

Original Research Article

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Development and Evaluation of Functional Biscuits from Under utilised Crops of Ladakh

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ABSTRACT

Multigrain biscuits were formulated by blending refined wheat flour with barley and buckwheat flours in the ratios of 100:0:0::WF:BF:BWF; 0:100:0::WF:BF:BWF; 80:20:0::WF:BF:BWF; 70:20:10::WF:BF:BWF; 60:20:20::WF:BF:BWF; 0:20:30::WF:BF:BWF; 40:20:40::WF:BF:BWF; 30:20:50::WF:BF:BWF. Incorporation of buckwheat flour led to increase in crude fibre, crude fat, ash, carbohydrate and antioxidant activity of multigrain biscuits, except moisture and crude protein contents where the reverse is true. Among the treatments, the highest mean moisture (4.20 %) mean crude protein (7.21 %) contents were observed in T₁ (100:0:0::WF:BF:BWF) and T₂ (0:100:0::WF:BF:BWF), respectively. The highest mean crude fibre (3.52 %), crude fat (23.34 %), ash (1.74 %), carbohydrate (73.68 %) and antioxidant activity (45.56 %) were observed in T₈ (30:20:50::WF:BF:BWF). Biscuits were stored for a period of 90 days during which there was a significant decline in nutritional as well as functional attributes. The blended biscuits were found to be within safe limits even after the storage for 150 days and the mean microbial count was found to be 27.28 x 10² cfu/g.

Keywords

Buckwheat, Barley, Multigrain biscuits, Apricot, Pseudo-cereal, DPPH scavenging activity

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Introduction

Buckwheat (*Fagopyrum esculentum* Moench), a highly nutritious pseudo-cereal known as a source of dietary fiber and starch (Skrabanja *et al.*, 2004), protein with favourable amino acids and vitamins (Bonafaccia *et al.*, 2003a), essential minerals (Steadman *et al.*, 2001) and trace elements (Bonafaccia *et al.*, 2003b). Phenolic compounds such as rutin, quercetin, orientin, vitexin, isovitexin, isoorientin, catechins and kaempferol-3-rutinoside (Dietrych-Szostak and Oleszek, 1999) are also

found in buckwheat. Compared to frequently used cereals, buckwheat has been reported to have higher antioxidant activity, mainly due to high rutin, catechins and other polyphenols. These components of buckwheat possess health benefits like reduction of high blood pressure, blood sugar control, lower blood cholesterol, prevention of fat accumulation, constipation (Kayashita *et al.*, 1996), colon carcinogenesis and mammary carcinogenesis (Liu *et al.*, 2001), strengthen capillary blood vessels and suppresses plasma cholesterol and gallstone formation (Tomotake *et al.*, 2000).

Another functionality of buckwheat is due its gluten-free characteristics making it suitable diet for celiac disease patients (Fessas *et al.*, 2008).

Barley (*Hordeum vulgare* L.) is considered as a functional grain because it contains β -glucan, B-complex vitamins, tocotrienols, tocopherols and has significant antioxidant potential (Sharma and Gujral, 2010a). Barley has higher amount of phenolic compounds and antioxidant activity as compared to the more widely consumed cereals, wheat and rice (Sharma *et al.*, 2012). Studies have shown that barley flour has high content of dietary fiber and high proportion of soluble fiber especially β -glucan.

Health effects of β -glucans are suggested to lower plasma cholesterol, improving lipid metabolism, reducing glycemic index and boosting the immune system. Insoluble fiber is known for reduction in the risk of colon cancer (Potty, 1996). In barley most of the free phenolics are flavanols and tocopherols, whereas the bound phenolics are mainly phenolic acids (ferulic acid and pcoumaric acid) (Holtekjolen *et al.*, 2006).

Due to changing lifestyle, the people have started demanding ready to cook or ready to serve convenience foods. Various epidemiological studies have shown that diet lacking fiber and minerals may be the cause of various gastrointestinal and cardiovascular diseases (Kumari and Grewal, 2007). Hence, incorporation of fibre rich ingredients in the baked products such as biscuits will improve their nutraceutical properties and help to cater the health needs of various cross sections of the population. Keeping in view, the tremendous benefits of the selected underutilized crops, i.e. buckwheat and barley, the current study was undertaken to assess the nutritional and nutraceutical properties of the developed product.

Materials and Methods

Raw materials

Raw grains of buckwheat (*Fagopyrum esculentum*) and barley (*Hordeum vulgare*) and dried apricot (*Prunus armeniaca*) were procured from Leh, Ladakh, India. Refined wheat flour (*Triticum aestivum*), ghee (vegetable fat), sodium bicarbonate and cane sugar were purchased from local market of Jammu. Cane sugar was grounded into fine powder using grinder (Philips, Model: HL 1632, New Delhi, India). Aluminium laminated pouches used for packaging of multigrain biscuits were obtained from Vishwas Traders, Jammu.

Development of biscuits

The multigrain flours of wheat, barley and buckwheat were blended together in different ratios as per the treatments given below. The process for preparation of biscuits was standardized using creaming method given by Whitley (1995). The ingredients used for the preparation of biscuits were flour: 70g, apricot powder: 30g, ghee: 30g, sugar: 30g, sodium bicarbonate: 1.5g and water: 30ml. The fat was creamed with sugar and hot water. To this, all the other ingredients *viz.* composite flour, apricot powder and sodium bicarbonate were added, mixed and kneaded to form a dough and then rolled and cut into shape with the help of cutter and baked at 160 °C till done. The biscuits were then cooled and packed.

Chemical properties and statistical analysis

Moisture, protein, ash and fat contents were measured according to AOAC (2002). The carbohydrate content was calculated by difference method by subtracting the sum of moisture, fat, protein and ash contents from 100. All the analyses were the means of three

replicates. The antioxidant activity was determined by DPPH (1,1, diphenyl-2-picrylhydrazyl) scavenging activity (Brand-Williams *et al.*, 2002). Microbial count (total plate count) was recorded by spread plate technique, described by Palczar and Chan, 1991, using Potato dextrose agar (PDA). All the experiments were performed in triplicates. Data collected from aforesaid experiments was subjected to ANOVA (statistical analysis) with the help of factorial completely randomized design (Gomez and Gomez, 2010) and using the OP Stat software package.

Results and Discussion

Proximate composition of multigrain biscuits

Moisture content

The minimum and maximum moisture contents of 3.13 and 4.20 % was recorded in T₂ (0:100:0::WF:BF:BWF) and T₁ (100:0:0::WF:BF:BWF), respectively. With the incorporation of the buckwheat-barley flours, there was reduction in moisture content (Table 1) which might be due to low levels of protein content in these flours (Mustafa *et al.*, 1986). Jan *et al.*, (2015) also reported that the moisture content of cookies made from the blends of wheat flour and buckwheat flour, decreased with the increase in the ratio of buckwheat flour. Similar findings were reported by Gupta *et al.*, (2011) in biscuits prepared from wheat flour incorporated with barley flour. There was significant increase in the mean moisture content from 3.07 to 4.46 % during 90 days storage period. The gain in moisture content was also supported by Nagi *et al.*, (2012) who reported that higher moisture pick up of biscuits during storage could be due to greater hygroscopicity of the product and storage environment (temperature and relative humidity). Jainudin and Hasnah (1991) also noticed increase in moisture

content in coconut blended biscuits upon storage.

Crude protein

Supplementation of refined wheat flour with composite flour led to the decrease in crude protein of the multigrain biscuits from 7.06 % in T₁ (100:0:0::WF:BF:BWF) to 5.18 % in T₈ (30:20:50::WF:BF:BWF) (Table 2). The decrease in protein content might be the result of the appreciably lower protein contents of the composite flour as well as due to dilution of gluten content of wheat flour in biscuits. Baljeet *et al.*, (2010) reported decrease in protein content in biscuits incorporated with buckwheat flour from 7.20 ± 0.05 % (control) to 5.60 ± 0.06 % (40 % BWF). There is decrease in protein content during storage which might be due to hydrolysis of peptide bonds with the help of protease enzyme that cause splitting of protein molecules. Similar behaviour of crude protein was also observed by Nwabueze and Atuonwu (2007) in African bread fruit seeds incorporated biscuits which confirm our findings.

Crude fiber

Highest crude fiber content of 3.52 % was recorded in treatment T₈ (30:20:50::WF:BF:BWF) followed by 3.12 % in T₂ (0:100:0::WF:BF:BWF) and 2.16 % in T₇ (40:20:40::WF:BF:BWF) and the lowest of 1.50 % was recorded in T₁ (100:0:0::WF:BF:BWF) (Table 3). Thus incorporation of composite flour resulted into the increase in fiber content in biscuits which might be due to the higher fiber content in barley and buckwheat flours as compared to wheat flour. Baljeet *et al.*, (2010) also reported increase in crude fiber in cookies, with the incorporation of buckwheat flour. As the storage period advanced, the crude fibre content decreased significantly from initial levels of 2.36 to 1.92 %.

Table.1 Effect of treatments and storage period on moisture content (%) of multigrain biscuits

Treatments	Storage period (days)				
	0	30	60	90	Mean
T ₁ (100:0:0::WF:BF:BWF)	03.42	03.82	04.35	05.23	04.20
T ₂ (0:100:0::WF:BF:BWF)	02.56	02.89	03.32	03.78	03.13
T ₃ (80:20:0::WF:BF:BWF)	03.24	03.62	04.01	04.64	03.87
T ₄ (70:20:10::WF:BF:BWF)	03.16	03.48	03.91	04.48	03.75
T ₅ (60:20:20::WF:BF:BWF)	03.10	03.46	03.89	04.47	03.74
T ₆ (50:20:30::WF:BF:BWF)	03.06	03.38	03.85	04.43	03.67
T ₇ (40:20:40::WF:BF:BWF)	03.03	03.33	03.80	04.36	03.63
T ₈ (30:20:50::WF:BF:BWF)	03.00	03.27	03.74	04.30	03.57
Mean	03.07	03.40	03.85	04.46	

Effects		C.D. (p ≤ 0.05)
Treatment	0.02	
Storage		0.01
Treatment x Storage	0.04	

Table.2 Effect of treatments and storage period on crude protein (%) of multigrain biscuits

Treatments	Storage period (days)				
	0	30	60	90	Mean
T ₁ (100:0:0::WF:BF:BWF)	07.22	07.17	07.04	06.82	07.06
T ₂ (0:100:0::WF:BF:BWF)	07.34	07.32	07.20	06.99	07.21
T ₃ (80:20:0::WF:BF:BWF)	07.26	07.21	07.10	06.91	07.12
T ₄ (70:20:10::WF:BF:BWF)	07.06	07.02	06.90	06.69	06.91
T ₅ (60:20:20::WF:BF:BWF)	06.97	06.94	06.84	06.61	06.84
T ₆ (50:20:30::WF:BF:BWF)	06.73	06.69	06.57	06.35	06.58
T ₇ (40:20:40::WF:BF:BWF)	06.41	06.38	06.24	06.05	06.27
T ₈ (30:20:50::WF:BF:BWF)	05.34	05.31	05.12	04.96	05.18
Mean	06.79	06.75	06.62	06.42	

Effects		C.D. (p ≤ 0.05)
Treatment	0.02	
Storage		0.01
Treatment x Storage	NS	

Table.3 Effect of treatments and storage period on crude fiber (%) of multigrain biscuits

Treatments	Storage period (days)				
	0	30	60	90	Mean
T ₁ (100:0:0::WF:BF:BWF)	01.67	01.60	01.50	01.23	01.50
T ₂ (0:100:0::WF:BF:BWF)	03.29	03.23	03.13	02.85	03.12
T ₃ (80:20:0::WF:BF:BWF)	01.81	01.75	01.64	01.4	01.65
T ₄ (70:20:10::WF:BF:BWF)	01.94	01.87	01.75	01.49	01.76
T ₅ (60:20:20::WF:BF:BWF)	02.01	01.94	01.84	01.57	01.84
T ₆ (50:20:30::WF:BF:BWF)	02.15	02.09	01.99	01.71	01.98
T ₇ (40:20:40::WF:BF:BWF)	02.33	02.26	02.16	01.89	02.16
T ₈ (30:20:50::WF:BF:BWF)	03.69	03.63	03.53	03.25	03.52
Mean	02.36	02.29	02.19	01.92	

Effects		C.D. (p ≤ 0.05)
Treatment	0.02	
Storage		0.01
Treatment x Storage	NS	

Table.4 Effect of treatments and storage period on crude fat (%) of multigrain biscuits

Treatments	Storage period (days)				
	0	30	60	90	Mean
T ₁ (100:0:0::WF:BF:BWF)	20.82	20.75	20.50	20.02	20.52
T ₂ (0:100:0::WF:BF:BWF)	21.21	21.12	20.89	20.44	20.91
T ₃ (80:20:0::WF:BF:BWF)	20.90	20.82	20.58	20.11	20.60
T ₄ (70:20:10::WF:BF:BWF)	21.03	20.94	20.71	20.22	20.72
T ₅ (60:20:20::WF:BF:BWF)	21.67	21.57	21.35	20.88	21.36
T ₆ (50:20:30::WF:BF:BWF)	22.73	22.64	22.41	21.94	22.43
T ₇ (40:20:40::WF:BF:BWF)	23.12	23.05	22.8	22.34	22.82
T ₈ (30:20:50::WF:BF:BWF)	23.65	23.54	23.33	22.85	23.34
Mean	21.89	21.80	21.57	21.10	

Effects		C.D. (p ≤ 0.05)
Treatment	0.02	
Storage		0.01
Treatment x Storage	NS	

Table.5 Effect of treatments and storage period on ash (%) of multigrain biscuits

Treatments	Storage period (days)				
	0	30	60	90	Mean
T ₁ (100:0:0::WF:BF:BWF)	01.32	01.29	01.25	01.11	01.24
T ₂ (0:100:0::WF:BF:BWF)	01.17	01.16	01.09	00.98	01.10
T ₃ (80:20:0::WF:BF:BWF)	01.26	01.22	01.18	01.06	01.18
T ₄ (70:20:10::WF:BF:BWF)	01.33	01.31	01.27	01.10	01.25
T ₅ (60:20:20::WF:BF:BWF)	01.42	01.42	01.34	01.20	01.34
T ₆ (50:20:30::WF:BF:BWF)	01.56	01.54	01.46	01.35	01.47
T ₇ (40:20:40::WF:BF:BWF)	01.67	01.63	01.59	01.48	01.59
T ₈ (30:20:50::WF:BF:BWF)	01.82	01.81	01.74	01.62	01.74
Mean	01.44	01.42	01.36	01.23	

Effects		C.D. (p ≤ 0.05)
Treatment	0.02	
Storage		0.01
Treatment x Storage	NS	

Table.6 Effect of treatments and storage period on carbohydrate (%) of multigrain biscuits

Treatments	Storage period (days)				
	0	30	60	90	Mean
T ₁ (100:0:0::WF:BF:BWF)	73.06	73.13	73.28	73.65	73.27
T ₂ (0:100:0::WF:BF:BWF)	70.79	70.86	71.01	71.38	71.01
T ₃ (80:20:0::WF:BF:BWF)	72.87	72.94	73.09	73.46	73.09
T ₄ (70:20:10::WF:BF:BWF)	72.94	73.01	73.16	73.53	73.16
T ₅ (60:20:20::WF:BF:BWF)	73.06	73.13	73.28	73.65	73.28
T ₆ (50:20:30::WF:BF:BWF)	73.19	73.26	73.41	73.78	73.37
T ₇ (40:20:40::WF:BF:BWF)	73.34	73.41	73.56	73.93	73.56
T ₈ (30:20:50::WF:BF:BWF)	73.47	73.54	73.69	74.06	73.68
Mean	72.84	72.91	73.05	73.40	

Effects		C.D. (p ≤ 0.05)
Treatment	0.02	
Storage		0.01
Treatment x Storage	0.04	

Table.7 Effect of treatments and storage period on antioxidant activity (%) of multigrain biscuits

Treatments	Storage period (days)				
	0	30	60	90	Mean
T ₁ (100:0:0::WF:BF:BWF)	34.62	33.03	30.30	24.30	30.56
T ₂ (0:100:0::WF:BF:BWF)	46.81	44.53	42.55	37.58	42.86
T ₃ (80:20:0::WF:BF:BWF)	36.40	35.64	32.52	27.73	33.07
T ₄ (70:20:10::WF:BF:BWF)	38.34	36.89	33.39	29.59	34.55
T ₅ (60:20:20::WF:BF:BWF)	41.40	40.29	37.19	32.82	37.92
T ₆ (50:20:30::WF:BF:BWF)	43.87	42.48	40.52	35.40	40.56
T ₇ (40:20:40::WF:BF:BWF)	46.50	44.30	41.49	37.41	42.42
T ₈ (30:20:50::WF:BF:BWF)	48.93	47.78	44.83	40.73	45.56
Mean	42.10	40.61	37.84	33.19	

Effects		C.D. (p ≤ 0.05)
Treatment	0.01	
Storage		0.02
Treatment x Storage	0.04	

Table.8 Effect of treatments and storage period on microbial count (c.f.u/g) of Multigrain biscuits

Treatments	Storage period (days)			
	30	90	150	Mean
T ₁ (100:0:0::WF:BF:BWF)	2.95 x 10 ²	15.98 x 10 ²	38.11 x 10 ²	19.01 x 10²
T ₂ (0:100:0::WF:BF:BWF)	2.50 x 10 ²	14.53 x 10 ²	26.39 x 10 ²	14.47 x 10²
T ₃ (80:20:0::WF:BF:BWF)	2.76 x 10 ²	15.80 x 10 ²	34.16 x 10 ²	17.57 x 10²
T ₄ (70:20:10::WF:BF:BWF)	2.55 x 10 ²	15.61 x 10 ²	29.12 x 10 ²	15.76 x 10²
T ₅ (60:20:20::WF:BF:BWF)	2.41 x 10 ²	14.46 x 10 ²	24.91 x 10 ²	13.92 x 10²
T ₆ (50:20:30::WF:BF:BWF)	2.31 x 10 ²	10.35 x 10 ²	23.72 x 10 ²	12.12 x 10²
T ₇ (40:20:40::WF:BF:BWF)	2.24 x 10 ²	09.29 x 10 ²	21.08 x 10 ²	10.87 x 10²
T ₈ (30:20:50::WF:BF:BWF)	2.02 x 10 ²	11.06 x 10 ²	20.75 x 10 ²	11.27 x 10²
Mean	2.46 x 10²	13.38 x 10²	27.28 x 10²	

Effects	C.D. (p ≤ 0.05)
Treatment	0.02
Storage	0.01
Treatment x Storage	0.03

Treatment details for multigrain biscuits

Treatments	Wheat flour	Barley flour	Buckwheat flour
T ₁	100	0	0
T ₂	0	100	0
T ₃	80	20	0
T ₄	70	20	10
T ₅	60	20	20
T ₆	50	20	30
T ₇	40	20	40
T ₈	30	20	50

The decrease in fibre content might be due to the heat and moisture stabilizers which degrade pectic substances. The relevance of our findings with respect to the fiber content is also supported by Butt *et al.*, (2007) in vitamin A fortified cookies.

Crude fat

In the current study, the crude fat content increased significantly from 20.52 % to 23.34 % with the increase in the ratios of barley and buckwheat flours in multigrain biscuits, (Table 4).

The reason behind the increase was probably due to the oil retention ability of buckwheat flour during baking process. While studying the quality assessment of gluten free crackers based on buckwheat flour, Sedej *et al.*, (2011b) reported that the fat content of the wholegrain buckwheat crackers was significantly higher in comparison to wholegrain wheat crackers. The fat content of biscuits decreased significantly ($P \leq 0.05$) from initial mean level of 21.89 % to 21.10 % during 90 days of storage. The decrease in fat content might be due to the lipolytic activity of the enzymes i.e. lipase and lipoxidase. These findings are in accordance with the findings of Singh *et al.*, (2008) who reported that during storage, the crude fat content decreased in biscuits supplemented with various levels of jaggery.

Ash

The ash content represents the total mineral content in the product. All the blends varied significantly in ash content resulting from differences among individual treatment ratios (Table 5). The ash content of multigrain biscuits increased from 1.24 % in T₁ i.e. control (0:100:0::WF:BF:BWF) to 1.74 % in T₈ (30:20:50::WF:BF:BWF). The increasing trend in ash content might be due to high minerals in composite flours as compared to that of wheat flour.

An increase in the ash content of different cereal products with the addition of buckwheat milling products was also reported by Atalay (2009) thus confirming the current study. A significant decrease in the ash content from 1.44 % to 1.23 % was noticed during 90 days of storage. Similar reports have also been reported by Nwabueze and Atuonwu (2007) while assessing organoleptic and nutritional evaluation of wheat biscuits supplemented with African bread fruit seed flour.

Carbohydrate

There was a significant ($P \leq 0.05$) increase in carbohydrate content of multigrain biscuits with the incorporation of composite flour (Table 6) and this might be attributed to its higher contents in composite flour than wheat

flour. Similar trend (Jan *et al.*, 2015) was found in biscuits incorporated with buckwheat flour. The highest carbohydrate content of 73.68 % was recorded in T₈ (30:20:50::WF:BF:BWF) whereas the lowest carbohydrate content of 71.01 % was recorded T₂ (0:100:0::WF:BF:BWF). With the progression of storage period, the carbohydrate content increased significantly from 72.84 to 73.40 % which might be due to the breakdown of insoluble polysaccharides into simple sugars. The reports of Varshney *et al.*, (2008) in defatted peanut biscuits are in agreement with our findings.

Antioxidant activity (DPPH scavenging activity)

In the current study, the DPPH inhibition potential or scavenging activity of the blended biscuits was higher than the wheat flour biscuits as the consequence of higher antioxidant activity of barley and buckwheat flours than wheat flour (Table 7). They might react with free radicals, particularly with the peroxy radicals, which are the major propagators of the auto-oxidation chain of fat, thereby terminating the chain reaction. Further increase in antioxidant activity of blended biscuits can be attributed to greater generation of melanoidins in buckwheat which is supported by higher non-enzymatic browning values of buckwheat cookies than wheat cookies.

Earlier Sharma and Gujral (2014) reported increase in the DPPH radical scavenging activity with the increase in barley supplementation in cookies and Jan *et al.*, (2015) also reported similar trend in buckwheat incorporated cookies. DPPH inhibition potential of the biscuits decreased from 42.10 and 33.19 % during 90 days of storage period. These results are in accordance with the findings of Reddy *et al.*, (2005) in biscuits enriched with plant extracts.

Microbial evaluation

Initially, the multigrain biscuits did not have any microbial contamination. After 30 days of storage period, (Table 8), the highest total plate count of 2.95×10^2 cfu/g was observed in treatment T₁ (100:0:0::WF:BF:BWF) and the lowest microbial count of 2.02×10^2 cfu/g was recorded in treatment T₈ (30:20:50::WF:BF:BWF). However, after 150 days storage, the same treatments recorded highest microbial load of 38.11×10^2 cfu/g and lowest load of 20.75×10^2 cfu/g, respectively. The mean microbial count during this period ranged from 2.46×10^2 to 27.28×10^2 cfu/g. The increase in microbial count might be due to the increase in moisture content during storage. Microbial studies indicated that the biscuits stored at room temperature up to 3 months had better stability as the microbial count remained within permissible limits of ISI specification (IS: 7463-1988). The results of our study corroborated with the findings of Nagi *et al.*, (2012) in cereal bran incorporated biscuits.

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