Research Article

Development of Gluten-Free Bread from Brown Rice-Potato Starch Composite Flour Incorporated with Red Seaweed Kappaphycus alvarezii powder

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ABSTRACT

Gluten free diets and lifestyles are rapidly increasing in popularity mainly due to the increase in consumer awareness about gluten related disorders and gluten intolerances. With increasing demand, the food industry strives to develop new food products that are gluten-free and able to be consumed by those who suffer from these conditions. The objective of this study was to develop a gluten-free bread from brown rice-potato starch composite flour incorporated with red seaweed Kappaphycus alvarezii (KA) powder. KA powder, ranging from 2% to 10%, was added, and the physical properties of the gluten-free bread were assessed. Sensory evaluation was conducted using two methods, the descriptive and hedonic tests. The amount of seaweed significantly ($p<0.05$) influenced the loaf volume, with F5 (10%), the highest concentration of seaweed, having the highest reduction of volume of 15.38% compared to the control F0 (without seaweed). With increasing seaweed concentration, the brightness and yellowness of each bread decreased. The texture profiles of KA-incorporated bread formulations were also affected with the firmness of bread being the most significant change. The descriptive test showed that F5 (10%) had the highest intensity of seaweed effect which affected the colour, texture, aroma, taste and aftertaste of the bread. The hedonic test showed that the most acceptable formulation was F1 (2%). From these formulations, the best formulation F1 (2%) was chosen for further nutrient analysis where it was compared to the control (without seaweed). It was shown that F1 had significantly ($p<0.05$) higher moisture, ash, fat, and dietary fibre content, but lower carbohydrate and protein content compared to F0 (without seaweed). In conclusion, the formulation of gluten-free KA-incorporated brown rice-potato starch bread had an impact on the bread texture, colour, volume, and nutrient content.

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1. Introduction

The rise of gluten-free foods initially emerged due to increased health complications in a specific population, caused by consuming gluten-containing wheat products, leading to various medical issues like autoimmune responses and allergies (Newberry et al., 2017). These conditions are now known as gluten-related disorders (GRDs), and affected individuals adopt a gluten-free diet (Hill et al., 2016). To cater to the growing number of diagnosed GRD cases, gluten-free foods were introduced (Demirkesen & Ozkaya, 2022).

A prime example of a gluten-free alternative is bread. Traditional white bread depends on the presence of gluten-forming proteins, specifically glutenin and gliadin, to impart desirable rheological properties to the bread. Gluten proteins are restricted to the grains of wheat, species of the genus Triticum (Shewry, 2019). However, gluten-free dough lacks elasticity, resulting in a crumbly texture and lighter colour. To improve gluten-free bread quality, various ingredients, both synthetic and natural, are added (Houben et al., 2012). These include hydrocolloids of cereal and non-cereal origins, seeds, and modified proteins (Šmídová & Rysová, 2022).

In gluten-free products, starch enhances various properties, such as structure and moisture retention (Horstmann et al., 2017). In this study, potato starch was chosen for its texture-enhancing properties and is incorporated into gluten-free brown rice bread. Brown rice, preferred over white rice, offers more nutrients, including fibre and minerals, due to its bran layer (Wu et al., 2016).

Research on seaweed, particularly Kappaphycus alvarezii (KA), has gained traction due to its agar-forming properties and health benefits. Seaweed is a staple food in many Asian diets, and used in many different traditional dishes and prepared in various methods, including drying for chips or incorporation into biscuits (O'Conner, 2017). KA, one of the most cultivated seaweeds globally, is a significant source of carrageenan, a hydrocolloid used in the food industry (FAO, 2018). While KA powder's potential as a food ingredient remains underexplored (Rudke et al., 2020; Aganduk et al., 2023), its inclusion in the study is expected to yield firmer bread due to carrageenan's presence. The study aims to develop gluten-free bread using brown rice-potato starch flour with KA incorporation and evaluate its physical properties, sensory acceptance, and nutrient composition.

2. Materials and Methods

2.1 Seaweed Powder Preparation

Fresh cultivated red seaweed, KA, brown tambalang variety were harvested by the Sabah Fisheries Department's seaweed farmers from Silungan Island, Semporna, Sabah, Malaysia around October 2022. The seaweed was then packed for transport in cooling storage and brought to Universiti Malaysia Sabah. The preparation of seaweed powder starts with cleaning the seaweed using distilled water to remove sand and debris. The seaweed was immediately subjected to drying in a drying cabinet oven (Termoline, TD-78T-SD) at 40°C for 72 hours. The seaweeds were then put through a universal grinder (Ban Hing Machinery CS-18, China/2850 rpm/1 hour) and ground into powder of approximately 125 µm particle size and further stored in a zip-lock bag (Glad®, Thailand) until needed (Neoh et al., 2021; Matanjun et al., 2010).

2.2 Gluten-free Bread Incorporated with KA Preparation

The gluten-free brown rice bread was prepared based on the formulation by Chase (2014) with slight modifications based on a preliminary study (unpublished data). The formulations comprised a blend of brown rice flour (Clean Eating, Malaysia), potato starch (E&G, Malaysia), and seaweed powder (Table 1). The other ingredients were calculated using Baker's percentage and are given as such relative to the weight of the flour: 3.6% sugar (CSR, Malaysia), 0.4% salt (Adabi, Malaysia), 7% eggs (QL, Malaysia), 3.6% sunflower oil (Sunlico, Malaysia), 0.7% instant yeast (Nona, Malaysia), 0.7% Xanthan gum (Keto Essentials, Malaysia), and 34% water. The baker's percentage can be calculated as the weight of the ingredient divided by the weight of the flour and then multiplied by 100%.

The bread-making process starts by separating the dry ingredients and wet ingredients. The dry ingredients include brown rice flour, potato starch, KA seaweed powder, sugar, salt, instant yeast, and xanthan gum while the wet ingredients include eggs, oil, and water. The dry ingredients were added into a mixing bowl and mixed to ensure all the ingredients were incorporated well using a mixer (KitchenAid

Inc., United States) fitted with a paddle attachment. The wet ingredients were combined in a separate mixing bowl, whisked and then added to the dry ingredients. The mixture was then mixed on low speed (speed 2) until it formed a loose batter. The loose batter was then transferred into a load pan of size 25cm x 15cm x 6cm (length x breadth x height) that had been greased with oil. The loaves were left to rise (35°C) for an hour. The loaves were then placed in the oven (Bakbar, New Zealand) on the middle rack and baked for an hour at 180°C.

Table 1 The amount of composite flour of brown rice flour, potato starch, and seaweed Kappaphycus alvarezii (KA) powder by percentage of weight.

Formulation	Ingredients (%)			Total flour (%)
	Brown rice flour	Potato starch	KA powder	
F0	70	30	0	100
F1	69	29	2	100
F2	68	28	4	100
F3	67	27	6	100
F4	66	26	8	100
F5	65	25	10	100

2.3 Bread Physical Characteristics

Bread physical characteristics that were determined were the loaf specific volume, crumb colour, and texture. These measurements were made two hours after the bread was removed from the oven. The physical properties of bread are conducted as soon as they are baked because they tend to stale easily and the staling process may affect the physical qualities of bread including developing a dense harder crumb (Chinachoti & Vodovotz, 2018).

2.3.1 Loaf Volume

The loaf volume of the bread was determined by the rapeseed displacement method based on the AACC method 10-91 (AACC, 2000). For this study, rapeseed was replaced with green beans, and the specific volume of each sample was obtained through calculation, dividing the volume by the loaf mass (Sangnark & Noomhorm, 2004).

2.3.2 Crumb Colour

The crumb colour was determined using a HunterLab Colorflex EZ (HunterLab, United States of America). The aspects of colour measured are lightness (L), redness (a), and yellowness (b). Each sample loaf was cut into slices about 2.5 cm in thickness and then crushed into crumbs. Five samples from the middle of the bread slice were taken and measured in their colour aspect and the average of the 5 samples was the representation of the colour measurement (Mamat et al., 2014).

2.3.3 Bread Texture

Bread texture was measured using a texture analyser (TA-XT2) based on the method of AACC (Method 74-09 (AACC, 1986)). Bread samples were sliced into slices of similar thickness (approximately 1.25cm) excluding the first slices from either end of the bread. Two slices were placed on top of each other and stacked and the amount of force needed to compress 25% of its original height was measured. The cylindrical probe used was of 7.5 cm diameter, pre-testing was at the speed of 2 mm s^{-1} and post-testing was at the speed of 10 mm s^{-1}. The actual testing speed was 1.7 mm s^{-1}. The measurements were triplicated for each bread sample with each maximum peak force value recorded and the force needed to compress 25% was the average of the triplicate results.

2.4 Sensory Evaluation

Two sensory analyses were conducted within 24 hours of bread baking. Bread samples were sliced into uniform sizes before being presented to the panellists. The first sensory test was an 8-point descriptive test with a total of 50 panellists. The characteristics measured in the sensory test are the intensity of seaweed that affects the texture, taste, after-taste, smell, colour, and overall acceptance. A score of 8 shows a strong intensity of seaweed presence while a score of 1 shows the opposite, which is not noticeable (1 = not noticeable, 2 = trace or not sure; 3 = faint; 4 = slight; 5 = moderate; 6 = definite; 7 = strong; 8 =very strong).

A 9-point hedonic scale test was also conducted at the same time as the descriptive test. Characteristics measured in the sensory test were how acceptable the texture, taste, aftertaste, smell, colour as well as the overall acceptance of the seaweed bread. A score of 9 shows a strong liking while a score of 1 will show the opposite, which is strong disliking (1 = dislike extremely, 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much, 9 = like extremely) (Lawless & Heymann, 2010). From the hedonic test, the best formulation was selected for proximate and dietary fibre content analysis.

2.5 Nutrient Analysis

The proximate composition of gluten-free bread of the control (F0) and best formulation (F1) from the sensory evaluation was evaluated according to AOAC (2013). The moisture content was determined by drying the sample in a universal oven at 105°C overnight (Binder FED 56, Germany) until constant weight. The ash content was determined after the sample was incinerated at 550°C overnight in a furnace (Carbolite, ELF, Germany). The fat content was determined by the Soxhlet extraction using petroleum ether (FOSS Soxtec™ 2050, Denmark). The crude protein content was determined using the Kjeldahl method in a Kjeldahl analyser (Kjeltec, Foss, Denmark), and was calculated using the conversion factor of N=6.25. The carbohydrate was calculated from the sum of the percentages of moisture, protein, ash, fat and dietary fibre subtracted from 100 based on the Malaysian Guide for Nutrition Labelling and Claims (Ministry of Health Malaysia, 2010). The enzymatic gravimetric method, which is based on AACC, 32.07.01 (AACC, 2000) was used to determine the dietary fibre content.

2.7 Statistical Analysis

Statistical analysis was performed by using one-way ANOVA. The comparison of data was based on Tukey's significant difference test ($p<0.05$). All data were presented as mean and standard deviation. All statistical analysis used the software of Statistical Package for Social Science (SPSS) version 28.

3. Results and Discussion

3.1 Bread Physical Analysis

The appearance of gluten-free brown rice bread with and without incorporating KA powder is shown in Figure 1.

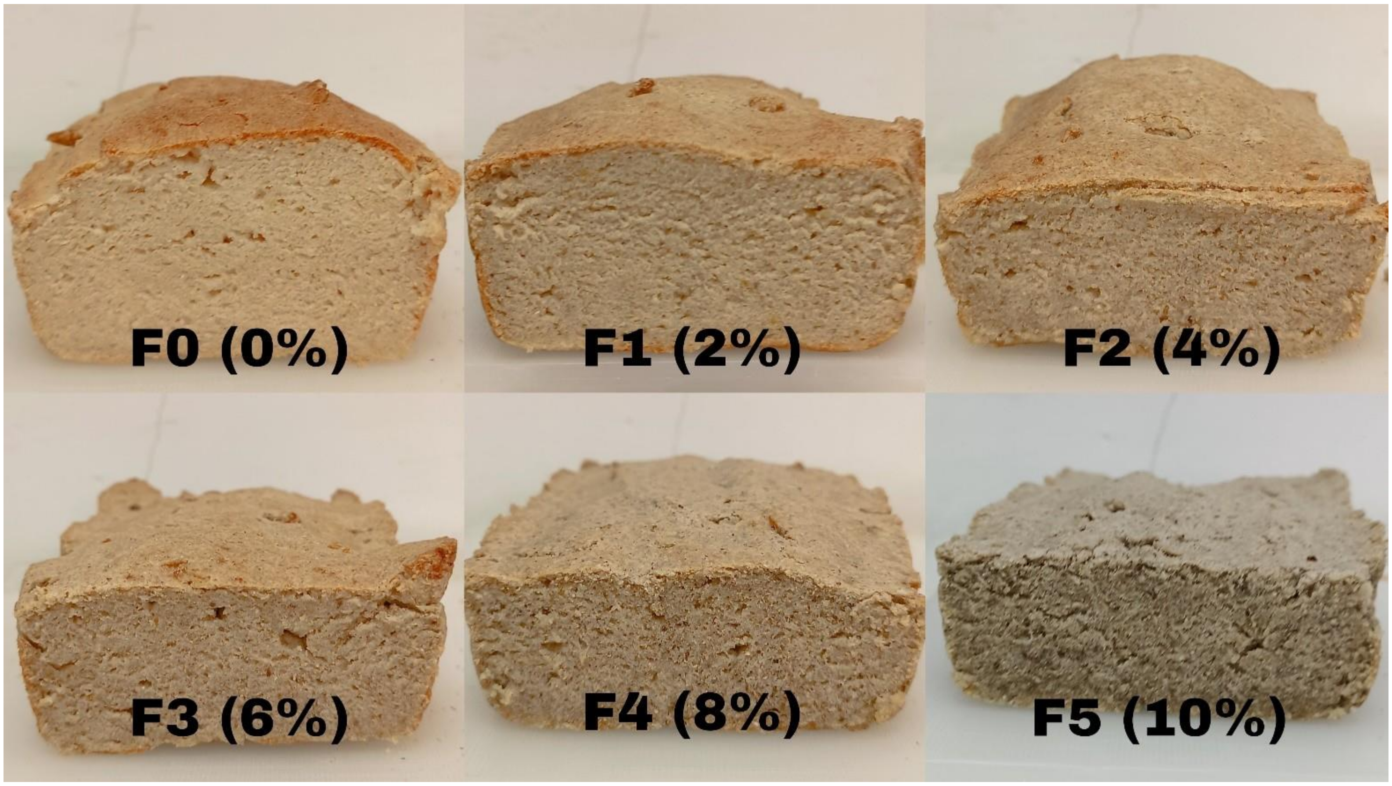

Figure 1 Gluten-free brown rice-potato starch bread without seaweed (F0) and with seaweed Kappaphycus alvarezii (KA) of various concentrations (F1=2%, F2=4%, F3=6%, F4=8%, F5=10%).

3.1.1 Loaf volume

The loaf volume of the control and all formulations of KA-incorporated brown rice-potato starch loaves of bread are presented in Table 2. Loaf volume measurements for bread are important as they reflect how dense the bread is. The results show that the loaf volume of brown rice bread incorporated with KA powder decreased significantly ($p<0.05$) with increasing KA powder.

Table 2 Loaf volume of gluten-free brown rice-potato starch bread incorporated with seaweed Kappaphycus alvarezii.

Formulation	Loaf Volume (cm^3)
F0	$953.33±2.89^{a}$
F1	$946.67±2.89^{ab}$
F2	$945.00±5.00^{ab}$
F3	$938.33±2.89^{b}$
F4	$891.67±2.89^{c}$
F5	$806.67±2.89^{d}$

Values are expressed as mean ± standard deviation, n=6
Values in the same column with different superscripts are significantly different ($p<0.05$)
F0=0%, F1=2%, F2=4%, F3=6%, F4=8%, F5=10% seaweed powder

Among the formulations with KA seaweed added, the formulation with the least seaweed (2%) had the highest volume while the formulation with the most seaweed (10%) had the lowest volume. The amount of seaweed significantly ($p<0.05$) influenced the loaf volume, with F5 (10%), the highest concentration of seaweed, having the highest reduction of volume of 15.38% compared to the control F0 (without seaweed). This study showed that loaf volume is affected by the quantity of KA seaweed powder added to the flour used for bread making. This could be attributed to seaweed powder having no gluten content and thus upon hydration, it is unable to produce the cohesive elastic dough that is capable of forming the open foam structure of bread (Sciarini et al., 2010). This is aligned with the study by Onyango et al. (2021), where wheat bread was substituted with KA resulting in bread with lower specific volumes. According to Onyango et al. (2021), the decrease in loaf volume could also be due to the water retention ability of kappa-carrageenan, which is found abundantly in KA. With kappa-carrageenan retaining water during baking, it also retains the steam produced while baking disrupting the formation of bread volume.

3.1.2 Crumb Colour

The crumb L, a, and b values were measured (Table 3), and it is evident that the L, a, and b values were affected significantly ($p<0.05$) by the amount of KA powder added. In terms of L values, the control (F0) had the highest value of 75.49 while the formulation with 10% seaweed had an L value of 63.80.

Table 3 Crumb colour of gluten-free brown rice-potato starch bread incorporated with seaweed Kappaphycus alvarezii.

Formulation	Parameter		
	Brightness (*L)	**Redness (*a)**	**Yellowness (*b)**
F0	75.49 ± 0.01^{a}	4.65 ± 0.02^{a}	20.15 ± 0.02^{a}
F1	73.71 ± 0.01^{b}	4.03 ± 0.02^{bc}	19.47 ± 0.02^{b}
F2	70.87 ± 0.02^{c}	4.10 ± 0.06^{b}	19.13 ± 0.05^{c}
F3	68.88 ± 0.02^{d}	3.71 ± 0.07^{e}	18.20 ± 0.07^{e}
F4	66.25 ± 0.01^{e}	3.89 ± 0.01^{d}	18.19 ± 0.01^{e}
F5	63.80 ± 0.01^{f}	3.94 ± 0.02^{cd}	18.73 ± 0.03^{d}

Values are expressed as mean ± standard deviation, n=6
Values in the same column with different superscripts are significantly different ($p<0.05$)
F0=no incorporation of seaweed powder, F1=2%, F2=4%, F3=6%, F4=8%, F5=10%

The crumb colour results show that with the increasing addition of seaweed, there is a decrease in the brightness of the bread. The darker crumb colour could be due to the addition of the red seaweed powder which contains phycobiliprotein pigment naturally found in KA. This phycobiliprotein is the protein pigment responsible for giving the seaweeds their signature brownish-red hue. Yellowness or b value of the bread also shows a decrease with increasing concentration of seaweed powder added. These results are in line with the study by Mamat et al. (2014) and Onyango et al. (2021), where there was a significant decrease in brightness and yellowness with increasing KA powder added to wheat bread formulations.

3.1.3 Bread Texture

TPA analysis such as hardness, cohesiveness, elasticity, and chewiness are usually the most important parameters to look for in bread products (Rahman et al., 2021). The results of the bread's physical texture analysis are shown in Table 4.

Table 4 Texture profile analysis of gluten-free brown rice-potato starch incorporated with seaweed Kappaphycus alvarezii.

Formulation	Parameters			
	Hardness (g)	**Chewiness**	**Cohesiveness**	**Resilience**
F0	117.33±11.44^{c}	0.43±0.12^{a}	0.63±0.03^{a}	0.45±0.09^{a}
F1	119.57±21.80^{c}	0.45±0.11^{a}	0.64±0.32^{a}	0.37±0.01^{a}
F2	121.03±26.73^{c}	0.68±0.21^{a}	0.66±0.74^{a}	0.37±0.07^{a}
F3	150.27±15.55^{bc}	0.58±0.32^{a}	0.67±0.05^{a}	0.34±0.05^{a}
F4	176.63±12.35^{b}	0.69±0.37^{a}	0.71±0.03^{a}	0.33±0.22^{a}
F5	235.53±10.01^{a}	1.07±0.71^{a}	0.76±0.12^{a}	0.32±0.06^{a}

Values are expressed as mean ± standard deviation, n=6
Values in the same column with different superscripts are significantly different ($p<0.05$)
F0=no incorporation of seaweed powder, F1=2%, F2=4%, F3=6%, F4=8%, F5=10

Table 4 showed a significant increase ($p<0.05$) in the hardness of bread with a higher concentration of seaweed in the formulation. This increase could be due to the KA powder, which contains kappa-carrageenan, a hydrocolloid with the ability to absorb and retain water as well as promote starch recrystallization, even after baking as steam is trapped during baking and hardening the crumb structure (Onyango et al., 2021). There was no significant difference ($p>0.05$) in chewiness, cohesiveness and resilience among the formulations with the control.

3.2 Sensory Evaluation

3.2.1 Descriptive Test

Table 5 shows the descriptive test and this test is important to provide a qualitative measurement of the intensity of the seaweed effect on the bread's sensory attributes.

Table 5 Descriptive sensory analysis scores of gluten-free brown rice-potato starch bread incorporated with seaweed Kappaphycus alvarezii.

Formulation	Parameters				
	Colour	**Aroma**	**Taste**	**Texture**	**Aftertaste**
F0	4.48±2.36^{c}	4.38±1.85^{b}	4.04±1.81^{c}	4.60±1.70^{c}	4.06±1.97^{b}
F1	4.82±1.79^{bc}	4.86±1.71^{ab}	4.96±1.80^{bc}	5.18±1.84^{ab}	4.82±1.80^{ab}
F2	5.32±1.42^{abc}	5.14±1.39^{ab}	5.16±1.63^{ab}	5.50±1.62^{ab}	5.36±1.48^{a}
F3	5.70±1.61^{ab}	5.28±1.49^{ab}	5.70±1.58^{ab}	5.42±1.46^{ab}	5.34±1.53^{a}
F4	5.58±1.58^{ab}	5.22±1.69^{ab}	5.64±1.61^{ab}	5.62±1.55^{a}	5.34±1.73^{a}
F5	5.96±1.64^{a}	5.50±1.66^{a}	6.08±1.43^{a}	5.92±1.55^{a}	5.46±1.96^{a}

Values are expressed as mean ± standard deviation, n=50
Values in the same column with different superscripts are significantly different ($p<0.05$)
F0=no incorporation of seaweed powder, F1=2%, F2=4%, F3=6%, F4=8%, F5=10%
(1 = not noticeable, 2 = trace or not sure; 3 = faint; 4 = slight; 5 = moderate; 6 = definite; 7 = strong; 8 =very strong)

In terms of colour, F2, F3, F4 and F5 showed a mean score of 5 which indicated a moderate intensity of seaweed colour in the bread. This increase in seaweed intensity on the colour of bread is due to increasing concentrations of phycobiliprotein pigment with increasing KA added. This pigment, especially abundant in red seaweeds is the cause of some wheat bread's reduction in brightness and yellowness while baking (Mamat et al., 2014). The results can also be supported by the results of the crumb colour analysis where there was a significant difference ($p<0.05$) in the brightness and yellowness of the crumb where increasing seaweed content, decreased both of these colour parameters.

The intensity of the seaweed aroma is not significantly different ($p>0.05$) between formulations. The concentration of seaweeds may not influence the aroma of the bread as much after baking. However, a trend of increasing mean score is seen in linear to increasing concentrations of seaweed. In a study conducted by Hall et al. (2010), the findings were similar where there was no significant difference in aroma between seaweed-enriched bread and the control. This could be an effect of using brown rice flour when baking that gives out a strong aroma that could mask the seaweed smell (Nespeca, 2020).

In terms of the intensity of seaweed in taste, as the concentration of KA powder rises, there is a significant ($p<0.05$) increase in the intensity of KA taste in the bread when comparing F0 (control) and F5 (10%). The intensity of a seaweed-y taste increases as concentration increases indicating that the concentration is linear to the intensity of seaweed taste in the bread. These results are similar to the study by Mamat et al. (2018), where the sensory evaluation of seaweed muffins also showed that the quality of the taste of muffins decreased with increasing percentages of seaweed powder.

The panellists were able to differentiate textures of F0 (control) and F5 (10%), ranking the latter as harder than the control, an effect of the addition of seaweed powder in the formulation. Since KA is known for its high kappa-carrageenan content that has gelling abilities, its presence is hypothesised to affect the final texture of the bread.

The results showed there was a moderate intensity of seaweed in the aftertaste of the bread during the sensory evaluation. This result is significantly ($p<0.05$) different than the control F0 (control) which only showed a mild intensity. Seaweed in general is known to have a very distinctive aftertaste, and it can be inferred that a higher concentration of seaweed imparted more aftertaste on the bread.

3.2.2 Hedonic Test

A hedonic 9-point test was conducted with the participation of 50 panellists. The result of this test is shown in Table 6.

Table 6 Hedonic acceptability sensory analysis scores of gluten-free brown rice-potato starch bread incorporated with seaweed Kappaphycus alvarezii.

Formulation	Attributes					
	Colour	**Aroma**	**Taste**	**Texture**	**Aftertaste**	**Overall Acceptance**
F0	$7.02±1.57^{a}$	$6.90±1.53^{a}$	$5.82±1.95^{a}$	$5.88±1.95^{a}$	$6.08±1.79^{a}$	$6.26±1.77^{a}$
F1	$7.06±1.43^{a}$	$6.44±1.55^{ab}$	$5.62±2.01^{a}$	$5.96±1.82^{a}$	$5.80±1.75^{ab}$	$6.28±1.68^{a}$
F2	$6.44±1.45^{ab}$	$6.04±1.43^{abc}$	$5.18±2.01^{ab}$	$5.44±1.85^{a}$	$5.34±1.83^{abc}$	$5.66±1.64^{ab}$
F3	$6.04±1.84^{bc}$	$5.70±1.69^{bc}$	$5.50±1.87^{ab}$	$5.38±1.76^{a}$	$5.42±1.79^{abc}$	$5.76±1.66^{ab}$
F4	$5.70±1.78^{bc}$	$5.46±1.79^{c}$	$5.14±2.04^{ab}$	$4.88±1.92^{a}$	$4.94±1.94^{bc}$	$5.18±1.78^{bc}$
F5	$5.44±2.01^{c}$	$5.18±1.69^{c}$	$4.40±1.96^{b}$	$5.42±7.69^{a}$	$4.42±1.76^{c}$	$4.56±1.86^{c}$

Values are expressed as mean ± standard deviation, n=50
Values in the same column with different superscripts are significantly different ($p<0.05$)
F0=no incorporation of seaweed powder, F1=2%, F2=4%, F3=6%, F4=8%, F5=10%
(1 = dislike extremely, 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much, 9 = like extremely)

Colour is an important attribute in the sensory evaluation of a food product because not only does it influence the initial impression before consumption but also serves to entice the consumer to consume the food product. In combination with results from the crumb colour analysis indicated that a brighter colour crumb may be preferred among the panellist and increase their likeability. This is evident when comparing panellists ranking the intensity of seaweed in bread in the descriptive test. From the two sensory analyses, it is evident with a higher intensity of seaweed colour affecting the bread, the likeability of the panellist towards the bread decreases. According to Mollakhalili-meybodi (2023), the colour of baked goods is an important factor in consumer acceptability as it is often associated with the freshness of the bread. A brighter colour of bread is usually a sign it is fresher and more delicious.

Aroma is also one of the main attributes contributing to the likeability of a food product. Aroma imparts flavours in the form of smell, and it has been frequently made known that around 75% to 95% of taste is affected by smell (Spence, 2015). The results showed that the likeability of aroma attribute for F0 (control), F1 (2%) and F2 (4%) is higher, scoring a mean score of 6 on the hedonic scale while F4 (8%) and F5 (10%) significantly ($p<0.05$) scored lower. KA has a very distinct fishy aroma. This odour in seaweed is due to the presence of amines and other specific compounds such as dimethyl sulfide (DMS). DMS when present in small amounts gives a pleasant smell of the sea, but when available in large amounts, it can form a disagreeable smell that is not favourable in food products (Mouritsen, 2013).

There is no significant difference ($p>0.05$) in likeability in terms of the texture of the bread between formulations. This indicates that even though there is a significant difference in terms of texture, especially in hardness results from the TPA analysis due to increasing seaweed concentration, it does not affect the likeability of the bread in the panellist. The panellist still scores it as "neither like nor dislike". There is a significant difference in the average aftertaste likeability with F5 scoring lower (dislike slightly) than F0 (like slightly). The results of the aftertaste parameter showed that the increasing amount of KA powder in the formulation affected the likeability of the bread. There is a significant difference in the average aftertaste likeability with F5 scoring lower (dislike slightly) than F0 (like slightly). The results of the aftertaste parameter showed that the increasing amount of KA powder in the formulation affected the likeability of the bread.

The overall acceptance mean score showed that F0 (control) and F1 (2%) scored better and ranked as "liked slightly" in terms of overall acceptance on the hedonic scale. The overall acceptance of KA-incorporated brown rice-potato starch bread could mainly be based on its colour, aroma and aftertaste as these parameters showed some significant differences between scores of likeability and acceptance. This result is aligned with a review by Mollakhalili-meybodi (2023) which states that the odour and colour of baked goods ultimately give consumers a guess about their quality and affect how they accept the product.

3.3 Selection of Best Formulation

From the sensory evaluation results, the best formulation is F1, the formulation which contains 2% KA powder. As well as being the best formulation in terms of overall acceptance, F1 (2%) also showed that it is the most favoured in terms of colour and texture, and second only to the control (without seaweed) in terms of aroma, taste and aftertaste. Nutrient analysis was conducted on F1 and control (F0) for comparison.

3.4 Nutrient Analysis

Table 7 shows the nutrient values of KA-incorporated gluten-free brown rice-potato starch bread compared to the control. There is a significant difference ($p<0.05$) between F0 (control) and F1 (2%) in moisture, crude protein, crude fat, ash, dietary fibre and carbohydrate content.

Table 7 Nutrient composition of gluten-free brown rice-potato starch bread incorporated with seaweed Kappaphycus alvarezii (KA).

Sample	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Ash (%)	Dietary Fibre (%)	Carbohydrate (%)
F0	38.68±1.84^{b}	5.36±0.34^{b}	1.91±0.67^{b}	1.17±0.10^{b}	6.17±0.15^{b}	46.71±1.85^{a}
F1	42.07±1.62^{a}	5.15±0.71^{a}	3.45±0.24^{a}	1.43±0.11^{a}	6.77±0.06^{a}	41.13±1.67^{b}

Values expressed as mean ± standard deviation, n=3
Values in the same column with different superscripts are significantly different (p<0.05)
F0=0% KA powder, F1=2% KA powder

F1 (2%) has significantly (p<0.05) higher moisture content compared to the control (without seaweed). This increase in moisture may be due to the high dietary fibre amounts due to the incorporation of KA powder (Maneju et al., 2011; Akhtar et al., 2008). Dietary fibre has high water-holding capacity and with the high amount of dietary fibre present in seaweed composite flours, it is not surprising that it can hold more moisture. The moisture present is bonded to hydroxyl groups existing within the dietary fibre structures (Mamat et al., 2014).

There is a significant (p<0.05) decrease in crude protein content in F1 as the main sources of protein in the formulation are brown rice, potato starch and egg. The brown rice flour and potato starch are part of the composite flour that when added with seaweed powder, decreased in volume which may be the main cause of a decrease in protein in the bread. F1 with 2% KA powder had a higher fat content compared to the control. This difference could be attributed to fat already present in the KA powder before mixing and baking. The main source of fat comes from the vegetable oil in both formulations, however, with the addition of KA powder, the fat amount increases. F1 has a significantly (p<0.05) higher ash content compared to the control. This could indicate a higher mineral composition in the batter due to the addition of KA powder. Ash content directly reflects the total amount of minerals in food (Park & Bell 2004).

F1 has a significantly (p<0.05) higher dietary content compared to the control. This result is consistent with several studies that show seaweeds have a high amount of dietary fibre in their composition which ranges from 33-75% (Ahmad et al., 2012; Matanjun et al., 2009). This can also be supported by several studies reporting an increase in dietary fibre content when added to different food products. Such examples are the incorporation of red seaweed Porphyra columbina into extruded maize products (Cian et al., 2014) and P. umbilicalis meat products (Cofrades et al., 2011). A study by Sholichah et al. (2021) also showed that the incorporation of KA into gluten-free pasta showed a significant increase in total dietary fibre. KA being rich in dietary fibre is no exception as many studies have proven that lred seaweed is abundant with this nutrient.

The substitution of the composite rice flour-potato starch with seaweed powder significantly (p<0.05) decreased the carbohydrate content in the bread. Brown rice, being the main source of carbohydrates in the bread, when reduced will affect the total carbohydrate in the bread. This would suggest that F1 (2%) has less energy content due to less carbohydrate available. This is in line with the study done by Makinde and Akinoso (2014), where the reduction of flour in bread formulation showed a significant decrease in carbohydrates.

4. Conclusion

The current study demonstrated that an increase in KA powder led to a reduction in loaf volume. Concerning crumb colour, there were significant (p<0.05) decreases in the L* (brightness) and b* (yellowness) values with an increase in KA powder added. TPA analysis indicated that the gluten-free bread's hardness increased with higher KA incorporation. Sensory evaluation results identified F1, comprising 2% KA powder, as the best formulation for gluten-free brown rice-potato starch bread. Nutrient analysis revealed that KA-incorporated brown rice-potato starch bread exhibited higher nutrient content compared to the control (without seaweed). Therefore, the inclusion of KA powder not only renders brown rice-potato starch bread gluten-free but also positions it as a more nutritious alternative, having higher ash, moisture, fat and dietary fibre content than the control.

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