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Effect of zinc-rice grit flour on the physicochemical, nutritional, and sensory properties of gluten-free biscuits

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Abstract

The utilisation of rice flour to produce gluten-free food applications is highly popular as consumers become more aware of celiac disease and safety for baby-led weaning products. The present work investigated the development of gluten-free biscuits made from rice grits flour (RGF). The physicochemical, nutritional, and sensory properties of the products were also evaluated. Rice grits used were from the Inpari 32 and Inpari IR Nutri Zinc types. Results showed that incorporating RGF into biscuit ingredients showed a lot of potential since RGF retained its nutritional value, especially vitamin A and microminerals such as zinc and iron, as well as protein (8.4 - 9.6%), which are all needed to prevent stunting. The Inpari IR Nutri Zinc flour had less amylose (21.12%) and smaller particles than the Inpari 32 flour (amylose: 23.42%) and commercial flour, which resulted in lower hardness level and lower final viscosity. Vitamin A content of biscuits produced from the Inpari IR Nutri Zinc was lower (367 IU/100 g) as compared to the Inpari 32 (412 IU/100 g). The amounts of zinc in the final product were 15 and 12 ppm for the Inpari IR Nutri Zinc and Inpari 32, respectively. Both the Inpari IR Nutri Zinc (19 ppm) and Inpari 32 (73 ppm), which were RGF biscuits, had less iron than commercial biscuit (119 ppm). There were no significant differences among biscuit products in terms of sensory analysis.

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Introduction

More than half of the world's population rely on rice (*Oryza sativa* L.) as a source of staple food (Xiang *et al*., 2015). Rice is one of the most suitable cereals for gluten-free product formulations due to its nutritional quality, hypoallergenic properties, easily digestible carbohydrates, and excellent sensorial properties (Yano *et al*., 2020). Rice milling produces approximately 80% head rice in Indonesia, where 10 - 15% were broken rice and rice grits/brewers' rice (Widowati and Luna 2019). Broken rice results from mechanical tensions caused by rice processing (Moraes *et al*., 2014), and are also known as rice grits (Omran and Hussien, 2015). In Indonesia, rice grits are mostly used as feed, and never considered as valuable by-products. However, rice grits are rich in nutrition. The classifications of rice grading and rice grits are depicted in Figures 1A and 1B. A previous study reported that rice wine was produced from the fermentation of broken rice or rice grits (Li *et al*., 2014); therefore, some researchers also called this as brewers' rice.

In order to utilise this by-product and develop it into a more profitable and value-added product, a rice bio-industry concept was used, in which all the paddy plants will produce zero-waste. To successfully incorporate rice grits or flour into products, the properties of the materials must first be understood because they affect the product quality, consistency, and consumer satisfaction (Leewatchararongjaroen and Anuntagool, 2016). Rice grits/brewers' rice is milled by the wet-milling method to gain fine flour. The flour is then used to make gluten-free biscuits for baby-led weaning.

Biscuits are a commercially important type of

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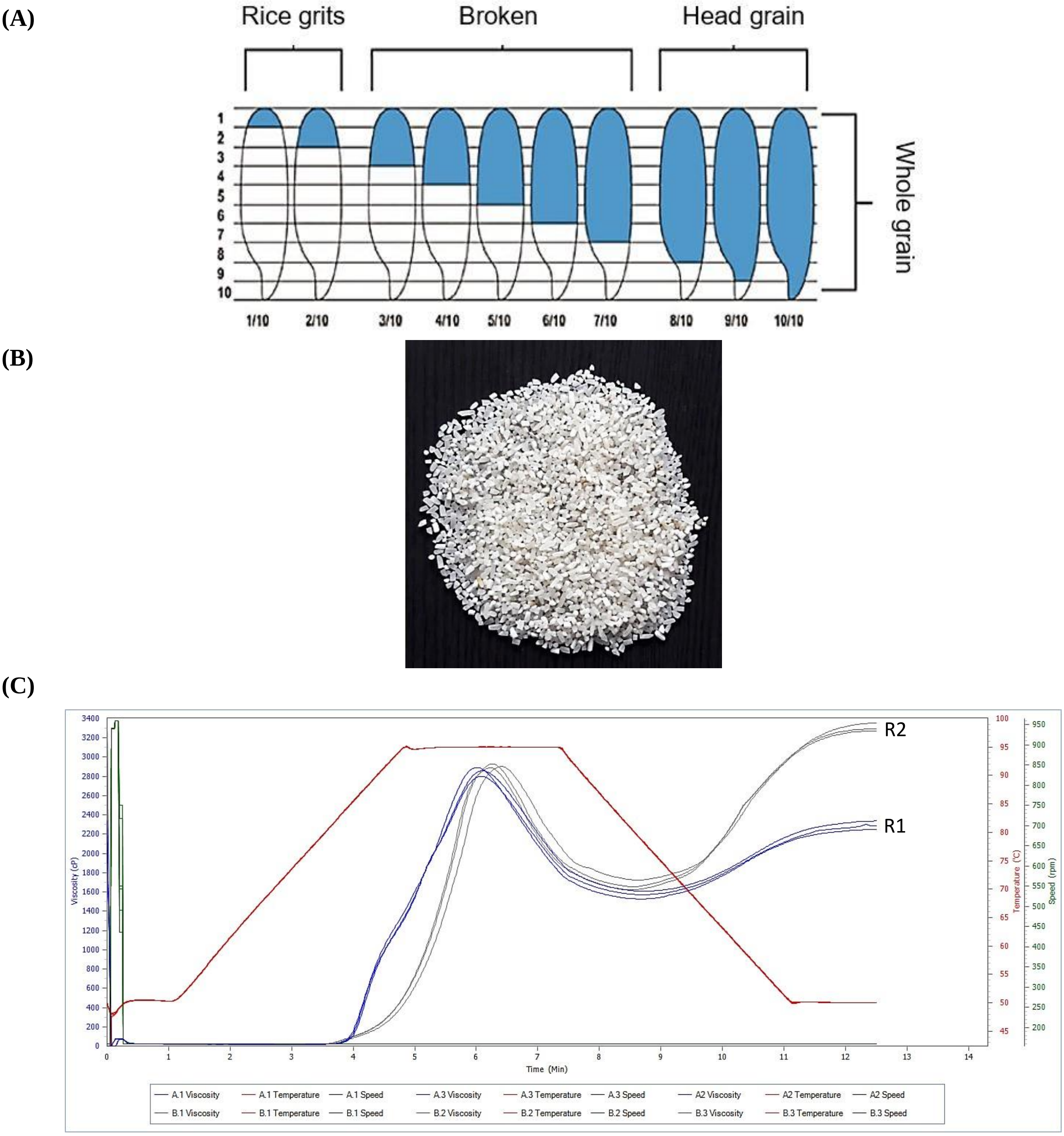


Figure 1. Rice grading classification **(A)**, rice grits **(B)**, and pasting properties of rice grits flour **(C)**; Inpari IR Nutri Zinc **(R1)** and Inpari 32 **(R2)**.

gluten-free bakery food due to their ease of consumption, good nutritional content, availability of a wide variety of biscuit types, long shelf-life, and relatively low selling price (Jothi *et al*., 2014; Cannas *et al*., 2020). Furthermore, as compared to bread or pasta production, biscuit production has fewer technological issues because gluten build-up is non-existent, thus making the process of replacing gluten-containing flours with gluten-free counterparts much simpler (Laguna *et al*., 2011). Nonetheless, at least two significant problems impede the production of gluten-free bakery products: low sensorial acceptability and an unbalanced nutritional profile caused by lower levels of several essential nutrients, including minerals (iron, zinc, magnesium, and calcium), dietary fibres, and vitamins (folate and B12) (Bolarinwa *et al*., 2019).

The Inpari 32 and Inpari IR Nutri Zinc are recognised as Indonesian rice varieties, and they contain zinc minerals which help prevent stunting in Indonesia. The Inpari IR Nutri Zinc variety contains zinc mineral of 34.51 ppm higher than the Ciherang variety (Suharna, 2019). The Inpari 32 rice contains 17 ppm zinc as reported by Indonesia Center for Rice Research. By using these two rice varieties, apart from utilising the by-product, it also increases the availability of gluten-free products including toddler biscuits for baby-led weaning. Our approach in the present work was to substitute wheat flour in biscuits with rice grits in order to increase the zinc content. The present work aimed to develop gluten-free biscuits made from rice grits high in zinc, and evaluate the physicochemical, nutritional, and sensory properties of the resulting product.

Materials and methods

Materials

Rice varieties, namely the Inpari 32 and Inpari IR Nutri Zinc, were obtained from the Indonesian Center for Rice Research (ICRR), Subang, West Java, Indonesia.

Wet milling processing of rice grits flour (RGF) of the Inpari 32 and Inpari IR Nutri Zinc

Approximately 1 kg of rice grits were soaked overnight in water before it was ground using a stone mill under continuous addition of water to obtain rice slurry. The slurry was filtered through a filter bag to obtain rice cake. The rice cake was dried overnight in a tray dryer at 40°C. The dried rice cake was ground and sieved through a 100-mesh sifter to obtain rice flour (RGF). The flour samples were then packed in polypropylene plastic bags, and stored in a desiccator at room temperature for subsequent analyses (Varavinit *et al.*, 2003).

Dough formulation and biscuit preparation

The main ingredient of this formulation was rice flours of two varieties (the Inpari 32 and Inpari IR Nutri Zinc). Other ingredients used were unsalted butter and eggs purchased from a local groceries store (Bogor, Indonesia). Commercial baby biscuit (made from mixed wheat and arrowroot flour) was purchased for comparison. The final formulation consisted of 45% rice flour, 20% unsalted butter, 20% powdered sugar, and 15% eggs from the total dough weight. All ingredients were stored at room temperature, except the unsalted butter and eggs, which were refrigerated at 4°C until use. The biscuit dough was prepared using a previous method described by Laguna *et al*. (2014) with slight modification.

Physical properties

The pasting properties of rice flour were analysed by Rapid Visco Analyser (Perten 4500 model) following a previous method from Marta *et al.* (2019). The texture of biscuit samples was measured by texture analyser (Texturepro CT V1.2 Build 9, Brookfield Engineers Lab. Inc.) (Luna *et al.*, 2021). The colour of biscuits samples was measured using a Spectrophotometer CM 5 (Konica Minolta Co., Osaka, Japan) with Spectra Magic software. The colour measurement included L* (lightness, 0 = black / 100 = white), a* (+a* = redness / -a* = greenness), b* (+b* = yellowness / -b* = blueness), and hue. The calculation of the whiteness value was based on a previous study by Marta *et al.* (2019).

Chemical properties

Moisture content was determined following the Association of Official Analytical Chemists method with slight modification (AOAC, 2005), by drying 2 g of sample at 105°C in an air oven for 24 h. The water content was then gravimetrically determined. Protein content was determined using the Kjeldahl method from the Association of Official Analytical Chemists method (Nielsen, 2010), while fat content was determined using a Soxhlet extractor.

Vitamin A was determined using HPLC following the Association of Official Analytical

Chemists method (AOAC, 2005). Sample was prepared by extracting vitamin A from 5 g of sample with 20 mL of ethanol, 5 mL of KOH 50%, and 0.5 g of pyrogallol. The sample was later heated on a hotplate at 80°C for 20 min, vigorously shaken until reached room temperature, and added with 5 mL of acetic acid anhydrous. All the mixture was put into a 50-mL volumetric flask, and diluted with a combination of ethanol:THF of 1:1. It was then refrigerated overnight. Then, the supernatant was filtered with ashless filter paper. The filtrate was filtered using a 0.45 µm PTFE syringe filter into a glass vial. And later, 20 µL of sample was injected into HPLC.

Iron and zinc were measured using the Association of Official Analytical Chemists spectrophotometric method. Standard addition was performed after the digestion of samples, and blanks were performed. A series of solutions for standard addition measurements were made containing 0, 0.5, and 1 µg/mL of Fe(III) and Zn(II) standards, respectively. After adding an appropriate amount of analyte, the samples were diluted with deionised distilled water to 25 mL, and flame atomic absorption spectrometry (FAAS) was performed for the determination of iron and zinc. Graphs of the standard addition method were plotted in terms of absorbance versus iron or zinc added (µg/mL). Iron and zinc contents in biscuit samples were calculated by extrapolation. Results were expressed as mean of at least three independent digestion procedures.

Particle's morphology

Particle's morphology of biscuits was determined using scanning electron microscopy (Carl Zeiss, EVO MA 10 at 16 kV, UK). The sample was sprayed onto an aluminium plate, and coated with gold at 8 - 10 mA for 10 - 15 min. Representative digital images of biscuit morphology were obtained at 250× magnification.

Sensory evaluation

Sensory evaluation of the biscuit samples was carried out following a previous method by Dewi *et al.* (2020) with slight modification. Twenty-five infants/toddlers aged 6 - 24 months with their mothers were the panellists selected from Tanah Sareal, Bogor, Indonesia. Biscuits samples of various proportions were evaluated for colour, texture, aroma, and taste. Determination of biscuits with RGF incorporation acceptability was obtained by using a hedonic test with five scales, namely 1 = extremely dislike; 2 = dislike, 3 = neutral; 4 = like; and 5 = like extremely.

Statistical analysis

Data were expressed as mean ± SD of triplicates. The data were analysed by one-way analysis of variance (ANOVA) to compare physicochemical, nutritional, and sensory properties between flour and biscuits from two varieties, and submitted to the mean comparison test by Duncan at a significance level of 5% ($p < 0.05$). Whereas independent-samples *t*-test (two-sample *t*-test) was used to compare the physicochemical, nutritional, and sensory between different forms (flour and biscuit forms). One-way ANOVA, independent-samples *t*-test, and Kruskal Wallis test for organoleptic properties analysis were analysed using Minitab 19.0 Statistical Software Programme.

Results and discussion

Pasting properties rice grits flours

The pasting properties of RGF from two Indonesian varieties are presented in Figure 1C and Table 1. Results showed that pasting properties were influenced by the varieties (Zhu *et al.,* 2020). Each variety of rice has unique physicochemical properties, and the type of starch present influences cooking quality (Nawaz *et al.,* 2016). The Inpari 32 flour showed a rapidly increasing viscosity as compared to the Inpari IR Nutri Zinc at temperatures lower than 95°C. These results suggested that Inpari 32 starch granules swell when heated at a relatively low temperature.

The RGF of the Inpari 32 had amylose ranging from 22.67 to 23.42%, while the Inpari IR Nutri Zinc had lower amylose ranging from 19.97 to 21.12%. Both varieties of rice were categorised as medium amylose content. The ratio of AM/AP of the Inpari IR Nutri Zinc was lower than the Inpari 32. This parameter is one of the essential qualities determining starch functionality. It can be observed from Table 1 that the ratios of AM/AP, peak temperature, final viscosity, and setback were significantly different ($p < 0.05$) among all pasting behaviour. However, the peak viscosity (PV) was not significantly different ($p > 0.05$).

Amylose, lipid content, and branch chain length distribution of amylopectin influence the pasting properties of flour (Tester and Morrison,

Table 1. Parameters of pasting characteristics of RGF.

Sample	Amylose	AM/AP	PT (ºC)	PV (cP)	HPV (cP)	BD (cP)	FV (cP)	SB (cP)
A	21.12 ± 0.02^b	0.46 ± 0.02^b	83.57 ± 0.25^a	2828.70 ± 46.1^a	1548 ± 42.6^a	1289.70 ± 74.6^a	722 ± 6.56^b	-558.7 ± 73.1^b
B	23.42 ± 0.02^a	0.54 ± 0.03^a	82.40 ± 0.53^b	2889 ± 22.6^a	1648 ± 37^a	1241 ± 52.1^a	1639.33 ± 10.07^a	398.3 ± 45^a

Values are mean ± standard deviation of triplicates (*n* = 3). Means followed by the same lowercase superscripts in a column are not statistically different at $\alpha = 0.05$. AM/AP: ratio of amylose to amylopectin, PT: pasting temperature, PV: peak viscosity, HPV: hot paste viscosity, BD: breakdown (PV-HPV), FV: final viscosity, and SB: setback (FV-HPV). A: Inpari IR Nutri Zinc, and B: Inpari 32.

1990). This difference could be attributed to the difference in starch granule size and starch component in the flour. Starches with large granules gelatinise relatively faster than starches with smaller fractions, owing to the less molecular bonding, which causes them to swell and break down more quickly (Chen *et al.*, 2003).

The disintegration of starch granules and restructuring of polymers occur during the cooling phase of the pasting process, thus resulting in a decrease in viscosity. It was observed that the Inpari 32 starch could maintain the paste viscosity as compared to the Inpari IR Nutri Zinc. As a result, the RGF of the Inpari 32 was more stable and transparent, and had a higher tendency to retrograde or recrystallise the starch molecules in solution. Based on previous research, the gelation and retrogradation of the amylose and amylopectin fractions, respectively, have been attributed to the short- and long-term development of crystallinity in starch (Soykeabkaew *et al.,* 2015; Alcázar-Alay and Meireles, 2015). This pasting behaviour is essential to determine the suitability of flour in biscuits. It can affect the biscuits characteristics, and the interaction of starch with other components in the dough.

Chemical composition of biscuits

The chemical compositions of all biscuits are presented in Table 2. The moisture content of all biscuits ranged from 3.77 to 6.02% (w/w). The moisture content of the biscuits was all less than 10%, thus implying a lower risk of spoilage by microorganisms, and as a result, a longer shelf life. It has been determined that most of the chemical compositions presented by biscuits produced with RGF met the Indonesia National Standard (SNI) for baby biscuits. The fat content of the two formulations was higher than the specification requirements for baby biscuits, which is 18%. A high-fat level in baby biscuits was due to the unsalted butter and egg yolk. The use of unsalted butter and egg yolk was the same in the two formulations; therefore, the increase in fat content was influenced by the fat content of the flour. The commercial biscuit contained composite arrowroot and wheat flour.

Protein content in the biscuits produced with RGF ranged from 8.43 - 9.59% (w/w), which was higher than the commercial biscuits (Table 2). This result was in accordance with Okpala and Egwu (2015), who reported that biscuits produced from broken rice flour contained 8.86% protein. This might have been due to the addition of egg in the formulation on top of flour, sugar, and fat (butter or vegetable shortenings), as reported by Klunklin and Savage (2018).

Commercial baby biscuits had the lowest protein and highest carbohydrate content. This could be due to the high carbohydrate level available in arrowroot, as described in a previous study. Okpala and Egwu (2015) reported that carbohydrates are predominant nutrients in roots and tubers. It had a dilution effect on the protein content in the biscuits since amino acids of the roots and cereals reinforced each other. When the entire nutritional state of poor sectors of the population is considered, including stunting issues, the development of high-

Table 2. Chemical composition of baby biscuits produced with RGF, and commercial biscuits.

Parameter	A	B	C	Indonesia National Standard for Baby Biscuits (SNI 01-7111.2-2005)
Moisture content (% w/w)	4.31 ± 0.25^b	3.77 ± 0.12^c	6.02 ± 0.12^a	Max 5%
Ash content (% w/w)	0.41 ± 0.07^b	0.30 ± 0.15^b	2.04 ± 0.06^a	Max 3.5%
Protein (% w/w)	8.43 ± 0.15^b	9.59 ± 0.34^a	6.36 ± 0.16^c	Min. 6%
Fat (% w/w)	22.19 ± 0.96^a	22.71 ± 0.26^a	13.96 ± 0.81^b	Max. 18%
Carbohydrate (% w/w)	64.65 ± 0.76^b	63.62 ± 0.35^b	71.62 ± 1.06^b	Min. 30%
Vitamin A (IU/100 g)	367.67 ± 2.52^a	412.58 ± 2.91^b	na**	Max. 700 IU
Fe content (ppm)**	19.50 ± 0.66	73.24 ± 6.52^b	119.87 ± 6.52^c	Min. 0.5 ppm
Zinc content (ppm)**	15.35 ± 0.57^a	12.17 ± 0.48^b	14.32 ± 0.74^a	Min 0.25 ppm

Values are mean ± standard deviation of triplicates (*n* = 3). Means followed by the same lowercase superscripts in a row are not statistically different at α = 0.05. na: not analysed. A: Inpari IR Nutri Zinc, B: Inpari 32, and C: commercial baby biscuits.

protein biscuits is a practical strategy as baby-led weaning products. Comparing the data on moisture, ash, and protein contents, there was a significant difference ($p < 0.05$) in the biscuit's chemical composition, thus suggesting a substantial role of a variety of flour and other ingredients on the biscuit's characteristics.

Minerals, iron, and zinc were available in all biscuits. Zinc is an essential mineral to prevent stunting in Indonesia (Table 2). The Inpari IR Nutri Zinc variety was released to prevent stunting in Indonesia due to its higher zinc content, at an average of 29.54 ppm, than other rice varieties. It was observed that biscuits produced with the Inpari 32 RGF had the lowest zinc content among all biscuits with significant difference ($p < 0.05$), thus suggesting a substantial role of a variety of flour on the biscuit's characteristics. Concerning iron and vitamin A contents, biscuits produced with the Inpari 32 RGF had higher iron and vitamin A contents with significant differences ($p < 0.05$). It had been reported that six months of supplementation with iron+zinc or iron+zinc+vitamin A resulted in an increased length of 1 cm when compared with placebo and supplementation with zinc alone (Beal *et al*., 2018). Therefore, biscuits produced with RGF can reduce the incidence of stunting in Indonesia.

Nutritional recommendation of biscuits produced with RGF was based on a 725 calories diet of Percent Daily Values of infants. The intake may be higher or lower, depending on the calories needed. The serving size of RGF biscuits would be five pieces (50 g). The total energy per serving would be 247 calories. The daily percentage of total fat, protein, total carbohydrate, vitamin A, iron, and zinc of RGF biscuits was 31, 28, 14 - 16, 14 - 16, and 20 - 26%, respectively. A protein-, essential fatty acid-, and micronutrient-rich diet have been shown to improve birth weight, growth, and cognitive development while lowering child mortality (Burchi *et al.*, 2011; Niaba *et al.*, 2013).

Particle's morphology of RGF and biscuits

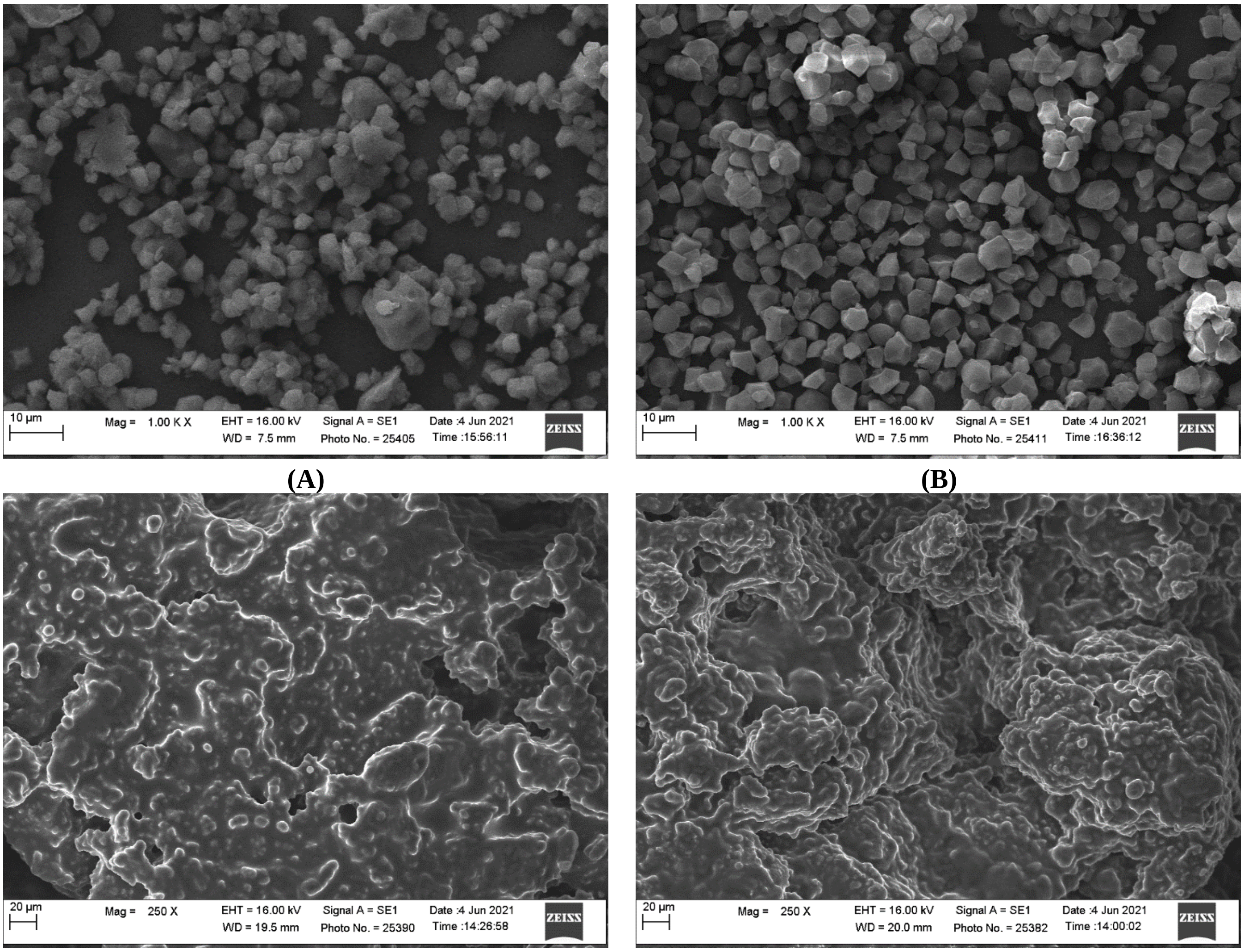
Particles morphology of RGF and biscuits was observed by Scanning Electron Microscope (SEM), and presented in Figure 2. It was observed that the starch granules in RGF were polygonal. This is similar to the rice flour structure in the previous study by Han *et al.* (2021). The Inpari 32 flour was harder, with denser surface, and more uniform than the Inpari IR Nutri Zinc, thus suggesting that the latter with fine particles was produced by grain with lower hardness (Figures 2C and 2D). The particle morphology of the two biscuits was also confirmed with the hardness value (Table 3) and final viscosity (FV) of pasting properties of RGFs (Table 1). Due to the loose structure of the Inpari IR Nutri Zinc of chalky endosperm, cells and starch granules separate more easily during milling, thus resulting in fine particles as reported by Ashida *et al.* (2009).

On the other hand, the biscuit morphology showed that the Inpari 32 biscuits structure had more incorporated structure than the Inpari IR Nutri Zinc biscuits, thus suggesting that the Inpari 32 flour was well blended with other ingredients of biscuits. The Inpari IR Nutri Zinc biscuits had an open structure with gaps between material layers. It has been reported that the expansion of gas bubbles due to increasing temperature, which also increases the water vapour pressure within them, may cause an increase in tensile stress in the membrane, thus resulting in rupture and the formation of holes and tunnels in the product through which gas escapes to the outside (Mamat and Hill, 2014).

Physical and mechanical properties of biscuits

The hardness, springiness, deformation at target, and total work were determined using the biscuit samples. The force required to achieve a given deformation is defined as hardness. The rate at which a deformed material returns to its undeformed state after the deforming force is removed is springiness. The mechanical properties of biscuits are presented in Table 3. Biscuits produced with RGF were not significantly different ($p > 0.05$) in terms of springiness, deformation at target, and total work parameters, thus suggesting no significant flour difference on those mechanical characteristics. However, it was significantly different ($p < 0.05$) for hardness of the biscuits, meaning that adding a different variety of rice grits flour to the biscuit dough formulations affected the hardness of biscuits. The difference in the results could probably be due to the size, concentration, and synergistic of all components in the dough (Luna *et al.*, 2021).

For colour, it was determined that both RGF were not significantly different ($p > 0.05$), while significantly different with the commercial biscuits (p



(A) **(B)**

(C) **(D)**

Figure 2. SEM of rice grits flours and biscuits: Inpari IR Nutri Zinc flour **(A)**; Inpari 32 flour **(B)**; Inpari IR Nutri Zinc biscuits **(C)**; and Inpari 32 biscuits **(D)**. M: 1,000× and 250×, respectively.

Table 3. Physical and mechanical properties of biscuits.

Sample	Hardness (N)	Springiness (mm)	Deformation at Target (%)	Total Work (mJ)	Whiteness	Lightness	Hue
A	7.29 ± 1.81^{b}	6.97 ± 1.62^{b}	88.73 ± 3.35^{a}	12.19 ± 7.32^{b}	68.80 ± 0.16^{a}	80.32 ± 0.32^{a}	88.94 ± 0.36^{a}
B	10.59 ± 2.5^{ab}	12.20 ± 3.22^{b}	91.43 ± 2.44^{a}	11.66 ± 2.16^{b}	67.74 ± 0.56^{a}	80.40 ± 0.94^{a}	88.71 ± 0.65^{a}
C	14.69 ± 1.57^{a}	25.23 ± 3.73^{a}	93.07 ± 1.69^{a}	36.87 ± 1.53^{a}	43.22 ± 0.16^{b}	54.43 ± 0.16^{b}	65.85 ± 1.3^{b}

Values are mean ± standard deviation of triplicates ($n = 3$). Means followed by the same lowercase superscripts in a column are not statistically different at $\alpha = 0.05$. A: Inpari IR Nutri Zinc, B: Inpari 32, and C: commercial baby biscuits.

< 0.05). The higher whiteness and L* value might be due to the lower phenolic content, as Marta *et al.* (2019) reported. The lower L* values were also validated by the particle size of starch as analysed by SEM. The leaching out of smaller particle size of starch may be hindered due to high molecular bonding, as observed by Chen *et al.* (2003); thus, the colour component may be lesser in the surface area. It can be seen in Table 1 that RGF A had smaller final viscosity than RGF B, since the particle size of RGF A was lesser than RGF B as shown in Figures 2A and 2B.

Sensory evaluation of biscuits

Table 4 shows the sensory scores of biscuits. It was observed that panellists scored the Inpari IR Nutri Zinc higher than the Inpari 32 biscuits. However, the commercial biscuits remained with the highest scores. The preference for all sensory attributes of the biscuits was not significantly different ($p > 0.05$). The obvious panellists' preference was the colour and the taste of biscuits. The darker biscuit was probably preferred due to the brown colour of the biscuit, which is associated with chocolaty flavour. These attributes were predominating indications of the acceptability of baby-led weaning biscuits.

Table 4. Sensory evaluation scores of Inpari Nutri Zinc and Inpari 32 biscuits.

Response	**N**	**Median**			**Mean Rank**			**Kruskal-Wallis test**
Sensory Attribute		**A**	**B**	**C**	**A**	**B**	**C**	**(*p*-values)**
Colour	75	4	4	4	35.3	34.4	44.3	0.208
Aroma	75	4	4	4	33.4	40	40.6	0.438
Taste	75	4	4	4	33.5	36.2	44.3	0.190
Texture	75	4	4	4	36.6	38.5	38.9	0.928
Overall	75	4	4	4	32.2	39.2	42.6	0.231

A: Inpari IR Nutri Zinc, B: Inpari 32, and C: Commercial baby biscuits. The test shows that the preference for the biscuits is not significantly different ($p > 0.05$). 1 = extremely dislike; 2 = dislike; 3 = neutral; 4 = like; and 5 = like extremely.

Conclusion

The present work demonstrated that incorporating RGF into biscuit ingredients had a great potential because it retained nutritional value, particularly microminerals such as zinc and iron, and also protein which are all needed to prevent stunting. The Inpari IR Nutri Zinc biscuit contained low amylose content and smaller particle size, which yielded lesser hardness level and lower final viscosity as compared to the Inpari 32 biscuit and the commercial biscuit. The final product had zinc contents of 15 and 12 ppm for the Inpari IR Nutri Zinc and Inpari 32, respectively. The iron contents of both RGF biscuits were 19 and 73 ppm for the IR Nutri Zinc and Inpari 32, respectively, where both values were observed with lower iron content than that in the commercial biscuit. There were no significant differences among biscuit products in terms of the sensory analysis. The rice grit flour is a potential ingredient for manufacturing biscuits since it contains excellent nutritional values such as zinc and iron.

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