\text{G}^{\prime} \text{max}}$	$0.33 \pm 0.04^a$	$0.23 \pm 0.01^a$	$0.23 \pm 0.01^a$
$G'_{95\degree}$	$264.17 \pm 23.05^a$	$405.33 \pm 56.47^{b**}$	$456.20 \pm 59.25^{b*}$
$G''_{95°}$	$76.58 \pm 2.23$ <sup>a</sup>	$88.88 \pm 14.52$ <sup>a</sup>	$98.74 \pm 0.71$ <sup>b**</sup>
tan $\delta_{\text{qsc}}$	$0.29 \pm 0.02$ <sup>a</sup>	$0.22 \pm 0.01^{b*}$	$0.22 \pm 0.01^{b*}$
Cooling			
$G'$ <sub>35°C</sub>	$329.53 \pm 12.26^a$	$474.27 \pm 69.27$ <sup>b**</sup>	$529.03 \pm 37.37$ <sup>b*</sup>
$G''_{35\degree C}$	$93.62 \pm 2.82$ <sup>a</sup>	$111.29 \pm 11.82$ <sup>b**</sup>	$127.97 \pm 6.33^{b*}$
tan $\delta_{35\degree}$	$0.28 \pm 0.00^a$	$0.24 \pm 0.01^{b*}$	$0.24 \pm 0.01^{b*}$

<span id="page-5-1"></span>Data presented as mean  $\pm$  standard deviation of duplicate determinations. Values with different letters in the same group are significantly different (\* *p* < 0.01, \*\* *p* < 0.05).



**Fig. 6** Dynamic viscoelasticity of IR65 as a function of frequency.

elastic properties indicate a more vital intermolecular interaction between the fortifying iron (II) and the amylopectin chains. Ultrasonication altered the molecular architecture of starch by disrupting hydrogen bonds in amylose/amylopectin and thus loosening the starch structures<sup>[\[30](#page-7-12)]</sup>. In effect, the structural changes in starch caused by ultrasonication facilitate the interaction of fortifying iron (II) ions with starch molecules, acting as a physical cross-linker between them.

model  $\eta^* = K^* \dot{\gamma}^{n^*-1}$  was applied to further parameterize the mechanical spectr[a-c](#page-6-14)onsistency c[oeffici](#page-5-1)ent  $(\eta^*)$  and flow behavior index  $(n^*)^{[15]}$  $(n^*)^{[15]}$  $(n^*)^{[15]}$ . Results show ([Fig. 6](#page-5-1)) that all rice samples The frequency sweep test was performed on the cooked rice gels to gain more structural insights into the starch-iron fortificant network formation. The test was within a range of 0.1 to 100 rad·s−1 at an oscillatory stress of 0.1 Pa at 35 **°**C to examine the viscoelastic nature of formed hydrogels after heating and cooling. Moreover, for the frequency sweep data, the power have similar mechanical spectra (G', G", tan *δ*). Generally, the storage moduli (G') were higher than those of the loss moduli (G"), characterizing a solid-like attribute over the range of measured frequencies. Sonicated iron-fortified rice has the highest G' values throughout the working frequency, indicating a more robust network and agreeing with the temperature ramp data. Similarly, ultrasound-treated rice flo[ur](#page-7-13) produced st[ronger a](#page-6-17)nd more elastic gel than native rice flour<sup>[\[31\]](#page-7-13)</sup>.

[Table 2](#page-6-17) shows the parameterized data showing the derived values for the consistency coefficient (K) and flow behavior index (n). Since the data indicate that the G′ primarily dominates over the G", the complex modulus (G\*) of the system and thus, the elasticity of these gels can be effectively represented by K. Results showed that the sonicated iron-fortified rice had the highest K value (13.0  $\pm$  1.33), which indicates the most elastic behavior among the rice gels, this result agrees well with other rheological parameters such as  $G'_{max}$ ,  $G'_{95°C}$ , and  $G'_{35°C}$  of sonicated iron-fortified rice, which supports the formation of the proposed strong starch-iron fortificant network, additive molecules such as rutin have been shown to increase the elastic behavior of rice gel by facilitati[ng](#page-7-11) physical interactions between the additive, starch, and water through hydrogen bonding during heating and cooling<sup>[[29](#page-7-11)]</sup>. Similarly, ultrasonication and iron addition in rice notably influence the mechanical attributes of rice by forming stronger intermolecular interactions between iron and rice components.



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#### <span id="page-6-17"></span>Incorporating iron fortificant in ultrasonicated waxy rice

**Table 2.** Derived parameters from frequency sweep experiment of IR65 rice samples.



Data presented as mean ± standard deviation of duplicate determinations. Values with different letters in the same group are significantly different.

# **Conclusions**

In conclusion, the findings of this study demonstrate that ultrasonic treatment can effectively enhance iron absorption in waxy rice grains. The modified rice showed a significant increase in iron uptake by 13.2%, and improved retention after washing and cooking (99.33%) may have substantial implications for combating iron deficiency, a global nutritional issue. Furthermore, concerning textural attributes, the rice fortified with nutrients reduced its firmness and increased its stickiness, enhancing the characteristics of rice-derived food items such as traditional rice cakes. These changes resulted from the forming of a stronger amylopectin-iron network within the rice grains. The increased elastic (G') and viscous (G") moduli observed in temperature ramp and frequency sweep experiments further support these findings. However, sensory evaluation of the fortified rice by human panels is still needed to confirm the impact of these textural changes on the overall eating experience. Overall, the outcomes of this study present promising prospects for improving the nutritional value and textural characteristics of rice and other grains, justifying additional exploration in both academic and industrial contexts.

# **Author contributions**

The authors confirm contribution to the paper as follows: study conception and design: Bonto A, Camacho D, Sreenivasulu N; data collection & analysis, interpretation of results: Bonto A; draft manuscript preparation: Bonto A. All authors read and approved the submitted version.

# **Data availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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# **Conflict of interest**

The authors declare that they have no conflict of interest.

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