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Unveiling the Quality Traits and Longevity of Biscuits Formulated with Black Rice Flour [*Oryza sativa* (L.) *indica*]

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ABSTRACT

Background: The study was aimed at producing biscuits made from black rice flour and analysing their proximate composition, mineral, antioxidant, glycaemic, organoleptic evaluation and shelf-life characteristics.

Methods: Established methods were used to evaluate the proximate composition, mineral composition, antioxidant, glycaemic, sensory evaluation and shelf-life characteristics of the biscuits.

Result: According to the results, the BRFB3 had a significantly higher content of dietary fibre and protein while it had a much lower content of carbohydrates, moisture and fat. Mineral content ($p<0.05$) was significantly different between the variants. BRFB2 scored higher in the sensory evaluation. All three biscuit formulations were found to have low GI values, the strongest antioxidant activity compared to the control sample. The biscuit formulations had a significant impact on the control and could be stored for a maximum of 30 days.

Key words: Antioxidant, Biscuits, Black rice, Physiochemical.

INTRODUCTION

Oryza sativa L., the rice species, includes black rice, which is mostly grown in Asia. Because of this rice's excellent nutritional content and antioxidant qualities, food scientists have been paying it a lot of attention lately. Because of its lovely organic food colour and its reputation as a nutritious food, this rice is becoming more and more popular in the USA and Europe (Richa *et al*., 2021). Manipur's black aromatic rice is distinct from other varieties of the same rice cultivated elsewhere in the world because of its attractive colour and aroma as well as its stickiness (Raleng *et al*., 2022).

Biscuits are a popular food product consumed by people all over the world. They are typically small, flat and crispy cakes made from dough or batter. The application of heat transforms the dough into a light, porous and appetising product. A convenient and inexpensive snack that stays fresh for a long time, biscuits come in a variety of sizes, shapes, textures, compositions, tenderness, flavours and colours, making them ideal for travelling (Chandra *et al*., 2015).

The most important ingredient used in the production of biscuits is wheat flour, along with other ingredients such as margarine, sugar, yeast, egg, milk and salt. However, biscuits are high in carbohydrates, fat and calories and low in fibre, making them an unhealthy choice for daily consumption. Manipulating the ingredients used in biscuits with potentially safer and healthier nutritional ingredients would be beneficial to improve the nutritional quality of baked goods (Gurung *et al*., 2016).

Biscuits are also a popular snack that is consumed around the world and are generally made from wheat flour, fat and sugar. However, traditional biscuits are deficient in various nutrients, phytochemicals and fibre, making them unhealthy (Okpala *et al*., 2013). Recent research has focused on fortifying biscuits with various food ingredients or plant materials that are considered good sources of nutrients and phytochemicals to overcome such limitations. Fortification of foods or formulation of new food products with health-promoting effects such as anti-diabetic, anti-inflammatory, anti-cancer and antioxidant properties is on the rise (Sadaf *et al*., 2013).

The quality and composition of their diet is now a serious concern. Foods that lead to weight loss, low-calorie sugars and fats and nutritious products are highly valued. The partial or complete substitution of hydrogenated fats with alternative fats/oils, especially unrefined and non-hydrogenated fats from locally under-utilized plants, is a step towards the development of healthy and nutritious food products (Mancebo *et al*., 2015).

Commercial biscuits are not suitable for daily consumption (Ismail *et al*., 2014) as they are rich in carbohydrates, lipids and calories but low in dietary fibre,

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minerals and vitamins. The nutritional value of biscuits can be improved by fortification and supplementation with a variety of protein, fat and mineral sources. Replacing wheat with rice flour from broken grains will produce diversified foods with unique qualities while adding value to the domestic rice value chain (Bhat *et al*., 2020).

Black rice has gained attention for its sensory properties, nutritional benefits and functional health attributes (Fig 1). It is not only a good source of carbohydrate and fibre, but also contains abundant nutritional and bioactive components such as essential amino acids, functional lipids, dietary fibre, vitamins (B complex, A and E), minerals (K, Fe, Zn, Cu, Mg, Mn and P), anthocyanins, phenolic compounds and phytosterols (Ito *et al*., 2019). Furthermore, black rice grain has been recognised as a diabetes controller in Chinese folk medicine, although white rice has been a major contributor to glycaemic load in many rice-consuming communities (Aalim *et al*., 2021). This study aims to formulate black rice flour biscuits and evaluate the nutrient composition, antioxidant activity, glycaemic response, sensory quality and shelf life of the biscuits to provide a healthier and more nutritious snack option.

MATERIALS AND METHODS

The research was conducted during the year of 2023 at the Department of Nutrition and Dietetics, Periyar University, Salem, Tamil Nadu and the Nutritional Analysis has been carried out at the Global Lab and Consultancy Services, Salem, Tamil Nadu.

In the formulation of biscuits a 100% wheat flour was used as a control and different blends of the raw black rice flours were prepared with BRFB1 25% (BRFB- Black Rice Flour Biscuits), BRFB2 50% and BRFB3 75% as shown below.

Formulation of biscuits

A 100% wheat flour was used as a control for the formulation of the biscuits and different blends of the raw black rice flours were prepared according to the blends as shown below. All the formulations were stored in air-tight containers at room temperature for the production of the biscuits as shown in (Table 1) and (Fig 2).

Methods

Standard methods were used to evaluate the proximate composition, mineral composition, antioxidant, glycaemic, sensory evaluation and shelf-life characteristics of the biscuits.

Statistical data analysis

T-test and analysis of variance (ANOVA) Duncan's multiple range (DMR) test with a 5% significance level were used to

Fig 1: Black rice.

Table 1: Ingredients for biscuit preparation.

Ingredients	Control	BRFB1	BRFB2	BRFB3
Wheat flour (g)	100	75	50	25
Raw black rice flour (g)	-	25	50	75
Brown sugar (g)	40	40	40	40
Olive oil (ml)	30	30	30	30
Water (ml)	25	25	25	25
Salt (g)	1	1	1	1
Sodium bicarbonate (g)	0.5	0.5	0.5	0.5

BRFB- Black rice flour biscuit.

Table 2: Proximate composition of black rice flour biscuits.

Proximate composition	Control	BRFB1	BRFB2	BRFB3	T value
Moisture (%)	6.19±1.34^{b}	5.52±1.02^{a}	4.21±1.68^{ab}	4.17±1.31^{c}	19.27**
Total CHO (%)	75.53±4.62^{ab}	75.51±3.41^{b}	73.03±1.68^{c}	69.74±4.15^{ac}	102.67**
Protein (%)	6.00±1.65^{a}	6.23±1.62^{ab}	6.75±1.69^{c}	7.02±1.30^{b}	54.29**
Total fat (%)	16.38±3.16^{ab}	13.05±3.16^{ac}	12.96±3.47^{c}	12.75±3.12^{a}	30.38**
Dietary fibre (%)	1.53±0.86^{b}	1.54±0.87^{a}	1.59±0.95^{ab}	1.71±0.62^{ac}	71.65**
Caloric value (kcal/100 g)	444.49±4.60^{ac}	440.79±4.60^{c}	436.84±4.68^{b}	433.13±4.38^{a}	342.44**
Ash (%)	0.94±0.12^{b}	1.02±0.21^{ab}	1.47±0.32^{a}	1.53±0.62^{c}	15.96**

$^{a-c}$Mean values with different superscripts within a column are significantly different ($p<0.05$); BRFB- Black rice flour biscuit.

compare the results of three replicates of the data collection.

RESULTS AND DISCUSSION

Proximate composition

Table 2 presents the chemical characteristics of the raw black rice flour biscuits and shows significant differences ($p<0.05$) in moisture, ash, carbohydrates, protein, fat and fiber. Our black rice flour biscuits had a moisture content between 1% and 5%, making them less perishable and increasing their shelf-life quality. The ash content ranged from 0.94% to 1.53%, with BRFB1 having the highest ash content of 1.53%. This indicates that raw black rice flour is a good source of calcium, sodium, magnesium, potassium and iron. The carbohydrate content of the biscuits varied from 69.74% to 75.53% and showed statistically significant differences ($p<0.05$). The range of protein content was between 6.0% and 7.02%. The addition of 25%, 50% and 75% black rice flour led to increase in protein content compared to the control biscuits. The fat content ranged from 12.75% to 16.38%. BRFB3 had the lowest comparing to the control. Butter was the fat used in all of the biscuits, but it can be replaced, at least in part, with extra virgin olive oil. The range of dietary fiber levels was between 1.53% and 1.71%. BRFB3 had the highest fiber content. Black rice flour considerably enhanced the amount of fiber in the biscuits while leaving the fat content unchanged. The caloric value of the formulated biscuits ranged from 433.13 kg to 444.49 kcal, with BRFB1 having the highest caloric value and BRFB3 having the lowest. The control and formulated black rice flour biscuits had the same range of caloric values with statistically significant differences at the 5% level.

Minerals composition

Table 3 displays the mineral composition of black rice flour biscuit formulations. BRFB1 showed a significant difference of 1% level compared to the control, whereas no significant difference was found in BRFB2 and BRFB3. The main mineral, phosphorus, varied between 5.2% and 9.18% (BRFB3). The potassium content in the biscuit formulations ranged from 11.25% to 18.45%. The variation in the results could be due to differences in raw materials. Phosphorus works together with calcium to provide structure and strength and is required for several metabolic processes, including energy production and regulation (Odimegwu *et al*., 2019). The high level of potassium in all biscuit formulations makes the development of biscuit

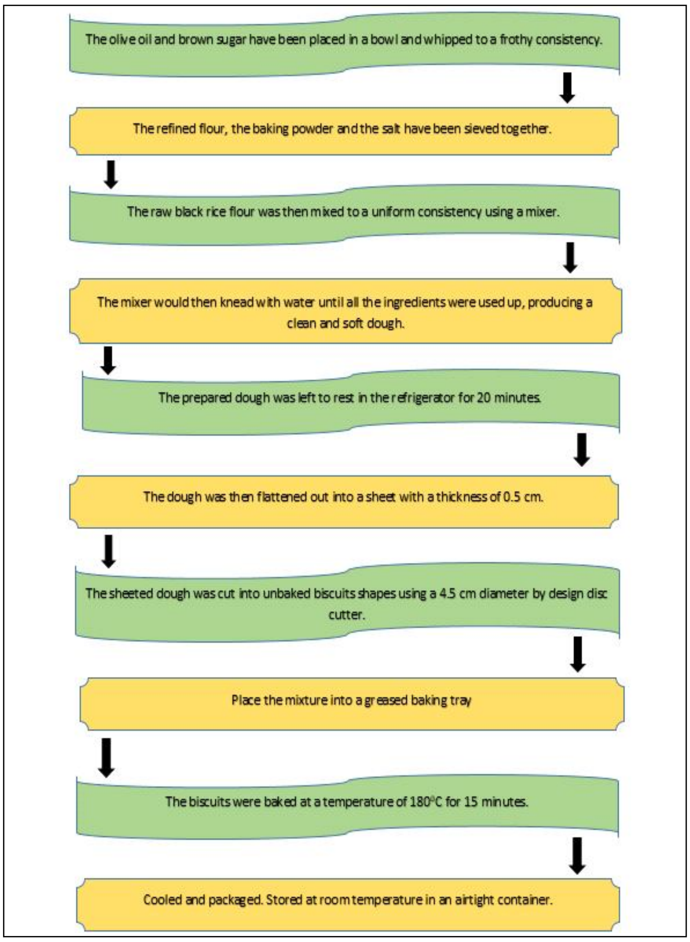

Fig 2: Preparation of black rice flour incorporated biscuits.

products potentially suitable as a health food for the prevention of hypertension and diabetes (Greer *et al*., 2020). The calcium content of the biscuits varied from 14.24% to 57.35%. Calcium is vital for muscle contraction and nerve impulse transmission. It also plays an important role in blood clotting and hormone metabolism. Calcium deficiency can lead to rickets in growing children and osteomalacia in adults (Murray *et al*., 2000). The magnesium concentration in the biscuits ranged from 12.56% to 50.23%. However, BRFB2 showed a significant difference at the 5% level compared to the control biscuit. The sodium content ranged from 5.12% to 9.21%, significantly lower in all the formulated biscuits than in the control biscuits. The results of our study are lower than those reported by Ponka *et al*. (2020). The BRFB2 biscuit (50%) is recommended for people with diabetes. The iron concentration ranged from 6.30mg to 12.64 mg in all biscuit formulations, which was relatively higher. The iron concentration of the biscuits varied significantly ($p<0.05$). A similar result for iron was reported by Chinma *et al*. (2012). Zinc concentration ranged from 6.21 to 12.82 mg/100 g, while copper content ranged from 5.13 to 16.12 mg/100 g. At 75% (BRFB3) incorporation of black rice flour, biscuits had high zinc (12.82 mg) and copper (16.12 mg) compositions, comparatively lower in the control biscuits (6.21 mg; 5.13 mg). Zinc plays a catalytic and metabolic role, forming the active site of almost 300 enzymes. It is also involved in the storage and release of insulin and the secretion of digestive enzymes (EFSA, 2014).

Antioxidant activities

The complete outcomes of the antioxidant components found in the formulated biscuits obtained from different levels of substitution of black rice flour are displayed in Table 4. The total flavonoid concentration ranged from 0.06 mg to 72.54 mg, with BRFB3 exhibiting the highest TFC content (72.54 mg), linked with the control biscuit. The TPC content of the biscuits ranged from 0.08 mg to 17.13 mg, with BRFB3 having the highest TPC value of 17.13 mg and BRFB2 having a slightly lower TPC value of 16.27 mg. Whole-colored cereal grains contain significant amounts of gallic acid (GAE), a primary phenolic acid, as well as other phenolic acids in their free, soluble conjugated and insoluble bound forms. Jang and Xu (2009) and Loypimai *et al*. (2016) proposed that purple rice is an intriguing source of phenolic compounds due to the dark purple color of anthocyanins. However, at 75% incorporation (BRFB3), peonidin-3-glucoside (P3G), tocopherol and the C3G level show the highest values, which could be due to an anthocyanin contain in the black rice. Cyanidin-3-O-glucoside (C3G), which has been shown to have an anti-diabetic effect in rats with type 2 diabetes, was the major anthocyanin of purple rice investigated in this study (Hosseinian *et al*., 2008). DPPH radical scavenging activities was found to be highest in BRFB3 (5.12%) and the lowest in BRFB1 (2.56%). These results showed that replacing wheat flour with black rice flour resulted in strong DPPH free radical scavenging activity.

Table 3: Minerals composition of black rice flour biscuits.

Mineral compositions	Control	BRFB1	BRFB2	BRFB4	T value
Phosphorus (%)	$5.2±0.65^{b}$	$6.23±0.0.89^{a}$	$7.25±0.01^{b}$	$9.18±0.32^{ab}$	15.57**
Potassium (%)	$11.25±0.36^{c}$	$12.35±0.85^{ab}$	$15.26±0.96^{b}$	$18.45±0.78^{b}$	16.99**
Calcium (%)	$14.24±0.56^{ac}$	$24.31±0.48^{c}$	$31.56±0.82^{b}$	$57.35±0.59^{a}$	8.12**
Magnesium (%)	$12.56±0.36^{ab}$	$32.54±0.54^{ab}$	$41.36±0.28^{b}$	$50.23±0.92^{a}$	8.61**
Sodium (%)	$9.21±0.64^{ac}$	$8.16±0.72^{b}$	$6.14±0.90^{a}$	$5.12±0.21^{b}$	14.72**
Iron (mg/100 g)	$6.30±0.20^{ab}$	$8.24±0.43^{c}$	$9.56±0.29^{b}$	$12.64±0.60^{c}$	13.20**
Zinc (mg/100 g)	$6.21±1.21^{a}$	$11.42±2.25^{ac}$	$12.65±2.41^{ab}$	$12.82±2.36^{ab}$	13.28**
Copper (mg/100 g)	$5.13±1.35^{a}$	$14.23±3.25^{c}$	$15.45±2.10^{ab}$	$16.12±2.62^{ac}$	9.50**

$^{a-c}$Significant differences ($p<0.05$) exist between mean values with different superscripts within a column, BRFB- Black rice flour biscuit.

Table 4: Antioxidant activities of black rice flour biscuits.

Biscuit variations	TFC (mg/100 g)	TPC (mg/100 g)	Cyanidin-3-glycoside (mg/100 g)	Peronidin-3-glycoside (mg/100 g)	Tocopherol (mg/100 g)	DPPH (%)
Control	$0.06±0.01^{a}$	$0.08±0.01^{b}$	$0.12±0.02^{ab}$	$0.08±0.01^{a}$	ND	$0.56±0.02^{ac}$
BRFB1	$32.45±2.56^{b}$	$12.74±1.57^{c}$	$15.78±1.65^{c}$	$1.12±0.45^{b}$	$0.06±0.01^{a}$	$2.56±0.95^{a}$
BRFB2	$48.24±2.67^{c}$	$16.27±1.57^{a}$	$15.67±1.62^{a}$	$3.58±1.02^{ac}$	$0.14±0.01^{b}$	$3.78±1.13^{b}$
BRFB3	$72.54±3.54^{ab}$	$17.13±1.69^{ab}$	$18.12±1.69^{b}$	$4.74±1.23^{c}$	$0.21±0.01^{c}$	$5.12±1.68^{c}$
T value	3.15**	1.65**	2.86**	3.46**	1.69**	2.64**

$^{a-c}$Significant differences ($p<0.05$) exist between mean values with different superscripts within a column, BRFB- Black rice flour biscuit; **-Significant at 1% level.

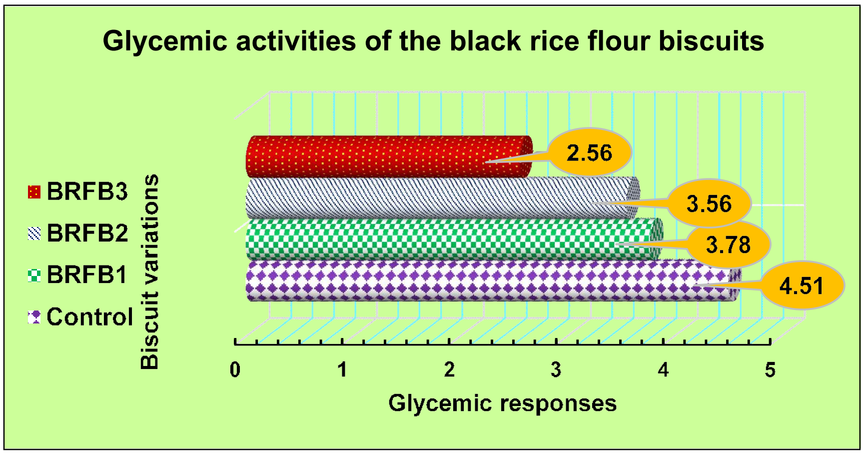

Fig 3: Glycemic activities of black rice flour biscuits.

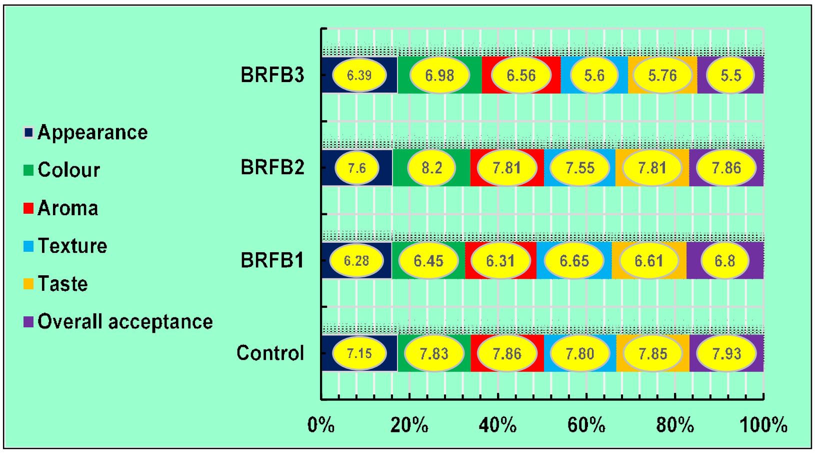

Fig 4: Sensory analysis of black rice flour biscuits.

Table 5: Shelf-life analysis of black rice flour biscuits.

Biscuit variations	Shelf-life analysis	0 day	15th day	30th day
Control	Total plate count (cfu/g)	Nil	0.2×10^2	1.5×10^2
	Yeast and Mold (cfu/g)	Nil	<10#	<10#
BRFB1	Total plate count (cfu/g)	Nil	0.3×10^2	0.7×10^2
	Yeast and Mold (cfu/g)	Nil	<10#	<10#
BRFB2	Total plate count (cfu/g)	Nil	0.32×10^2	0.72×10^2
	Yeast and Mold (cfu/g)	Nil	<10#	<10#
BRFB3	Total plate count (cfu/g)	Nil	0.33×10^2	0.75×10^2
	Yeast and Mold (cfu/g)	Nil	<10#	<10#

cfu- Colony forming units; #-acceptable limits.

Glycaemic activities

The incorporation of black rice flour in all the biscuit formulations resulted in a notable decrease BRFB3 (2.56%) in the glycaemic index in comparison with the control (4.51%) in Fig 3 by Similä *et al*. (2011) is consistent with the findings of our study.

Sensory analysis

The sensory evaluations of the biscuits were analysed in and Fig 4. The shape of the food product had a strong influence on the sensory characteristics and product acceptability. The black rice flour substitution responses ranged from 6 to 8, indicating acceptable to moderately satisfactory scores. Significant differences were observed between the control, BRFB1 and BRFB3 formulations at the 5% level. Nwatum *et al*. (2020) asserted that the state of the biscuit's ingredients, including fiber, starch and protein, as well as the amount of water absorbed during dough combining, all have an impact on the end product's quality during baking (temperature and time factors). In this analysis, we discovered that black rice flour biscuits also satisfied these requirements and that the composition of BRFB2 was superior to other formulations.

Shelf-life analysis

The results of the shelf-life analysis of black rice biscuits are presented in Table 5. Total viable counts were used to evaluate the level of overall microbial contamination and assess the microbiological quality. These biscuits could be kept in acceptable condition for 6 weeks at ambient temperatures of 30-1°C and RH of 75-80%. In summary, biscuits made with black rice flour ranging from 25% to 75% were successfully stored for up to 30 days, indicating that they could be commercialized based on previous studied by Peter-Ikechukwu *et al*. (2018). Thivani *et al*. (2016). Different packaging materials will be explored to protect the health benefits of such commodities.

CONCLUSION

According to the study, adding more black rice flour enhanced the biscuit's with high nutrients with low amount of fat. Further investigation of the consequences showed that the best overall result in terms of sensory quality was made with 50% raw black rice flour. Increased addition of the raw black rice flour greatly boosts the antioxidant activities in biscuits. The biscuits' lower GI emphasis and appropriateness for a diabetic patient's replenishment were demonstrated by the glycaemic response. Black rice flour biscuits are capable of being preserved for up to 30 days with proper packing, according to shelf-life parameters. New opportunities for using black rice flour in food formulations have emerged as a result of this work. According to the above studies, black rice flour biscuits could potentially be a good choice for people dealing with degenerative issues because they revolve around a lot of antioxidants that may be helpful in maintaining human health.

Conflict of interest

There are definitely not any potential disagreements of interest from the authors.

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