

RESEARCH ARTICLE

GLUTEN-FREE BISCUIT PREPARED USING JACKFRUIT (*Artocarpus heterophyllus* L.) SEED FLOUR: AN APPROACH TOWARDS UTILIZATION OF UNEXPLOITED FOOD SOURCE

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ABSTRACT

Jackfruit (*Artocarpus heterophyllus* L.) seeds are an underutilized, readily available source in Sri Lanka that encounters a huge postharvest loss. Jackfruit seeds are a significantly rich source of gluten-free (GF) flour that can be identified as a potential flour source for producing GF flour confectionaries. The present study evaluated the potential of using jackfruit seed flour (JSF) in GF biscuit production. Jackfruit seeds were processed into flour. Several sets of biscuit samples were prepared by incorporating JSF with three flour types: finger millet flour (FMF), white rice flour (WRF) and red rice flour (RRF). In addition, 100% JSF biscuits were prepared. The most accepted product was selected through a series of sensory evaluations and the physicochemical, nutritional, microbial, and sensory properties were evaluated. 100% JSF biscuit was selected as the most accepted biscuit. According to proximate analysis, 100% JSF biscuit contained $3.88 \pm 0.16\%$ moisture, $5.93 \pm 0.55\%$ crude proteins, $1.2 \pm 0.02\%$ crude ash, $18.31 \pm 0.62\%$ fat, $3.1 \pm 0.04\%$ crude fiber and $66.94 \pm 1.36\%$ total carbohydrates. The sugar content was $40.3 \pm 0.3\%$, and the salt content as sodium chloride was $0.57 \pm 0.01\%$. Dietary fiber content was detected as $4.1 \pm 0.02\%$. According to the study, JSF was identified as a successful substitute for wheat flour in biscuit production.

Keywords: Biscuits, Gluten-free, Jackfruit seed flour, Underutilized, Value-added

INTRODUCTION

Biscuits are one of the daily consumed flour confectioneries and are favored worldwide (Nilugin *et al.*, 2015). Some reasons for the wide popularity are their ready-to-eat nature, affordable cost, decent nutritional quality, assortment, and longer shelf life (Arepally *et al.*, 2020; Naseeha *et al.*, 2023). Biscuits are flour-based baked food products that are usually flat and unleavened. The perpetual main ingredient of biscuits is wheat flour (Rao *et al.*, 2018).

Celiac disease is an auto-immune, chronic disease that occurs in the gastrointestinal tract. The prime characteristic of celiac disease is permanent gluten intolerance. A strict gluten-

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free (GF) diet is considered the only available countermeasure for celiac disease (Aljada *et al.*, 2021). Various GF products have been introduced to the market to aid the community with celiac disease. The demand for GF goods is growing, and the range of products offered to consumers is widespread. Nevertheless, the need to enhance the quality of GF goods is often highlighted (Cairano *et al.*, 2018). Though the global demand for GF bakery products, including biscuits, is high, the Sri Lankan market for GF products is still in its infancy.

Jackfruit (*Artocarpus heterophyllus* L.) belongs to the family Moraceae and is abundantly found in most parts of South Asia,

including Sri Lanka. Jackfruit is a multiple fruit having edible bulbs and seeds surrounded by the rind. It comprises hundreds of berries rich in carbohydrates, complex B vitamins, minerals, and phytonutrients (Jagdale *et al.*, 2021). Unripe jackfruit bulbs are usually cooked, and ripe bulbs are consumed fresh or processed into snacks, juices, jam or canned fruits. Roasted, boiled, or steamed seeds are usually consumed as a snack (Madruga *et al.*, 2014). Nonetheless, most of the seeds are discarded as a waste. Jackfruit seeds are an underutilized rich source of starch that has the potential to be used in different food applications, such as flour confectionary and the bakery industry (Akter & Haque, 2018; Kaushalya & Wijesinghe, 2022).

Altogether, the ultimate objective of this study was to formulate a GF biscuit using jackfruit seed flour (JSF) to add value to unexploited jackfruit seeds and to evaluate the physicochemical, nutritional, microbial, and sensory properties of the formulated biscuit. Finger millet (*Eucenea coracana*) flour and rice (*Oryza sativa* L.) flour (white rice flour and red rice flour) were used to incorporate with jackfruit seed flour during the formulation of GF biscuits.

MATERIALS AND METHODS

Jackfruit seeds were collected from the Welimada area in Badulla district, Sri Lanka, on the prior day of seed flour preparation. All the other ingredients, finger millet flour, white rice flour, red rice flour, margarine, vanilla extract, baking powder and eggs, were purchased from local supermarkets in Sri Lanka.

All the other chemicals and reagents used during the study were of standard analytical grade.

Preparation of Jackfruit seed flour (JSF)

JSF was prepared using a previously described method with slight modifications (Maduwage *et al.*, 2019). In brief, good quality jackfruit seeds from mature unripe and mature fully ripened fruits (with yellowed arils, flattened spikes, and aromatic odor) were collected. The rinds were removed. Lye peeling was used to

remove the testa (seed coat) of jackfruit seeds. Accordingly, seeds were soaked in 5% sodium hydroxide (m/v) solution for 2 minutes and then soaked in 5% citric acid (m/v) solution for another 2 minutes. Then, the seeds were washed thrice with potable tap water until the brown seed coat was removed. Excess water was allowed to drain off. The lye-peeled jackfruit seeds were sliced and weighed. Seed slices were then steam blanched for 4 – 5 minutes. Blanched slices were immediately dipped in cold water, and excess water was allowed to drain off. Next, the slices were laid on cotton clothes, keeping the cut surface exposed to the hot air, and dried at 55°C for 18 hours. The dried jackfruit seed slices were ground using an electric mixer grinder (PHILIPS Classic, HL1606) and sieved through a 100µm standard sieve to get a fine flour. Flour was packed in triple laminated pouches and stored in a cool and dry place until further use.

Determination of physicochemical properties of JSF

Yield Percentage

The weights of fresh sliced jackfruit seeds (W1) and the weight of sieved JSF (W2) were measured. The yield percentage of JSF was calculated by using the equation 01 (Mog & Fox, 1991; Noor, 2014).

$$\text{Yield percentage (\%)} = \frac{W_2}{W_1} \times 100\%$$

...Equation 01

Where W1: weight of fresh sliced jackfruit seeds and W2: weight of sieved JSF

Moisture content

The moisture content of JSF was determined according to the method described in AOAC 925.10 using the moisture analyzer.

Water activity (a_w)

The water activity was measured according to the AOAC 978.18 modified method using the dew point water activity meter.

Formulation of biscuits

Biscuits were first formulated by incorporating JSF with finger millet flour

(FMF), white rice flour (WRF), and red rice flour (RRF) in three different flour ratios of JSF (25%, 50%, and 75%). In addition, biscuits with 100% JSF were developed. Formulations are given in Table 1.

Table 1: Flour combinations used for biscuit preparation

Incorporations	JSF	FMF	WRF	RRF
JSF	100%	-	-	-
JSF + FMF	75%	25%	-	-
	50%	50%	-	-
	25%	75%	-	-
JSF + WRF	75%	-	25%	-
	50%	-	50%	-
	25%	-	75%	-
JSF + RRF	75%	-	-	25%
	50%	-	-	50%
	25%	-	-	75%

JSF: Jackfruit seed flour; FMF: Finger millet flour; WRF: White rice flour; RRF: Red rice flour

Each biscuit formulation comprised constant amounts of flour, margarine, sugar, egg yolk, sodium bicarbonate and vanilla extract. Sugar was ground into a fine powder using a grinder and mixed with margarine for 5 minutes until it was made into a cream using an electric hand mixer (INNOVEX, IHM001, P.R.C). The egg yolk and vanilla extract was added while mixing. The flour incorporations were made after mixing well with baking powder (Sodium bicarbonate). The flour and the cream mixture were mixed well to form the dough. The dough was sheeted to a uniform thickness of 5mm and cut into shapes using a biscuit cutter of diameter 5cm. Shaped biscuit dough was baked at 165°C for 30 - 40 minutes in an oven. The biscuits were packed in a food-grade resealable multi-layer zip lock standup pouch.

Sensory analysis

A series of sensory evaluations were used to select the biscuit with the best sensory perceptions as the final product and to evaluate consumer acceptability. Appearance, color, aroma, flavor, texture, and overall acceptability of biscuits were assessed using the 9-point hedonic scale, where 9 = extremely like and 1 = extremely dislike.

Fifty untrained panelists equally representing males and females aged 20 - 40 years, and no issues with taste, odor, or flavor ability were chosen. All the sensory evaluations were done on the next day of biscuit preparation, and the samples were coded using 3-digit codes presented at room temperature under normal lighting conditions. A cup of potable water was provided for oral rinsing between evaluations (Jayamali *et al.*, 2022).

Four sensory evaluations were held to select the most accepted biscuit, and another sensory review was held to compare the sensory attributes of the selected biscuit with those of 100% wheat flour biscuits.

Proximate composition analysis

The moisture content, ash, crude protein, crude fat, and crude fiber content of the most accepted biscuit were determined as outlined in the AOAC methods (925.10, 900.02, 930.25, 920.39, and 978.10, 2016, respectively). The total carbohydrate content was determined according to the method described by Akande *et al.*, (2020) using the equation 02.

Total carbohydrate content (%) =

**100 - (% moisture + % ash + % protein
+ % fat + % fiber content)**

... Equation 02

Nutritional composition of the biscuits

Energy

The energy per 100g of the most accepted biscuit was determined according to the Akande *et al.* (2020) by using the equation 03.

Energy (kcal per 100 g) =

**4(% Protein + % Digestible carbohydrates)
+ 9(% Fat)**

... Equation 03

Fat, salt, sugar, and dietary fiber content of the biscuits

The acidity of the extracted fat and fatty acid profile was determined according to SLS 251:2010 and ISO 12966-2:2011(E),

respectively. The total sugar content was determined according to the SLS 586:1982, and salt content as Sodium Chloride was determined according to the ISO 2006a (Volhard method) with slight modifications. The total dietary fiber content of the most accepted biscuit was determined based on the AACC method 32-05.01 and AOAC method 985.29. All the determinations were carried out at room temperature and in triplicate for each parameter.

Physicochemical properties of the biscuits

The spread ratio was determined according to the AACC method, and the pH of the final product was determined according to the AOAC official method. The water activity was measured according to the AOAC modified method using the dew point water activity meter. Color was determined according to Cahyana *et al.* (2020) using the chromameter. The density of the biscuit sample was determined according to Dignity *et al.* (2018) with slight modifications. All the determinations were carried out at room temperature and in triplicate for each tested parameter.

Microbial analysis of the biscuits

The microbiological analyses were carried out using standard plate counts. Total aerobic plate count and yeast and mold count were determined according to SLS 516: Part 1 and SLS 516: Part 2 with slight modifications. Both tests were carried out just after and three months after biscuit preparation. All the microbial analyses were performed in triplicates.

Comparison with Sri Lanka Standards (SLS) for biscuits

Following SLS specifications for biscuits (251:2010), the chemical and microbial requirements to be fulfilled were compared (Specification for biscuits (Second Revision) Sri Lanka Standards Institution, 2010). Chemical requirements, moisture percent by mass and acidity of extracted fat were compared. As microbial requirements, aerobic plate count and yeast and mold count per gram were compared.

Statistical analysis

Data of proximate analysis of JSF biscuits and other physicochemical properties were analyzed using Microsoft Excel 2021. Three replicates of each experiment were done, and the mean values and standard deviations were calculated. Sensory evaluation data were analyzed using the non-parametric Friedman test with a 95% confidence level (5% significance level). Minitab 17 was used to analyze the sensory evaluation data.

RESULTS AND DISCUSSION

Physicochemical Properties of JSF

The percentage yield of JSF was $46 \pm 3\%$ out of cleaned and lye-peeled jackfruit seeds. One of the previous studies reported a comparatively higher yield percentage of JSF (75%), where JSF has gained from jackfruit seeds with testa without undergoing lye peeling (Islam *et al.*, 2015). However, the yield can vary on the botanical properties, maturity level, geographic distribution, maternal origin, growing habitat and agronomic factors of the mother tree (Khang *et al.*, 2020).

The average moisture content of JSF was $8.3 \pm 0.3\%$. Similar values of moisture content of JSF were reported earlier by Maduwage *et al.* (2019) and Kushwaha *et al.* (2021). The lower moisture content of JSF is a crucial indicator of the stability and long shelf life of flour, which ensures the storability and marketing of flour (Thanh *et al.*, 2020). The average water activity (a_w) of JSF was 0.4705 ± 0.04 . Water activity affects the development of microorganisms in food, affecting its storage and quality. The lower water activity of JSF (0.4705 ± 0.04) can be identified as within the safe limits of bacteria, yeast, and micromycetes and ensures the storability, high quality, and safety of prepared JSF.

Sensory Analyses

The results of sensory evaluations were as concluded in Figure 1, and 100% JSF biscuit (Figure 2) was selected as the most accepted biscuit formulation. In the sensory evaluation conducted to compare the sensory properties of 100% JSF biscuit and 100% wheat flour

biscuit, any significant difference in consumer acceptance regarding color, aroma, flavor, texture, and overall acceptability of two biscuits (100% JSF and 100% WF) was not found at 95% significance level ($p < 0.05$). Related to the appearance of biscuits,

there was a significant difference at a 95% significance level ($p = 0.001$), and 100% JSF biscuit was accepted with better appearance as of its higher estimated median. The relevant web diagram is given in Figure 3.

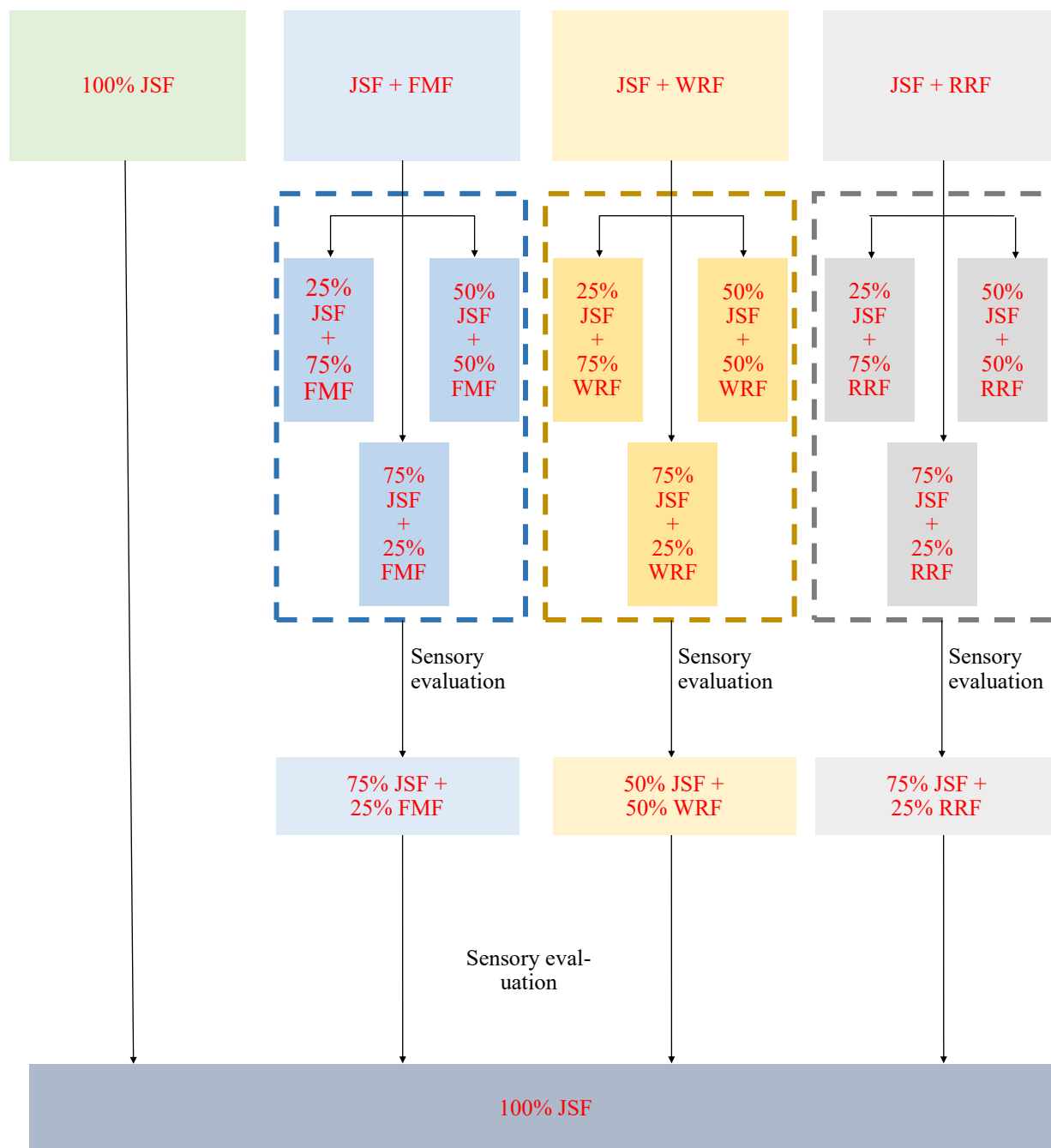


Figure 1: Synopsis of results of sensory evaluations where biscuit prepared from 100% JSF was selected as the final product

JSF: jackfruit seed flour; FMF: finger millet flour; WRF: white rice flour; RRF: red rice flour



Figure 2: Biscuits prepared from 100% JSF

JSF: jackfruit seed flour

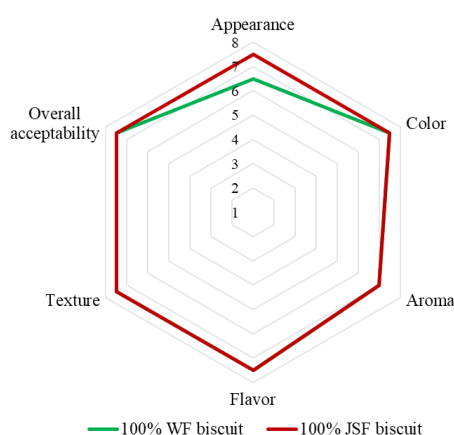


Figure 3: Radar diagram for sensory evaluation for comparing sensory properties of biscuit prepared using 100% JSF and biscuits prepared from 100% WF

WF: wheat flour; JSF: jackfruit seed flour

Proximate Composition of 100% JSF Biscuit

The proximate composition of 100% JSF biscuit and JSF are given in the Table 2. The proximate composition of JSF is compatible with the results of Maduwage *et al.* (2019). According to the results the highest composition was total carbohydrates which represents $67 \pm 1\%$ of the composition of the 100% JSF biscuit. The crude fat content of 100% JSF biscuit was $17.7 \pm 0.6\%$. The fat content of JSF is low which was reported as $2.77 \pm 0.05\%$. Therefore, the contribution of JSF for the fat content of 100% JSF biscuit is lower, and the higher content has come from margarine. According to Maduwage *et al.* (2019) the protein content of JSF was $13.4 \pm 0.09\%$ and the protein content of 100%

JSF biscuit was $5.9 \pm 0.5\%$ (Maduwage *et al.*, 2019). A considerable contribution of proteins from JSF to the 100% JSF biscuit can be expected.

Table 2: Proximate composition of biscuits prepared from 100% JSF and JSF

Parameter	100% JSF biscuit	JSF
Moisture (%)	3.9 ± 0.2^b	8.5 ± 0.2^a
Ash (%)	1.2 ± 0.02^b	2.4 ± 0.3^a
Crude fat (%)	17.7 ± 0.6^a	2.77 ± 0.05^b
Crude protein (%)	5.9 ± 0.5^b	13.4 ± 0.09^a
Crude fiber (%)	3.1 ± 0.04^b	2.9 ± 0.15^a
Digestible carbohydrates (%)	67 ± 1^b	69.9 ± 0.3^a

Values are the average of triplicate analysis \pm standard deviation.

The moisture content of the 100% JSF biscuit was $3.9 \pm 0.2\%$. Baking results the low moisture profile of the biscuit (Arepally *et al.*, 2020). Moisture content of the biscuit is crucial, and it act as an indicator in stability and shelf life of the biscuit. It is also one cognitive factor of the texture of biscuit where lower moisture content is affiliated with more crispiness of biscuits (Pico *et al.*, 2019).

Nutritional Composition of 100% JSF Biscuit

The energy per 100g of the product was 456.19 kcal/100g which represents the calorie amount gained by 100g of the biscuit when oxidized (Banureka & Mahendran, 2009). The acidity of extracted fat indicates the level of rancidity of fat in the biscuit. The acidity of extracted fat of 100% JSF biscuit was $0.4 \pm 0.01\%$, which was below the maximum allowable limit as declared by Specification for Biscuits (Second Revision), Sri Lanka Standards Institution, (251:2010): 1.0% by mass (as Oleic acid). Higher acidity of extracted fat can be a result of using rancid fat sources during processing or if the food product is expired (Duta *et al.*, 2019).

The fatty acid (FA) profile of fat extracted from 100% JSF biscuit is given in Table 3. The highest contribution of fatty acids was from saturated fatty acids, followed by monounsaturated and polyunsaturated fatty acids. The most abundant fatty acids in the

100% JSF biscuit were palmitic, oleic, and lauric (Table 3). Stearic acid, myristic acid and linoleic acid were also present but with lower percentages. Trans fatty acids or any other type of fatty acids were not detected. Palmitic acid is the primary saturated fatty acid found in the biscuit. It imparts the aerated form and smooth texture of biscuits where the functional unit is called β crystals. β Crystals of palmitic acid are stable compounds which enhance the shelf life of the final product (Mamat *et al.*, 2012).

Table 3: Fatty acid profile of biscuits prepared from 100% JSF

Fatty acid (FA)	Group of fatty acids	Composition (%)
Caproic acid	Saturated FA	0.03
Caprylic acid	Saturated FA	0.41
Capric acid	Saturated FA	0.34
Lauric acid	Saturated FA	3.29
Myristic acid	Saturated FA	1.40
Palmitic acid	Saturated FA	5.18
Palmitoleic acid	Monounsaturated FA	0.03
Heptadecanoic acid	Saturated FA	0.01
Stearic acid	Saturated FA	0.71
Oleic acid	Monounsaturated FA	4.88
Linoleic acid	Polyunsaturated FA	1.21
Arachidic acid	Saturated FA	0.04
g-Linolenic acid	Polyunsaturated FA	0.01
Cis-11-eicosaenoic acid	Monounsaturated FA	0.02
Linolenic acid	Polyunsaturated FA	0.02
Cis-11,14-eicosadienoic acid	Polyunsaturated FA	0.01
Behenic acid	Saturated FA	0.01
Methyl cis-8,11,14-eicosatrienoate acid	Polyunsaturated FA	0.01
Cis-5,8,11,17-eicosapentaenoic acid	Polyunsaturated FA	0.01

JSF: Jackfruit seed flour

The total sugar content, which can be defined as the combination of free sugars and naturally occurring sugar, was determined (Bernstein *et al.*, 2016). The sugar content of the biscuit was $40.3 \pm 0.3\%$, which can be expressed as $40.3 \pm 0.3\text{g}$ per 100g of biscuit. The salt content of the biscuit was determined

to be sodium chloride. It was $0.57 \pm 0.01\%$, which can be expressed as 0.57g per 100g of biscuit. The source of salt is the salted fat sources used as an ingredient, as salt was not added externally.

According to the Food Regulations 2019—No 26/1980 (Color Coding for Sugar, Salt, and Fat), 100% JSF biscuits were considered a high-fat product, where a red color code for fat was given, a high-sugar product, where a red color code for sugar was given, and a moderate-salt food, where an amber color code for salt was given in the label.

The dietary fiber content of the 100% JSF biscuit was $4.1 \pm 0.02\%$, which is higher than the commercially available wheat flour biscuits. The higher dietary fiber content of JSF biscuit is associated with the rich dietary fiber content of Jackfruit seeds (Brahma & Ray, 2023). Dietary fibers are responsible for the dough viscosity, hydration, adsorption, and solubility that aid in the success of using JSF in biscuit preparation (Wedamulla & Wijesinghe, 2021).

Physicochemical Properties of 100% JSF Biscuit

The spread ratio of biscuits is an important parameter for packaging biscuits. The spread ratio of a particular type of biscuit should be constant to have all biscuits with uniform shape and size. An inconsistent spread ratio can create trouble in the packaging of biscuits. Standard deviation was used to determine the variability of the spread ratio of biscuits. Accordingly, the standard deviation of 100% JSF biscuit was 0.00, manifesting its suitability over commercial production in terms of uniformity of biscuits and packaging. The spread ratio of 100% JSF biscuits was 4.3 ± 0.00 , where there is a lower spread ratio when compared to wheat flour biscuits in literature. The lower spread ratio could be associated with the higher water holding capacity of JSF and higher content of fiber in JSF, where the bounteous hydroxyl groups in fiber structure aid more water binding through hydrogen bonding (Arpit & John, 2015; Demirkesen, 2016; Khan *et al.*, 2016).

The weight of a biscuit with 5cm diameter and 5mm thickness was 8.3 ± 0.3 g. The average bulk density of 100% JSF biscuit was 0.7 ± 0.1 gcm⁻³. Bulk density is associated with the amount of carbohydrates provided by ingredients. From a nutritional perspective, low bulk-density foods allow high consumption of low-weight food, increasing the nutrient absorption of consumers (Bhatt *et al.*, 2021). Besides, bulk density also affects on the packaging, transportation, and storage of the product (Drakos *et al.*, 2019). The higher the bulk density of the biscuit, the cost of transportation and packaging material is lower and the lower the bulk density of the biscuit, the higher number of biscuits per weight is acquired (Ding *et al.*, 2020).

The average water activity of 100% JSF biscuit was 0.4302 ± 0.01 . Water activity effects on the development of microorganisms in food, thereby affecting the storage period and quality of food (Mahloko *et al.*, 2019). The lower water activity of 100% JSF biscuit (0.4302 ± 0.01 a_w) can be identified as within the safe limits of bacteria, yeast and micromycetes. This ensures the storability, high quality, and safety of prepared JSF biscuits. Furthermore, the water activity of the 100% JSF biscuit lies within the recommended water activity range for dry products (0.35 – 0.5), which is also partly responsible for the texture of the biscuit. The water activity, together with moisture content, affects the textural characteristics of the biscuit, such as crispiness (Benkadri *et al.*, 2018).

The average pH of the 100% JSF biscuit at 25°C was 7.15 ± 0.01 . The slight alkalinity of the product can be due to the presence of Sodium bicarbonate in baking powder. The color of 100% JSF biscuits was represented in L*, a*, and b* values in the Table 4.

Table 4: Color of biscuits prepared from 100% JSF

Color parameter	Value
L*	55.40 ± 0.06
a*	6.90 ± 0.10
b*	18.40 ± 0.40
ΔE	40.73 ± 0.08
HUE	1.20 ± 0.00

Values are the average of triplicate analysis \pm standard deviation.

The color can be affected by baking temperature and the baking time as the chemical reactions, such as the Maillard reaction, take place at higher temperatures in the presence of reducing sugars (Amarasinghe *et al.*, 2021).

Microbial analysis of 100% JSF Biscuit

During the study, total aerobic plate count and yeast and mold count were carried out 24 hours after preparation and 30 days after preparation of biscuits. The mean total plate count was less than 1 CFUg⁻¹ on both occasions. Any yeast and molds were not tested positive during the study. The low moisture content of the biscuit ensures the microbial stability of the product. Lower water activity level inhibits the growth of microorganisms and enzymatic activities. In addition, during biscuit baking, microbes are destroyed within the product itself due to higher temperatures in the oven. Accordingly, microbial results ensure the storability, high quality, and safety of prepared 100% JSF biscuits.

Comparison with SLS standards

The chemical properties and some microbial properties of the 100% JSF biscuit were compared with the SLS standards, and the comparison is given in Table 5. All the compared properties accomplished the SLS requirements.

Table 5: Comparison of biscuits prepared from 100% JSF with SLS specifications for biscuits

	Parameter	SLS requirement	100% JSF biscuit	Compliance
Chemical requirements	Moisture (%) by mass	Biscuits – 4.0 (max. limit)	3.9 ± 0.2	Yes
	Acidity of extracted fat (%)	1.0	0.4 ± 0.01	Yes
Microbial requirements	Aerobic plate count (CFU)	10^3 (min.) – 10^5 (max.)	< 30 CFUs/g	Yes
	Yeast and mold count	10^2 (min.) – 10^3 (max.)	Not detected	Yes

JSF: Jackfruit seed flour

CONCLUSIONS

Jackfruit seed flour is a rich source of flour that can be used in the production of flour confectionary rather than discarded as waste during fruiting seasons. This study has shown that jackfruit seed flour is a successful substitute for wheat flour in producing gluten-free biscuits. Physical properties such as spread ratio and chemical properties such as pH, moisture content, and acidity of extracted fat manifest the suitability of 100% JSF biscuit for commercial production. Nutritional properties are well compatible with the wheat flour biscuits, and the dietary fiber content is higher than that of the wheat flour biscuits on the market. Furthermore, the microbial properties (total plate count and yeast and mold count) and sensory properties of the 100% JSF biscuit prove the product successful.

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AUTHOR CONTRIBUTION

SDNK conceptualized the study, performed all the experiments, analyzed the data, visualized, and wrote the manuscript. WAJPW conceptualized, supervised the study, reviewed, and edited the manuscript.

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