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Quality assessment of gluten-free muffins from buckwheat flour and rice bran

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Abstract
Buckwheat is a highly nutritious pseudo-cereal and contains no gluten. It is rich source of dietary fiber andrutin that has potential antioxidant activity. As by- product, rice bran is a good source of proteins, fiber and minerals. Muffin is an acceptable popular snack food. The main objectives include standardization of muffin formulation with studies on its sensory, nutritional, texture and shelflife analysis. Three flour blends were prepared by mixing buckwheat flour with rice bran in the proportions of 95:5(B), 90:10(C) and 85:15(D), 100% buckwheat flour was used as control(A). The moisture, crude protein, crude fat and ash contents increased significantly from 20.07% to 23.67%, 12.04% to 13.10%, 1.57% to 3.77% and 1.46% to 2.41% respectively. The carbohydrate content was found to be decreased to 52.953 % from 67.112% and so the calorie value was also decreased from 456.698 Kcal to 424.23 Kcal. The springiness in texture was found to be decreased with 6 days of storage. The muffin containing 10% of rice bran is widely accepted among the composition.

Keywords: buckwheat, rutin, pseudo-cereal, muffin

Introduction
Buckwheat (*Fagopyrumesculentum* Moench) is highly nutritious pseudo cereal belong to polygonaceae family known as a dietary source of protein with favourable amino acid composition and vitamins, starch and dietary fibre, essential minerals and trace elements. In comparison to most frequently used cereals, buckwheat possesses higher antioxidant activity, mainly due to high rutin content, phenolic acids, flavonoids, phytic acid, vitamin B1, B2 and E, glutathione, carotenoids, phytosterols and as a gluten-free cereal can be widely used for producing gluten-free products (Wronkowska *et al*., 2010) [23]. Celiac disease (CD) is an inflammatory disease of the small intestine triggered by gluten proteins from wheat, barley and rye. Its prevalence is 1:100e1:200 in any population over the world (Schuppan, Junker, & Barisani, 2009) and CD patients must adhere to a lifelong gluten-free diet. Therefore, gluten-free products commercialization has grown at an annual rate of 28% in the last years (Glover, 2009) [8].
A biologically rich food, buckwheat offers a wide range of health benefits. It possesses high levels of dietary fibre, antioxidants and vitamins and is nutritionally superior to many of even the most healthful grains. The latest research indicates that buckwheat may even help with the management of diabetes and with the prevention of cancer and cardiovascular diseases.Buckwheat contains high amounts of antioxidants, which may offer protection from serious conditions such as cancer and heart disease. Buckwheat is useful in the management of diabetes and may help to lower blood glucose levels when eaten regularly.
Rutin has been shown to exhibit antioxidative, antihemorrhagic, and blood vessel protecting properties (Baumgertel, Grimm, Eisenbeib, & Kreis, 2003) [6]. Rutin is a complex sugar found in abundance in buckwheat, and also in lesser amounts in asparagus, rhubarb, and the peels and rinds of citrus fruit. There are a number of beneficial effects of rutin in the human body. Rutin counteracts platelet-activating factor (PAF), which causes blood clot formation and which triggers the inflammatory reactions of allergies. It also reduces the rate at which oxidized LDL cholesterol attracts the white blood cells that transform it into the plaque that hardens arteries, act as an anti-inflammatory agent and a strong antioxidant. And, most importantly,rutin strengthens the linings of blood vessels throughout the body, reducing bleeding and preventing collapse.
However, the application of buckwheat as functional food was still limited in certain degree for the followings reasons: the low digestibility of buckwheat proteins caused by buckwheat trypsin inhibitors and other protease inhibitors, and the unpleasant taste of buckwheat products. Moreover, the allergenic proteins of buckwheat were also a problem (Ikeda *et al.*, 1991; Park *et al.*, 1997) [13, 17].

Rice bran, a by-product of the milling process, contains the enzyme lipase, which rapidly degrades the oil making the bran rancid and inedible. Rice bran, the brown outer layer of rice kernel is mainly composed of pericarp, aleurone/sub-aleurone layers and germ. Currently it is discarded as a waste product during the process of rice milling in this part of the world (De and Korver, 1996) [8]. However, it is an excellent source of total dietary fibre ranging from 20% - 51%. It is also a good source of proteins, lipids, vitamins and minerals (Well, 1993). Chemically it contains 11% - 17% protein, 11% - 18% fat, 10% fibre, 9% ash and 45% - 65% nitrogen free extract (NFE). It is a rich source of B-vitamins and minerals such as potassium, calcium, magnesium and iron. The amino acid profile of rice bran has been generally reported to be superior to cereal grain proteins. Rice bran contains an array of micronutrients like oryzanols, tocopherols, tocotrienols, phytosterols, 20% oil and 15% protein, 50% carbohydrate (majorly starch) dietary fibers like beta-glucan, pectin, and gum (Hernandez, 2000) [11]. The low content of saturated fatty acids and high content of linoleic acid, polyunsaturated fatty acids plus tocopherol makes rice bran oil a health beneficial food. Hu *et al*, 1996 [12] have shown that rice bran and rice bran oil have potential health benefits in the prevention of diseases such as cancer, kidney stones, heart disease and hyperlipidaemia. This is attributed to the high content of gamma-oryzanol, which is a mixture of ferulate esters of triterpene alcohols.

The objective of this study includes, standardizing the muffin formulation from buckwheat and rice bran with acceptable taste and appearance, its proximate composition and texture analysis.

2. Materials and methods

Raw Materials

About 4.0 kg of parboiled rice bran was obtained from Lath Rice Mill, Bargarh, Odisha; while 5.0 kg of buckwheat flour and other ingredients like sugar, baking soda, spray dried milk powder, baking powder and essence(for flavour) were purchased from Local Market, Bargarh, Odisha.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), FolineCiocalteu’s reagent, protocatechuic acid, ferulic acid, quercetin, rutin, butylated hydroxytoluole(BHT), (þ)-a-tocopherol, (þ)-g-tocopherol, and (þ)-dtocopherol were obtained from Sigma (SigmaeAldrich). HPLC grade methanol and chemicals of analytical grade were purchased from Merck. Water was purified using Millipore water purification system, using Simplicity UV, Millipore.

Preparation of Muffin

Bake trials were conducted under laboratory conditions. Dough mixing, processing and baking were performed on laboratory-scale equipment. A straight dough process was used. The ingredients were weighed according to the proportions. Three flour blends were prepared by mixing buckwheat flour with rice bran in the proportions of 95:5, 90:10 and 85:15 using a mechanical blender, while 100% buckwheat flour was used as control. The four flour samples were packaged in black low density polyethylene bags and stored in 500 ml lidded plastic containers at room temperature from where samples were taken for analysis and muffin production.All ingredients were thoroughly mixed in a dough mixer to form dough, which was put into a baking pan greased with plasticized fat and covered with wrapper. The dough were fermented for 90 minutes at room temperature (28°C - 30°C) and baked at 250°C for 20 minutes. The muffins were packaged in low density polyethylene bags for consumption and stored at room temperature for future analysis.

Proximate composition

Proximate composition of muffin including protein, fat, total dietary fiber, ash and water contents were determined by standard methods of analysis (AOAC, 2000) [3]. The energy calculation was done according to their nutrients in t kcal value.

Preparation of ethanoic extracts

Buckwheat muffin dried powder (5 g) was mixed with 50 mL of ethanol/water (80/20, v/v). Extractionwas carried out by shaking the mixture at room temperature for 1 h, and left overnight. The extract was separated by filtering through filter paper (Whatman no.1), and procedure was repeated twice with 50 mL of solvent. Combined extracts were dried by vacuum-evaporator. The obtained yield was calculated based on the wet weight of the sample. The dried extract was redissolved in ethanol/water (80/20, v/v) to 10 mL volume. The extract obtained was used for investigation of total phenolics content and scavenging activity on DPPH.

Determination of total phenolic content

Total phenolic content of muffin extracts was determined spectrophotometrically by using Foline Ciocalteu’s reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999) [20]. Gallic acid was used as a standard and results were expressed as gallic acid equivalents (GAE) (mg GAE/100 g of sample on wet mass basis). The extract (0.1 mL) of crackers was diluted with pure water (7.9 mL). Foline Ciocalteu’s reagent (0.5 mL) and sodium carbonate solution (1.5 mL; concentration 20 g/100 mL) were added, and the reaction mixture was mixed thoroughly. The mixture was allowed to stand for 120 min with intermittent shaking, and the absorbance at 750 nm was measured (Systronics, UV/Vis spectrophotometer 104).

DPPH radical scavenging activity

Effect of the extracts on scavenging of 1, 1-diphenyl-2-picrylhydrazyl radicals (DPPH) was determined according to the modified method of Hatano *et al*., 1988 [10]. The concentration of the DPPH solution used in the assay was 90 mmol/L (22.5 mL 0.4 mmol/L DPPH solution (0.01577 g DPPH in 100 mL methanol) was diluted with 95 mL/100 mL methanol to 100 mL). An aliquot (1.0 mL) of the DPPH solution (90 mmol/L) was diluted in 2.9 mL methanol, and 0.1 mL of the extracts at various concentrations (0.50e30.0 mg/mL) was added. The mixture was shaken vigorously and left to stand for 60 min in the dark, then the absorbance was measured at 517 nm (Systronics, UV/Vis spectrophotometer 104) against the blank (mixture without extract). The IC50 value (mg/mL) was defined as the concentration of an antioxidant extract which was required to quench 50% of the initial amount of DPPH under the experimental conditions given. It wasobtained by interpolation from linear regression analysis.

Sensory evaluation

Sensory evaluation was carried out 24 h after baking by panelists. The panelists were selected from previously trained academic staff of the Dept of Food Science Technology and Nutrition, Sambalpur. The facilities for sensory analyses fulfilled requirements of ISO 8589 (2007) [14]. Drinking water was provided for palate cleansing between each sample. Attributes like taste, color, texture, flavor and overall acceptability was scored based on its intensity scaled. 9-Point Hedonic Scale has been used the purpose. The sensory score given by the panel have been evaluated for the sensory result, according to Tang, Hsieh, Heymann, and Huff (1999) [22], and Sikora, Kowalski, Krystyjan, Krawontka, and Sady (2007) [19]. Sensory evaluation included the selected representative properties of crackers. These properties were evaluated using a 9-points method. Marks were given based on the scale from 1 "unacceptable" to 9 "excellent". Each mark was described with words, using previously prepared standard cards (Pajin, 2000; Sikora *et al.*, 2007) [16, 19]. Texture was analyzed by the texture analyzer during its storage period at room temperature. The texture of the sample was graphically presented by the instrument and the effect of the storage was analyzed.

Statistical analysis

All analyses were performed in triplicate, except for sensory analysis and the mean values with the standard deviations (SD) are reported. Analysis of variance and Duncan's multiple range testwere used. Statistical data analysis software system SPSS Package (SPSS 20.0) was used for analysis. P values <0.05 were regarded as significant.

3. Result and discussion

Proximate composition of crackers

In order to create a gluten-free product, buckwheat muffins were formulated without wheat flour. According to our knowledge, such a product does not exist on the market. Proximate composition of raw ingredients were given in Table 1. Those results were expected as buckwheat flour was reported to contain higher protein and mineral content than wheat flour (Bonafaccia, Marocchini, & Kreft, 2003; Skrabanja *et al.*, 2004) [5, 21].

Table 1: Proximate contents of Buckwheat flour and Rice bran flour

Sr. No.	Constituents	Buckwheat Flour (%)	Rice Bran Flour (%)
1.	Moisture	9.74±0.5	10.2±0.2
2.	Carbohydrate	71.76±1.28	34.24±1.50
3.	Protein	13.02±1.42	23.04±1.22
4.	Fat	3.5±0.5	22.8±0.58
5.	Ash	1.98±0.4	9.72±0.66

Results are presented on dry weight basis. Values are means of three determinations _ SD. Values in each row with the same superscript are not significantly different (P <0.05).

The effect of supplementation of rice bran flour in 100:0, 95:5, 90:10 and 85:5 proportions with buckwheat flour have been studied. The effect of storage on texture and sensory attributes were analyzed. Effect on moisture, ash, fat and protein content of the product was studied. The muffin also under gone for sensory evaluation on color, taste, flavor, texture and overall acceptability analysis has been done. The texture and its degradation rate have also been studied. Three replications of parameters were made and consequently averaged and tabulated.

Table 2: Proximate contents of Buckwheat-Rice bran Muffin.

	Carbohydrate	Protein	Lipid	Ash	Moisture
Sample A	52.56±1.1^{a}	11.24±1.2^{b}	15.82±0.5^{c}	1.47±0.15^{d}	18.91±0.4^{c}
Sample B	50.03±1.7^{b}	12.04±0.5^{b}	16.98±0.3^{b}	1.84±0.05^{c}	19.11±0.3^{b}
Sample C	46.43±1.2^{b}	12.78±0.6^{a,b}	17.65±0.4^{a,b}	2.23±0.04^{b}	20.91±0.5^{a}
Sample D	42.88±1.0^{c}	13.86±1.0^{a}	18.52±0.8^{a}	2.68±0.11^{a}	22.06±0.2^{a}

Results are presented on dry weight basis. Values are means of three determinations _ SD. Values in each row with the same superscript are not significantly different (P <0.05).

Table 2 represents the proximate composition of developed muffin. There was increase in protein, lipid, ash and moisture with implementation of rice-bran where as the carbohydrate content get decreased. The mean values given with standard deviation. The values were found to be significant when analyzed in SPSS 20, at p<0.05%.

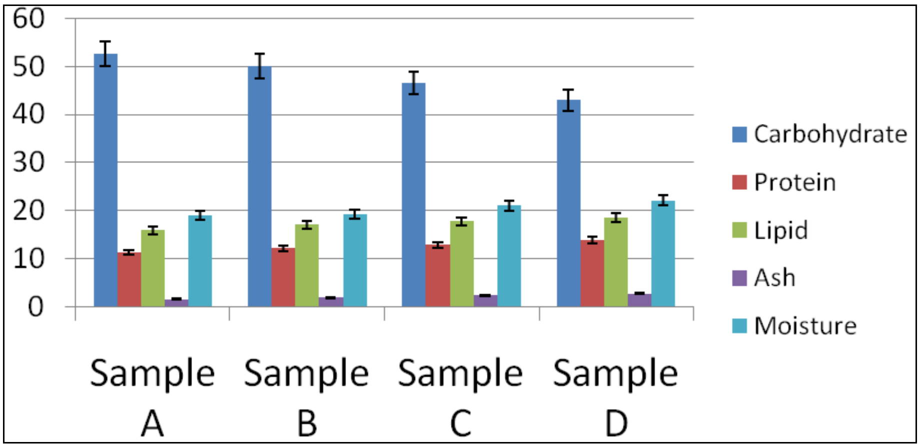

Fig. 3.1: Effect of rice bran supplementation on proximate compositionmuffin

Total phenolic content

Total phenolic content in muffins ranged from 84 mg GAE/100 g for combined formulations to 292 mg GAE/100 g for wholegrain buckwheat muffin. Phenolic compounds in buckwheat are, protocatechuic and ferulic acid whereas two flavonoids, rutin and quercetin were found in buckwheat crackers. Protocatechuic acid in buckwheat bread was reported by Alvarez-Jubete, Wijngaard, Arendt, and Gallagher (2010) [1], and syringic acid derivative and caffeic acid were quantified in buckwheat seeds.

DPPH radical scavenging activity

DPPH is a stable free radical and is usually used as a substrate to evaluate antioxidant activity of extracts obtained from plant materials. Results of DPPH scavenging activity of buckwheat muffins are presented in Table 3. Effectiveness of antioxidant activity inversely correlated with IC50 value. Wholegrain buckwheat muffins were the most effective as evidenced by their lowest IC50value. Results indicate that remarkable DPPH scavenging activity of the buckwheat muffins could have been partly due to the presence of rutin which possesses strong ability to scavenge DPPH (Yang, Guo, & Yuan, 2008) [25]. Also, significant positive correlation between total phenolic content and DPPH scavenging activity was obtained (0.96, P <0.05). The high DPPH scavenging activity of the whole buckwheat muffin may be related to their higher phenolic content compared to the muffins made with refined flour. These findings are in accordance with results of Lin *et al*., 2009 [15] who reported that methanolic extract of unhusked buckwheat enhanced bread was more effective than methanolic extract of husked buckwheat enhanced wheat bread in scavenging ability on DPPH.

Table 3: DPPH Radical scavenging activity of Buckwheat-Rice bran Muffin

	Sample A	Sample B	Sample C	Sample D
Scavenging activity on DPPH(IC50 mg/mL)	28.2 ±1.17c	21.3 ±0.77c	16.2 ± 1.85b	10.95 ± 0.17a

Results are presented on dry weight basis. Values are means of three determinations _ SD. Values in each row with the same superscript are not significantly different (P <0.05).

Sensory evaluation

Organoleptic characteristics observed in the freshly prepared muffin at different maturity condition are presented in Table 4. The color, flavor, appearance, taste and texture of the prepared muffins were graded as like extremely to dislike very much with score values as 9-1. The maximum score value of 9.0 were found in sample 90:10 combination. The changes noted in each quality attribute of the muffins had influenced the overall acceptability score values. From the data on the organoleptic evaluation of the muffins indicated that none of the raisins had overall acceptability score below 6. The sample C had a better appearance of scored better next to highest score because of its bright colour and texture.

Table 4: Sensory Evaluation of Buckwheat-Rice bran Muffin

	Sample A	Sample B	Sample C	Sample D
Color	5.9±0.99^{c}	5.8±0.42^{c}	8.1±0.56^{a}	7.4±0.69^{b}
Flavor	5.7±0.94^{c}	6.2±0.42^{c}	8.3±0.48^{a}	7.6±0.51^{b}
Appearance	4.5±0.52^{d}	5.7±0.48^{c}	8.4±0.51^{a}	7.3±0.48^{b}
Taste	6.5±0.52^{c}	7.5±0.52^{b}	8.3±0.48^{a}	7.4±0.51^{b}
Texture(Crisp)	7.4±0.51^{c}	8.2±0.42^{a}	8.0±0.51^{a,b}	7.7±0.56^{b,c}
OAA	6±0.82^{d}	6.68±0.45^{c}	8.25±0.52^{a}	7.52±0.55^{b}

Results are presented on dry weight basis. Values are means of three determinations _ SD. Values in each row with the same superscript are not significantly different (P <0.05).

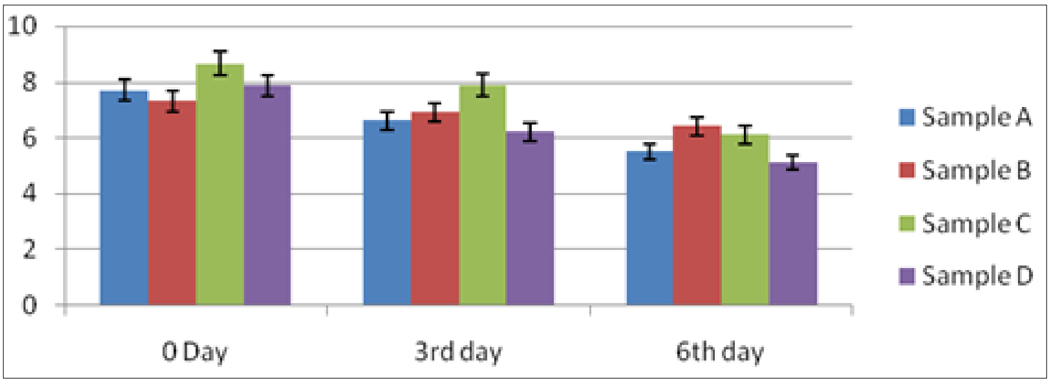

Fig 3.2: Effect of storage on Sensory analysis of muffin

Shelf life and texture deterioration

The buckwheat muffin was prepared without addition of any type of preservatives and stored in room temperature. Hence, it loses its moisture and simultaneously gets a stiff texture, which deteriorates its quality. On the other hand, when the sample stored in refrigeration condition, the product becomes soggy after 4days which was also not preferable as per quality point of view. The moisture loss and texture profile was shown in fig. 3.3 and 3.4.

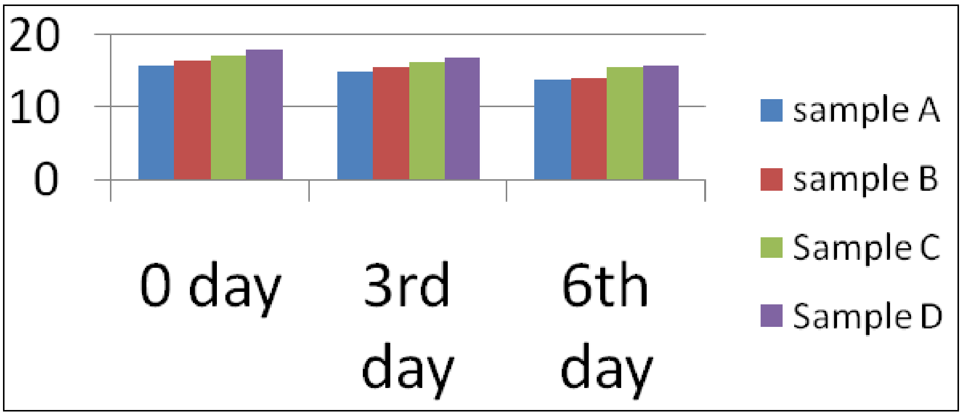

Fig 3.3: Effect of storage on Moisture content of muffin

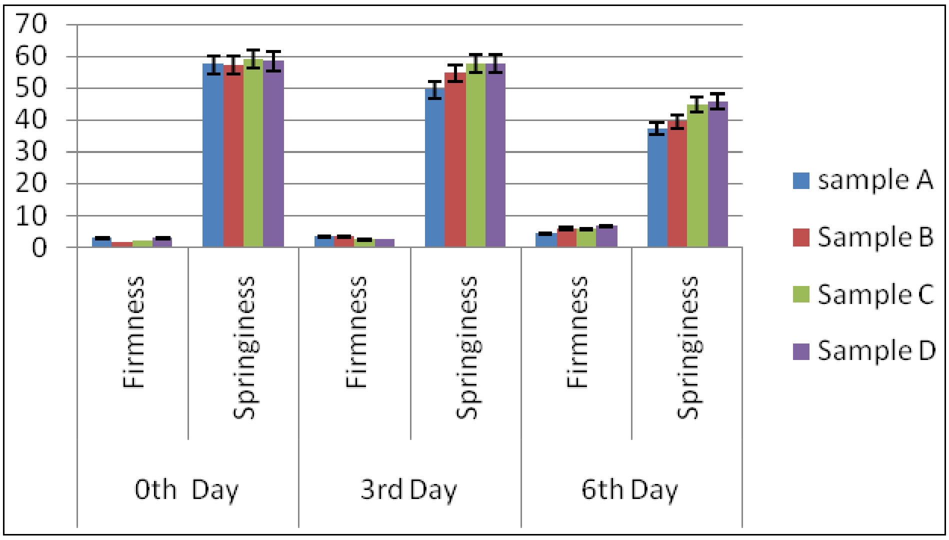

Fig 3.4: Effect of storage on Firmness and Springiness of muffin

Conclusion

The result obtained in the present study shows that the proximate contents of muffin are influenced by the supplementation of rice bran in buckwheat muffin. Results of the proximate composition of the muffin samples are presented in fig 4.6. The moisture, crude protein, crude fat and ash contents increased significantly from 18.07% to 22.67%, 11.04% to 13.10%, 15.87% to 18.7%and 1.46% to 2.61% respectively; with increased level of supplementation. The carbohydrate content was found to be decreased to 42.953 % from 52.112%. This is expected since there is very little carbohydrate left in rice bran after milling. Total phenolic content in muffins ranged from 84 mg GAE/100 g for combined formulations to 292 mg GAE/100 g for wholegrain buckwheat muffin and had a significant positive correlation between total phenolic content and DPPH scavenging activity. The muffins had an attractive and appreciable color and flavour. In sensory analysis, the OAA of 90:10 proportions of buckwheat flour and rice bran flour was mostly acceptable by score of 8.25. The texture analysis by the texture analyzer represents Springiness of the formulations varied from 57.411 to 59.44 on 0day, from 49.321 to 57.523 on 3rd day and 37.282 to 45.874 on 6th day. By analysis of texture it was found that formulation 90:10 has overall good texture, springiness and firmness. The shelf life of muffin was found to be 5 days because after that the sensory scale found to be degraded. The texture of muffin was sticky as compared to the first day in this period. From study it was found that muffin containing 10% of rice bran is widely accepted and found to be the better composition.

New formulations for gluten-free buckwheat muffins were developed under the laboratory conditions and compared. Muffins made from buckwheat flours have significantly higher ($P<$ 0.05) total phenolic content and radical scavenging activity. They were also characterized with significantly higher ($P<0.05$) protein content. Additionally, the presence of flavonoids in all types of buckwheat muffins would be beneficial and contribute to the functionality of products. Sensory quality of the muffins showed that buckwheat flours may be used in gluten-free muffin formulation without adversely affecting the sensory properties of muffins. The introduction of buckwheat muffins in the market would increase the diversity of functional bakery products and, even more importantly, of functional foods suitable for celiac disease patients.

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