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Article *in* Journal of Agriculture and Food Research · September 2022

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Journal of Agriculture and Food Research 10 (2022) 100394

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Nutritional and sensory attributes of biscuits enriched with buckwheat

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ARTICLE INFO

Keywords:
Buckwheat
Biscuit
Nutritional
Microbial
Sensory evaluation

ABSTRACT

Buckwheat is a promising food ingredient for the manufacture of different bakery foods because of its abundance of nutrients with health advantages, its gluten-free nature, and different polyphenol ingredients. In this study, buckwheat enriched biscuits (BEB) were formulated by blending wheat flour with buckwheat flour (BF) at the ratios of 100:0 (Control Biscuit), 90:10 (BEB-10), 80:20 (BEB-20), 70:30 (BEB-30), and 60:40 (BEB-40), and their nutrient, sensory, and microbiological analyses were investigated. The increment of buckwheat flour in formulated biscuits led to an increase in the mean protein, fat, fiber, and ash content, except carbohydrate. The iron, potassium, and zinc contents of the biscuits were also increased with the addition of buckwheat flour (BF). In terms of sensory evaluation, the overall acceptable scores for control biscuit (CB) and BEB-10, BEB-20, BEB-30, and BEB-40 were 7.56, 7.02, 6.98, 6.86, and 5.94, respectively. Therefore, BEB developed with up to 30% BF showed improved nutritional quality with acceptable sensory attributes. This study also concluded that BEB is microbiologically acceptable for up to six months.

1. Introduction

Biscuit or cookies are a well-known cereal food product, which is very popular among both rural and urban people of Bangladesh. Its popularity is due to its relatively low cost, varied taste, good eating quality, availability, and long shelf life [1]. The quality of biscuits depends on the nature and quantity of ingredients used. Biscuits are high in carbohydrates, fat, and calories but low in protein, fiber, vitamins, and minerals. The development of supplemented biscuits or other composite flour bakery products is the latest trend in the bakery industry. The growing interest in bakery products is due to their better nutritional properties and the possibility of their use.

Buckwheat (*Fagopyrum esculentum*) is a pseudo-cereal that has been cultivated in Bangladesh since ancient times and is locally known as "Dhemsi" in Bangladesh. It is cultivated in poor and marginal soils. It is short in duration, drought-tolerant, well adapted and very less susceptible to pests and diseases [2]. In Bangladesh, rice is our staple food, and at present, inhabitants also prefer wheat flour as a cereal along with rice. Buckwheat has not yet become a popular food in the country. The buckwheat growing area has rapidly depleted due to dense population, the introduction of high-yielding varieties of other cereals, and remarkable socio-economic changes in the country. Recently, buckwheat has become interesting due to its nutritional composition and pharmaceutical properties. It is a good source of complex carbohydrates, proteins, fibers, vitamins and minerals. Several flavonoids and polyphenols have been found in buckwheat [3,4]. It also possesses numerous nutraceutical compounds [5] and a vitamin B complex [6]. According to Holasova, Fiedlerova [7] buckwheat seeds have higher lipid stability protection factors than other cereals like oats and barley. Buckwheat dough helps to improve gastric motility, mucus production, mucosal blood flow, and other important biological functions [8]. It also prevents toxic nitrosamine formation in the digestive tract [9]. The functional properties of buckwheat have many health benefits, such as preventing excess fat accumulation, regulating uncontrolled blood pressure, blood sugar, and constipation, etc. [10]. Protein content in wheat flour is 7–14% with a lack of some amino acids such as lysine, while buckwheat flour is higher in protein quality, including certain amino acids viz. Leucine, lysine, histidine, and valine [11]. Therefore, several studies have focused on incorporating buckwheat flour into flour mixtures for bakery products like cakes, noodles, pies, spaghetti, and gluten-free bread production as consumer choices for ready-to-eat snacks [12,13]. Buckwheat flour contains relatively high levels of various ingredients, such as protein, fiber, certain minerals, and phytochemicals, compared to wheat. In general, buckwheat flour contains

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https://doi.org/10.1016/j.jafr.2022.100394
Received 18 July 2022; Received in revised form 24 August 2022; Accepted 15 September 2022
Available online 16 September 2022
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16.66 gm of protein, 3.42 gm of fat, 72.19 gm of carbohydrates, 0.58 gm of fiber, and 1.68 gm of ash per 100 gm of flour [14]. Keeping in view the functional properties and health benefits of buckwheat, the present study was designed to incorporate buckwheat flour into cookies (biscuit) preparation with different percentages and assess the improved nutritional value, considering sensory and microbiological attributes of the developed buckwheat enriched biscuits (BEB).

2. Materials and methods

2.1. Materials

Raw materials for buckwheat enriched biscuits (BEB) such as wheat flour, salt, sugar, eggs, milk powder, and other ingredients were collected from the local markets. Buffer peptone water (Hi-Media, India), plate count agar (Hi-Media, India), Lauryl sulfate tryptose broth (Hi-Media, India), EC broth (Hi-Media, India), and Brilliant green lactose bile broth (Hi-Media, India), Dichloran Rose Bengal Chloramphenicol (DRBC) and Dichloran 18% Glycerol (DG18) agar media were purchased from India. All the chemicals were of analytical grade (Sigma Aldrich, Germany).

2.2. Buckwheat flour (BF) preparation

Common buckwheat groats (*Fagopyrum esculentum*) were collected from the northern part of Bangladesh, especially the greater Panchagar district. Panchagarh, has the GPS coordinates of 26° 20′ 7.3572″ N and 88° 33′ 6.1092″ E. First, buckwheat groats were cleaned and washed. Then they were dried (Drying Oven, Brand: Memmert, Model: Memmert D-91126, Origin: Germany) at 50 °C for 6 h and milled into flour. Finally, the flour was sieved using 0.25 mm sieves (Analytical Shieve, Model No: FRITSCH analysette 3, Germany) to pass the powder. The buckwheat flour was packaged in airtight containers for further analysis.

2.3. Phytochemical screening of buckwheat flour (BF) extract

The standard methods proposed by Harborne [15] were used to determine the qualitative tests of various phytochemicals present in the buckwheat. The methanolic extract of buckwheat flour was used in this study to detect the presence of phytochemicals such as alkaloids, anthracene, anthraquinone, cardiac glycosides, coumarins, flavonoids, glycosides, phenol, phlobotannins, quinones, saponin, steroid, tannins and terpenoid compounds [16–19]. The qualitative results are expressed as (+) for the presence and (−) for the absence of phytochemicals.

2.3.1. Test for alkaloids

0.5 g of extract was diluted to 10 ml with acidified alcohol, boiled and filtered. To 5 ml of filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendroff's reagent to another. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendroff's reagent) was taken as positive for the presence of alkaloids.

2.3.2. Test for anthracene

The extract is shaken with volume of chloroform and allowed to separate. Brick-red precipitate is formed with anthracene.

2.3.3. Test for anthraquinone (Borntrager's test)

0.5 g of powder was boiled with dilute sulphuric acid (H_2SO_4). Filtered and cooled. The filtrate is extracted with chloroform or benzene and dilute ammonia is added to it. The ammonical layer becomes pink to red due to the presence of anthraquinone.

2.3.4. Test for cardiac glycosides

0.5 g of Extract was shaken with 5 ml distilled water. To this, 2 ml glacial acetic acid containing a few drops of ferric chloride ($FeCl_3$) was added, followed by 1 ml H_2SO_4 along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

2.3.5. Test for coumarins

0.5 g of the extracts was taken in a test tube. The tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

2.3.6. Test for flavonoids (shinoda test, alkaline reagent test)

Shinoda test: About 0.5 of extract was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. Hydrochloric acid (HCl). A pink, orange, or red to purple coloration indicates the presence of flavonoids.

Alkaline reagent test: 2 ml of extract was taken in a test tube and added few drop (2 ml) of dilute NaOH solution. A yellow color was appeared in the test tube. It became colourless when on addition of a few drop of dilute acid that indicated the presence of flavonoids.

2.3.7. Test for glycosides

5 ml of H_2SO_4 was added to each of the test extracts in separate test tubes. The mixture was heated in boiling water for 15 min. Fehling's solution was then added, and the resulting mixture was heated to boiling. A brick-red precipitate indicates the presence of glycosides.

2.3.8. Test for phenols

50 mg of extract was dissolved in 5 ml of distilled water, and the addition of a few drops of neutral 5% ferric chloride solution, followed by the development of a dark green color, was regarded as positive for phenolic compounds.

2.3.9. Test for phlobotannins

In a test tube, 2 ml of extract was boiled with 1% HCl acid. If the sample carries phlobatannins, a deposition of a red precipitate will occur; this will indicate the presence of phlobatannins.

2.3.10. Test for quinones

One ml of each of the extracts was treated separately with alcoholic potassium hydroxide (KOH) solution. Quinones give coloration ranging from red to blue.

2.3.11. Test for saponins

0.5 g of extracts was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable, persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, after which it was observed for the formation of an emulsion.

2.3.12. Test for steroids

0.5 g of extract was mixed with 2 ml of acetic anhydride followed by 2 ml of H_2SO_4. The color changed from violet to blue or green indicated the presence of steroids.

2.3.13. Test for tannins

About 0.5 g of the extract was boiled in 10 ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride were then added. Then, it was observed for brownish green or a blue-black coloration.

2.3.14. Test for terpenoids (Salkowski's test)

About 100 mg of extract was shaken with 2 ml of chloroform

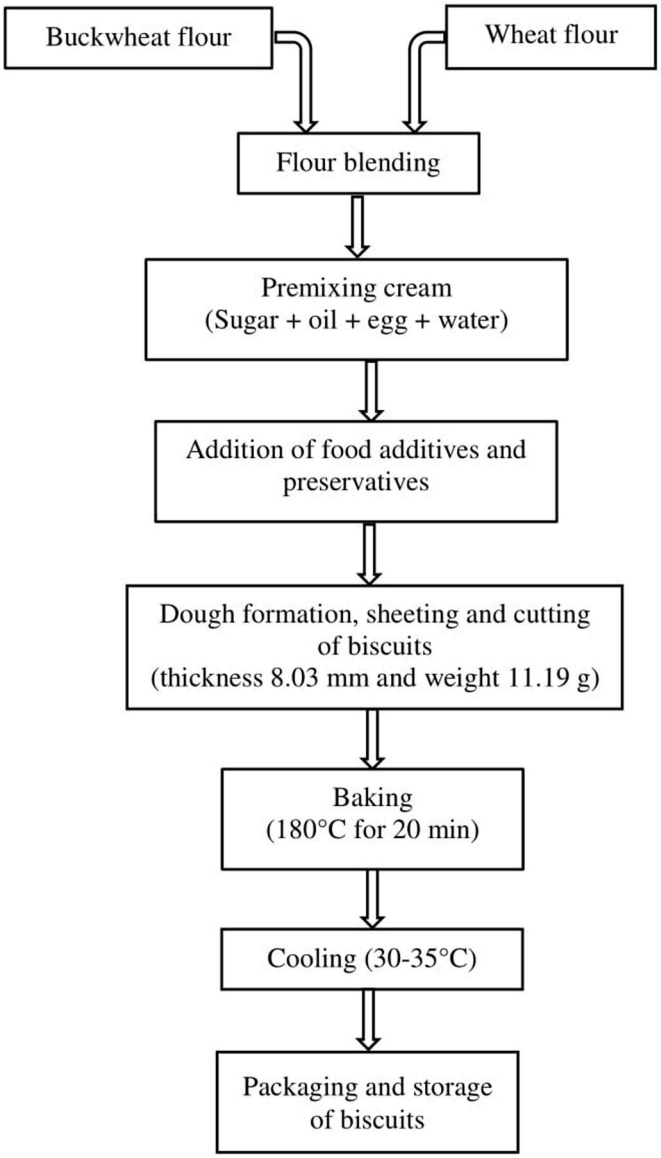


Fig. 1. Flow chart for buckwheat enriched biscuits (BEB) preparation.

followed by the addition of 2 ml of conc. H_2SO_4 along the side of the test tube; a reddish brown coloration of the interface indicates the presence of terpenoid.

2.4. Product development

Control biscuits (CB) and buckwheat enriched biscuits (BEB) were developed by slightly modifying the [20] method No. 10–91. CB and BEB were formulated by blending wheat flour with buckwheat flour (BF) at the ratios of 100:0 (CB), 90:10 (BEB-10), 80:20 (BEB-20), 70:30 (BEB-30), and 60:40 (BEB-40), and the dough was made with sugar (22%), salt (0.2%), oil (9%), egg (14%), baking powder (0.4%), milk powder (7.5%), cocoa powder (0.9%), sodium benzoate (0.07%) as preservative and water (Fig. 1). The prepared biscuits were stored in a polyethylene bag for 24 weeks (6 months) at 30 °C average temperature and 70%–80% relative humidity for microbial studies.

Table 1
Phytochemicals investigated in buckwheat extract.

Phytochemicals	Buckwheat extract	Phytochemicals	Buckwheat extract
Alkaloid	+	Phenol	+
Anthracene	–	Phlobotanin	–
Anthraquinone	+	Quinones	+
Cardiac Glycoside	+	Saponin	–
Coumarin	+	Steroid	+
Flavonoid	+	Tannin	+
Glycoside	+	Terpenoid	+

Here, (+) means presence, and (−) means absent.

2.5. Proximate nutrient analysis of BEB

The proximate nutrient content of BEB was determined according to the standard analytical methods of AOAC [21]. The carbohydrates and the energy value were determined by the methods of Farzana and Mohajan [22]. Sodium, potassium, and calcium were estimated by a flame photometer (JENWAY, Model: PFP7, Germany) [23]. Iron, zinc, copper, and manganese were determined by an Atomic Absorption Spectrometer (Thermo, Model: ICS-3000, USA) [24]. The working solutions were prepared by dilution of each mineral from the standard stock solutions (1000 μg/mL).

2.6. Sensory evaluation of BEB

The sensory evaluation of prepared biscuits was carried out by 11 trained panelists from BCSIR, Dhaka, Bangladesh. The parameters are evaluated, such as color and appearance, texture, flavor, taste, and overall acceptability. The sensory evaluations were collected on the 9-point Hedonic Scale [25] where the quality characteristics of each sample were rated as, 9- like extremely, 8- like very much, 7- like moderately, 6- like slightly, 5- neither like nor dislike, 4- dislike slightly, 3- dislike moderately, 2- dislike very much, 1- dislike extremely. Samples were evaluated in a day after baking under daylight illumination and in isolated booths within a sensory laboratory.

2.7. Microbiological quality analysis of BEB

About 25 g of biscuit samples were aseptically suspended in 225 ml of buffer peptone water (Hi-Media, India) and homogenized appropriately. Microbiological quality analyses were carried out in accordance with the procedure of bacteriological analytical manual [26–28]. Total mesophyllic aerobic bacteria, total coliforms, Staphylococcus sp., Bacillus cereus, yeasts and molds were evaluated by a quantitative method; conversely, a qualitative method was applied for salmonella sp. And *Escherichia coli*. For total mesophyllic aerobic bacteria, an aliquot (1 ml) of the sample was placed on plate count agar (Hi-Media, India) and incubated at 37 °C for 24 h. The prevalence of bacterial colonies was determined as the number of CFU (colony forming units) and reported as log CFU/g. Total coliforms and *E. coli* tests were carried out with Lauryl sulfate tryptose broth (Hi-Media, India), EC broth (Hi-Media, India), and Brilliant green lactose bile broth (Hi-Media, India) media along with an inverted Durham tube. For enumeration a table of the most probable numbers was used according to Feng, Weagant [26], and the results are expressed as MPN/g. Yeast and mold counts were determined with Dichloran Rose Bengal Chloramphenicol (DRBC) and Dichloran 18% Glycerol (DG18) agar media and incubated for 3–5 days at 30 °C. The prevalence of Bacillus sp. was determined by following Gdoura-Ben Amor [29]. A determination of Salmonella sp. was carried out according to Siala, Barbana [30]. The entire test panels were conducted in triplicates.

2.8. Statistical analysis

All experiments were carried out in triplicate and the results were reported as mean ± SD. One-way ANOVA was used to compare significant variations in nutrient content among experimental biscuits. Duncan's multiple range tests were used to determine the mean differences within the groups. All analysis was done by appropriate statistical

Table 2
Nutritive values for buckwheat enriched biscuits (per 100 gm dry basis).

Nutrients	Buckwheat enriched biscuit (BEB)				
	CB	BEB-10	BEB-20	BEB-30	BEB-40
Moisture (%)	3.28 ± 0.07	3.20^{b} ± 0.05	3.10^{b} ± 0.06	2.99^{b} ± 0.03	2.87^{b*}±0.07
Ash (gm)	1.39 ± 0.06	1.50^{a}±0.03	1.61^{a}±0.06	1.73^{a*}±0.07	1.84^{a*}±0.03
Protein (gm)	9.10 ± 0.05	9.63^{a}±0.07	10.27^{a}±0.05	10.92^{a*}±0.05	11.66^{a*}±0.05
Fat (gm)	9.87 ± 0.02	9.96^{a}±0.05	10.06^{a}±0.07	10.25^{a}±0.06	10.46^{a}±0.07
Crude fiber (gm)	0.1 ± 0.06	0.15^{a}±0.05	0.21^{a*}±0.02	0.27^{a**}±0.03	0.32^{a**}±0.06
Carbohydrate (gm)	76.26 ± 0.21	75.56^{b} ± 0.27	74.75^{b} ± 0.27	73.84^{b} ± 0.26	72.85^{b} ± 0.25
Energy (Kcal)	430.27 ± 0.27	430.4^{a}±0.22	430.62^{a}±0.41	431.29^{a}±0.11	432.18^{a}±0.2
Calcium (mg)	170.30 ± 0.15	174.94^{a*}±0.15	180.03^{a**}±0.22	185.28^{a**}±0.2	189.96^{a**}±0.15
Iron (mg)	2.70 ± 0.03	2.95^{a*}±0.02	3.21^{a**}±0.03	3.46^{a**}±0.07	3.71^{a**}±0.03
Potassium (mg)	202.81 ± 0.12	212.72^{a*}±0.03	223.05^{a**}±0.05	234.06^{a**}±0.05	244.27^{a**}±0.02
Sodium (mg)	296.07 ± 0.05	297.12^{a}±0.06	298.18^{a*}±0.03	299.25^{a*}±0.06	300.21^{a*}±0.06
Zinc (mg)	3.22 ± 0.03	3.68^{a}±0.02	4.21^{a*}±0.02	4.65^{a**}±0.02	5.02^{a**}±0.04
Manganese (mg)	0.86 ± 0.01	0.91^{a}±0.03	0.96^{a}±0.03	1.02^{a*}±0.04	1.07^{a*}±0.01
Copper (mg)	1.03 ± 0.03	1.12^{a}±0.02	1.22^{a}±0.01	1.33^{a*}±0.03	1.42^{a*}±0.03

Values are means of triplicates ± standard deviation. Superscript 'a' and 'b' in a row indicate higher & lower values respectively and were compared with that of CB (control). *indicates significantly different ($p < 0.05$) and **indicates highly significant ($p < 0.001$) as determined by Duncan's multiple range test.

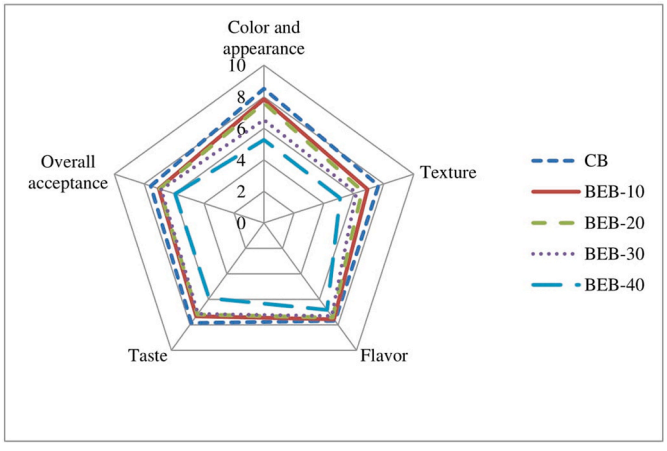
methods using R Studio (Version April 1, 1717) based on R (Version 4.1.0).

3. Results and discussions

3.1. Qualitative analysis of phytochemicals in buckwheat flour extract

The phytochemical screening of methanolic extracts of buckwheat samples revealed the presence of some phytochemicals such as alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, glycosides, phenols, quinones, steroids, tannins, and terpenoids, as shown in Table 1. However, anthracene, phlobotanins, and saponins were absent in buckwheat extract. This result was consistent with the previous study, where buckwheat contains flavonoids, polyphenols, alkaloids, anthraquinones, cardiac glycosides, coumarins, quinones, steroids, tannins, terpenoids, and many other phytochemicals [31,32].

The chemical constituents present in the extract have been reported to possess many therapeutic and medicinal values. For example, alkaloids have been shows biological activities like, anti-inflammatory, antimalarial, cytotoxicity and pharmacological effects [33,34]. Similarly, steroids have cardiotonic effect and also possess antibacterial properties [35]. Flavonoids have attracted attention due to their potential health benefits. Over the past few years, several studies have demonstrated the biological and pharmacological properties of many flavonoids, especially their antioxidant, antimicrobial, and anti-inflammatory properties, which are associated with free radical-scavenging action [18]. According to research, tannins are known to have antidiarrheal effect. They work by precipitating microbial proteins, thus making nutritional protein unavailable to them. Cardiac glycosides have been used to treat congestive heart failure. Their mode of action starts by inhibiting the Na+/K+ pump, which increases the level of calcium ion and reduces the distension of the heart [36].



Values are means of eleven panelists'± standard deviations.

Fig. 2. Sensory evaluations of buckwheat enriched biscuits (BEB).

3.2. Proximate and mineral composition of BEB

The proximate composition of buckwheat enriched biscuits (BSB) is given in Table 2. A significant difference was observed in the nutrients of BEB with the addition of buckwheat flour. The BEB showed lower carbohydrate and moisture content than the control biscuit (CB). The moisture content ranged from 3.28% (control) to 2.87% (BEB-40) and the carbohydrate was 76.26 gm/100 gm (CB) to 72.85 gm/100 gm (BEB-40), which is likely according to the report proposed by De Francischi, Salgado [37]. The decreased moisture content of BEB biscuits may be due to the low water absorption capacity of buckwheat flour rather than wheat flour [39]. The ash content of BEB increased significantly ($p \leq 0.05$) with the addition of buckwheat flour by more than 20%, and this may be due to the higher mineral content in buckwheat [11]. According to Jan, U. et al. [38] the ash content of cookies increased with the addition of BWF. The fat content of CB was 9.87 gm/100 gm and it increased to 10.46 gm/100 gm in BEB-40. The higher fat content in BEB biscuits may be due to the fact that buckwheat contains more fat than wheat flour [14] and that buckwheat flour has a higher oil absorption capacity than wheat flour [39] which improves the mouthfeel and flavor of the biscuits. Buckwheat is an excellent source of cereal proteins (8.5 gm/100 gm to 19 gm/100 gm) depending on the varieties, pesticides used, and fertilization [40]. This study also confirmed the protein content of biscuits gradually increased with the increment of buckwheat flour (BF) and ranged from 9.1 gm/100 gm (CB) to 11.66 gm/100 gm (BEB-40). The increased protein content may be due to the higher protein content of buckwheat flour than wheat flour and the addition of egg albumin. In the study, BEB-20 (0.21 gm/100 g), BEB-30 (0.27 gm/100 g), and BEB-40 (0.32 gm/100 g) biscuits had significantly (p 0.05) higher fiber content than CB (0.21 gm/100 g). Chopra et al. [40], also formed cookies with the incorporation of buckwheat flour in 50, 75, and 100% concentrations with wheat flour and measured only macronutrients. According to their investigation, the nutritional quality of cookies was enhanced up to a level of 75% concentration of buckwheat flour. Therefore, the previous study also accorded with the present study.

Minerals have important roles to play in many activities in the body, such as calcium, which is a vital element of bones and teeth and in

Table 3
Microbiological quality assessment of buckwheat enriched biscuits (BEB).

Tests	Results						
	Initial day	1st month	2nd month	3rd month	4th month	5th month	6th month
Mesophyllic aerobic bacteria, cfu/g	<10[a]	3.4×10^2	5.9×10^2	8.0×10^3	8.6×10^3	9.7×10^3	6.7×10^4
Total Coliforms, MPN/g	<0.3[b]	<0.3[b]	<0.3[b]	<0.3[b]	<0.3[b]	<0.3[b]	<0.3[b]
Escherichia coli, MPN/g	<0.3[b]	<0.3[b]	<0.3[b]	<0.3[b]	<0.3[b]	<0.3[b]	<0.3[b]
Yeasts and Molds, cfu/g	<10[a]	<10[a]	<10[a]	<10[a]	<10[a]	$2.0x10^2$	$2.7x10^2$
Bacillus sp., cfu/g	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salmonella sp., cfu/g	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Staphylococcus sp., cfu/g	<10[a]	<10[a]	<10[a]	<10[a]	<10[a]	<10[a]	<10[a]

[a] <10 indicate absence of test organisms in 1 g of sample.
[b] As per MPN (most probable number) chart, MPN <0.3 indicates absence of test organism in 1 g.

preventing osteoporosis; sodium and potassium are maintenance of osmotic balance; iron, copper, and zinc are important co-factors that are found in certain enzymes [41,42]. The data in Table 2 showed that the addition of buckwheat flour (BF) into wheat flour (WF) augmented the minerals like iron, sodium, potassium, calcium, zinc, manganese, and copper. The iron, potassium, and calcium content of BEB significantly ($p \leq 0.05$) higher than CB. The addition of 20% buckwheat flour significantly ($p \leq 0.05$) increased the sodium and zinc content of BEB when compared to CB, and the addition of 30% buckwheat flour significantly ($p \leq 0.05$) increased the manganese and copper content of BEB when compared to CB. This may be due to the higher ash content in buckwheat. The BEB developed with 30% (BEB-30) and 40% (BEB-40) buckwheat flour was nutritionally superior ($p \leq 0.001$) to the control biscuit (CB).

3.3. Sensory evaluations of BEB

The biscuits prepared from wheat flour and buckwheat flour were evaluated for their color and appearance, texture, flavor, taste and overall acceptability using 9-point hedonic scale (Fig. 2). The sensory quality of the biscuit was best in the control sample (CB) and gradually decreased with the increment of buckwheat flour in the BEB. This may be due to the lower lightness, higher yellowness, and redness values of buckwheat flour [43]. The color and appearance of the product make the first impression in the consumer's mind. The mean score for color and appearance for BEB ranged from 8.51 to 5.27. The control biscuit (CB) showed the highest score and the lowest score was found in BEB-40. The color and appearance of BEB up to 10% addition of BF was not significantly ($p < 0.05$) different from CB. The texture is another important sensory attribute that indicates the softness or hardness of the biscuit. The mean score of CB, BEB-10, BEB-20, BEB-30, and BEB-40 were found 7.64, 6.91, 6.56, 6.16, 5.08 respectively. In the study, the textural properties of BEB improved with the increasing amount of BF. The texture of BEB up to 20% addition of BF was not significantly ($p < 0.05$) different from CB. Flavour and taste determine the acceptability and market success of the biscuit. The mean score for flavour and taste for BEB ranged from 7.68 to 6.83 and 7.85 to 5.92 respectively. The flavour and taste of BEB up to 30% addition of BF was not significantly ($p < 0.05$) different from CB. The overall acceptability for CB and BEB-10, BEB-20, BEB-30, and BEB-40 were 7.56, 7.02, 6.98, 6.86, and 5.94, respectively and did not differ significantly BEB up to 30% addition of BF. Thus, biscuits prepared with 10–30% buckwheat flour suggest acceptability by consumers. This result also accords with the previous study proposed by Baljeet, Ritika [11].

3.4. Microbial analysis of BEB

In present study, WHO standard and good manufacturing practice (GMP) guidelines were used as reference for microbiological assessment. According to WHO standards (1994), the maximum acceptable limits on baked products (cake, bread, and biscuits) for total mesophyllic aerobic bacteria is 2.0×10^5 cfu/g, coliform bacteria <200 MPN/g, *E. coli* absent, yeast and mold is $< 1.0 \times 10^4$ cfu/g. The current analysis of the freshly prepared biscuit sample demonstrates no bacterial growth after 24 h. After different storage periods, the total mesophyllic aerobic bacterial count was within the acceptable limit up to six months. Total coliform and *E. coli* were absent, where the yeast and mold count were below the satisfactory limit for up to six months. Furthermore, pathogenic bacteria (Bacillus sp., Salmonella sp., Staphylococcus sp., and Pseudomonas sp.) were not found within six months. However, the total mesophyllic aerobic bacterial count exceeded the WHO standard by six months, determining the validity of this biscuit sample for up to six months. The results are presented in Table 3.

4. Conclusion

Buckwheat enriched biscuits (BEB) were nutritionally appreciable due to their considerable amount of protein, fat, fiber, iron, potassium, and zinc content. Buckwheat flour incorporation into wheat flour up to a level of 30% to yield biscuits showed improved nutritional quality with acceptable sensory attributes. This study also concluded that BEB is microbiologically acceptable for up to six months. Hence, the development of such fortified biscuits could help the nutritional status of the population and also encourage the food industry to develop food products using new croup source.

Declaration of competing interest

Authors declared that there is no conflict of interest to publish the article.

Acknowledgment

The authors are thankful to the laboratory of Quality Control Research Section, Institute of Food Science & Technology (IFST), Bangladesh Council of Scientific& Industrial Research (BCSIR), and Department of Applied Nutrition and Food Technology, Islamic University, Kushtia, Bangladesh.

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