



Research Article

Polyphenolic Content, Antioxidant Activity and Anti-Glycation Effect of Bread Enriched with Buckwheat Flour

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Abstract | The study aimed to determine the effect of buckwheat flour incorporation into bread on polyphenolic content, antioxidant activity and inhibitory potential against the formation of advanced glycation end products (AGEs). Buckwheat flour was substituted at 30%, 40%, and 50% incorporation levels with wheat flour to prepare buckwheat-containing bread (BWB) while control bread (CB) was made from 100% wheat flour. Antioxidant activity, total phenolic and flavonoid content of bread were determined by colorimetric assays (ABTS and DPPH), folin-ciocalteu method and aluminum chloride assay, respectively. Bovin serum albumin (BSA)-glucose assay was used to measure the inhibitory effect of bread AGEs formation. Total phenolic, flavonoid, rutin content, and antioxidant activity were significantly ($P<0.05$) higher at all incorporation levels compared to control bread. The incorporation of buckwheat flour into bread resulted in significantly higher ($P<0.05$) inhibition of AGEs at all incorporation levels compared to control bread. In conclusion the incorporation of buckwheat flour into bread resulted in a product with improved bioactive properties. The research is valuable for understanding the effects of dietary interventions on various biomarkers related to cardiovascular health and metabolic disorders, ultimately contributing to the development of dietary strategies for the prevention and management of cardio-metabolic diseases.

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Keywords | Buckwheat bread, Control bread, Advance glycation end products, Rutin, Polyphenols, antioxidants



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Introduction

Buckwheat is a member of the polygonance family and is produced in Gilgit-Baltistan, Pakistan, as a minor crop. The short growing season make it suitable for cropping sites in the far north of Pakistan. Buckwheat is a crop of cold, wet, temperate regions and is susceptible to high temperature and hot, dry winds therefore the topography and climate of

Gilgit-Baltistan favours its production (Facho, 2016). Around the world many varieties are grown, only 09 species have agriculture importance (Krkoskova and Mrazova, 2005). Generally, as a food only two species of buckwheat are used; common buckwheat is widely grown, while tartary buckwheat is grown in mountainous area (Bonafaccia *et al.*, 2003; Li and Zhang, 2001). Due to its high nutritional content, buckwheat has generated more attention from

consumers and food processors in recent decades (Gimenez-Bastida, 2018; Kreft and Mateja, 2008).

Buckwheat contains rutin, a secondary metabolite that has been found to have antioxidants, anti-inflammatory, anti-cancer properties (Kreft *et al.*, 2006; Zhang, 2012). Animal's studies mention that protein extracts contain anticancer and cholesterol-lowering properties (Tomotake, 2006). Buckwheat containing-bread has been demonstrated to significantly reduce postprandial blood glucose and insulin levels when compared to white wheat bread (Skrabanjan, 2001). D-Chiro-Inositol has been suggested by Kawa *et al.* (2003) to be beneficial for the treatment of type 2 diabetes (Burluc, 2012; Hatcher, 2008). Dietary advanced glycation end products (AGEs) are produced by the millard reaction in thermally processed foods (Bastos and Gulgiucci, 2015). Uribarri (2010) stated that dietary AGEs contribute significantly to produce in living things, which can lead to oxidative stress and increase the risk of diabetes and cardiovascular disease. One could presence of AGEs by adhering to the low-dietary AGEs diet recommended by (Kellow and Savage, 2013; Vlassare and Uribarri, 2014). Nonetheless, it was recommended to consume foods that naturally block AGEs (Peng, 2011). Common buckwheat has been reported to have a respectable quantity of rutin (Jiang *et al.*, 2007; Szawara-Nowak *et al.*, 2014; Fabajan, 2003).

The study hypothesized that incorporating buckwheat flour to wheat bread would increase the bread polyphenol content and antioxidant activity, potentially resulting in improved antioxidant defenses and against oxidative damage protection. Furthermore, the proposed research can contribute to the knowledge on functional foods, provide evidence for the potential benefits of incorporating buckwheat into bread, and guide future dietary interventions for the prevention and management of cardio-metabolic disease, offering individuals a tasty and nutritious dietary option to support their overall health and well-being.

Materials and Methods

Basic materials

Conventionally used baking ingredients that include sugar, oil, and yeast was procured. Refined wheat flour (Pakistan-2013) was procured from Bilour flour and general mills private limited Peshawar

while other baking ingredients were purchased from Peshawar local market. Buckwheat grains (raw dried) were purchased from Pakistan Council of Scientific and Industrial Research (PCSIR) Skardu, Gilgit-Baltistan Pakistan.

Flours preparation

Wheat and buckwheat flours were measured independently for flour manufacture using a digital electronic scale (Metra, model TL 600). First, three treatment bread samples of 30%, 40%, and 50% composition of buckwheat flour were prepared while control bread was prepared from 100 % wheat flour.

Bread preparation

According to Amendola (2002), the straight dough technique was used to produce the bread. For ten minutes, the mixture was kneaded by hand to create soft and consistent dough. After that, the dough was left in baking pans and allowed to ferment for 10 minutes at (28°C) temperature. After that, it was kept for 30 min at 28°C in an electronic oven (Panasonic Digital Oven). The baked bread was taken out of the baking pans right away, allowed to cool at room temperature, and then packed.

Samples preparation and storage

Bread samples were kept in an air oven for 24 hours at 50 degrees Celsius in order to conduct chemical analyses of various parameters. The jars were sealed with aluminum foil and refrigerated at 4 °C until analyzed.

Chemicals

Methanol, sodium carbonate, gallic acid, aluminum chloride, folin-ciocalteu reagent, potassium acetate, ABTS (2,2-azinobis zothiazoline-6-sulfonate), DPPH (2,2 -Diphenyl-1-picrylhydrazyl), radical cation, trolox, KOH, oxidase/peroxidase, pepsin, NaOH and sodium acetate buffer.

Sample extraction for determination of polyphenols and antioxidant activity

Extraction of polyphenols and antioxidant activity were collected from breads and raw flour and composite bread samples by following the method (Awika *et al.*, 2004). In short, 1 g dry sample was kept into pre-labeled plastic Greiner tubes, shaken for 2 hours in shaking water bath (1900 x g for 10 min at 25°C). The supernatant was kept in glass vials protected with aluminum foil.

Determination of total phenolic content

Gallic acid was used in the Folin-Ciocalteu (FC) technique to calculate the total phenolic content (Khan, 2013). Briefly, FC solution (1 ml) was placed in a beaker and diluted ten folds to make FC reagent solution. A test tube was filled with 0.8 ml of the Fc reagent solution, Fc solution (1 ml) and 0.2 ml of the sample extract for the experiment. After three minutes, two milliliters of 15% of sodium carbonate solution and two milliliters of deionized water (milli q) were put to the tube to bring the total volume to five milliliters. For one hour, the solution mixture was kept at room temperature the absence of light. The OD value of each sample was taken in quadruplicate after an hour.

Determination of total flavonoid content

Aluminum chloride (ALCL₃) colorimetric assay with a standard quercetin were used to determine total flavonoids (Bag *et al.*, 2015). the tubes containing the sample extract and solution combination were first filled with 0.5 ml of the sample extract and 0.1 ml of aluminum chloride (10%), then 0.1 ml of potassium acetate (1 M), 1.5 ml methanol (80%) and 2.8 ml water (distilled) was added. The OD at 415nm measured while the tubes were kept at room temprature in the dark. As a blank, distilled water was utilized. The resulted presented as mg quercetin equivalent (QE) per 100g dry sample.

Determination of rutin content

Rutin content was measured using the method by Kreft *et al.* (2006) outlined. A 100 mg buckwheat sample was placed in a falcon tube and combined with 10 ml (90%) methanol solution. The sample was centrifuged at 5000 rpm for 10 minutes after being in a water bath for an hour at 25°C and supernatant was taken out. Next, using pipette, 0.2 ml 5% Aluminum chloride mixed with 1ml sample extract. To make sample mixed 0.2 ml methanol with 1 ml sample extract. After 30 min the absorbance was measured at 420 nm. The concentration of Rutin was calculated from the difference of sample solution with AlCl₃ and sample with methanol.

Determination of antioxidant activity

To determine total antioxidant activity the First DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed to following by Cheung *et al.* (2003). 200µl sample extract mixed

with 2.85ml DPPH (0.1M) solution (10mg DPPH with 250ml methanol) to start the experiment. For thirty minutes, the sample solution was kept at room temperature in a dark environment. The OD was found out at 517nm using spectrophotometer (Gynesis 10). The dry sample findings were shown in µmol TE/g.

The second assay ABTS (2,2 aazinobis (3-ethylbenzothiazoline-6-sulfonate) used radical cation method of Re *et al.* (1999). First 7mM ABTS solution was prepared by taking 19.2 mg ABTS and dissolved in 5ml H₂O then weighed 33mg potassium persulphate and mixed with 50ml distilled water to make 2.45mM potassium persulphate solution. The obtained solution samples were diluted with methanol until optical density becomes 0.7- 0.8 at 420nm (Forni *et al.*, 1986). For assay 1ml ABTS solution was combined with 50 µl extract and stored in dark area for 30 min. Standard curve was prepared using 0-1mM Trolox solution.

Inhibition of advanced glycation end products (AGEs)

In vitro systems: (BSA-glucose) were evaluated for its inhibitory effect on advanced glycation end products by Szawara-Nowak (2014). At first, bread sample of 1 gm (grinded) was weighed and keep into a clean falcon tube. Then 5ml (80%) methanol was added and the tubes were horizontally placed along the test tube holder to ensure thorough mixing. The temperature was adjusted to 25 °C for 40 min at 100 rpm of the water bath to achieve proper mixing. Then after 40 minutes the tubes were taken out from the water bath and placed in a centrifuge machine at 5000 rpm. After 10 min of centrifugation, the tubes were removed from the centrifuge to collect the supernatant in the falcon tubes by pipette and then shifted to clean pre-labeled glass bottle containers (10ml). The glass containers containing the supernatant were covered and placed in oven for the removal of methanol for 36 hours. After that phosphate buffer 5ml (pH 7.4) was added to the glass containers. Taking 1 ml bovine serum albumin (BSA), 1 ml phosphate buffer (pH 7.4), and 1 ml D glucose solution in a separate, cleaned and pre-labeled glass container, the test samples were prepared in cleaned pre-labeled glass containers by adding 1 ml bovine serum albumin (BSA), 1 ml sample extract and 1 ml phosphate buffer (pH 7.4) to the containers, similarly, standard samples contained 1 ml bovin serum albumin, 1 ml rutin solution and

Table 1: *Ingredients used in control and treatment bread preparations.*

Samples	Ingredients						
	WF(g)	BF(g)	Oil(g)	Salt(g)	Sugar(g)	Yeast(g)	Water(ml)
Control	100	0	5	1	6	2	70
30% BF	70	30	5	1	6	2	70
40% BF	60	40	5	1	6	2	70
50% BF	50	50	5	1	6	2	70

CB: control bread (Wheat flour); BF: buckwheat flour

1 ml phosphate buffer pH 7.4. The samples were kept in an incubator for 48 hours at 40 °C. To avoid any fungal and microbial activity 0.6 mg sodium azide (NaN₃) was added to each well. For the experimentation a clean and sterilized microplate was taken having 96 well. Then 300 ul samples were poured in each well of the microplate through pipette, pattern for the sample in microplate was noted down on a blank page, and then fluorescence concentration in the microplate reader (ELISA reader ALLshang Model-100) was measured for all the samples with an excitation wave set to 330 nm and emission wave 410 nm.

Statistical analysis

Statistical analysis was performed by SPSS, Values were analyzed in duplicate, triplicate and reported in mean (standard deviation). One-way analysis of variance with LSD post hoc test for multiple comparisons was used to determine the effect of buckwheat bread incorporation on outcome parameters ($P < 0.05$) significance level was considered in all analysis.

Results and Discussion

Total phenolic content of flour and bread samples

Total phenolic content of raw materials and bread samples is given in Table 2. The total phenolic content (TPC) of buckwheat flour and BF-containing bread was significantly higher ($P < 0.05$) in comparison with wheat flour. Similarly, substitution of wheat flour with incorporated buckwheat augmented the total phenolic content compared to wheat flour depending on the level of incorporation; with 50% incorporation had the highest total phenolic content. Baking significantly ($P < 0.05$) reduces the phenolic content in bread samples. However, it remained higher ($P < 0.05$) in composite bread than CB.

Total flavonoid content of flour and bread samples

The total flavonoid content of raw materials and bread samples is presented in Table 2. Buckwheat flour had

a higher ($P < 0.05$) total flavonoid content as compared to wheat flour. Significant improvement was shown in the total flavonoid content of buckwheat containing bread samples. Buckwheat flour and bread samples with 50% incorporation had the maximum flavonoid content. Baking significantly ($P < 0.05$) reduced the flavonoid content of bread samples. Nevertheless, it was higher ($P < 0.05$) in bread samples compared to CB sample.

Table 2: *Total phenolic, total flavonoid and rutin contents of raw materials and bread samples (dry basis)*.*

Sample	Total phenolic content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Rutin content (µg/g)
Wheat flour	52.36 ± 7.26 ^e	32.47 ± 4.74 ^e	4.4±1.76 ^e
Buckwheat F	310.39 ± 8.42 ^a	126.38 ± 6.24 ^a	93.3±4.34 ^a
CB	34.28 ± 5.05 ^f	25.68 ± 4.26 ^f	2.2±5.30 ^e
30% BWB	87.52 ± 6.30 ^d	50.41 ± 5.37 ^d	17.9±3.88 ^d
40% BWB	116.67 ± 5.85 ^c	63.53 ± 5.56 ^c	31.7±1.55 ^c
50% BWB	133.84 ± 6.75 ^b	70.91 ± 4.63 ^b	40.0±3.18 ^b

*Values are means ± SD of triplicate analyses. Means in the same columns with different letters are significantly different ($P < 0.05$, LSD test). CB: control bread, BWB: buckwheat-containing bread.

Rutin content of flour and bread samples

The total rutin content of raw materials and bread samples is presented in Table 2. Buckwheat flour had a higher ($P < 0.05$) rutin content as compared to wheat flour. Similarly, Rutin content was significantly higher ($P < 0.05$) to incorporation of buckwheat flour to bread samples relative to control bread. 50% incorporation had the highest total rutin content, while, lowest rutin content was presented in control bread.

Antioxidant activity

The results of ABTS and DPPH assays for flour and bread samples are given in Table 3. Buckwheat flour had higher ($P < 0.05$) radical scavenging activity relative to wheat flour. Among both flours, buckwheat flour had the highest ($P < 0.05$) ABTS

and DPPH radical scavenging activity followed by wheat flour. The incorporation of buckwheat flour at different levels enhanced the antioxidant activity of the bread samples, with maximum incorporation exhibiting the highest antioxidant activity ($P < 0.05$). The baking process decrease the antioxidant activity ($P < 0.05$) of both control and incorporated breads but it was still higher in incorporated samples. With 50% incorporated buckwheat bread sample had highest antioxidant activity.

Table 3: Antioxidant activity of raw materials and bread samples (dry basis)*.

Sample	ABTS ($\mu\text{mol TE}/100\text{g}$)	DPPH ($\mu\text{mol TE}/100\text{g}$)
Wheat flour	51.05 \pm 5.69 ^e	79.47 \pm 10.48 ^e
Buckwheat flour	348.47 \pm 6.43 ^a	307.73 \pm 11.38 ^a
CB	29.61 \pm 4.17 ^f	40.30 \pm 9.24 ^f
30% BWB	101.61 \pm 8.74 ^d	84.70 \pm 11.69 ^d
40% BWB	118.54 \pm 6.33 ^c	107.31 \pm 10.43 ^c
50% BWB	147.17 \pm 8.91 ^b	136.57 \pm 11.33 ^b

*Values are means \pm SD of triplicate analyses. Means in the same columns with different letters are significantly different ($P < 0.05$, LSD test). CB: control bread, BWB: buckwheat-containing bread.

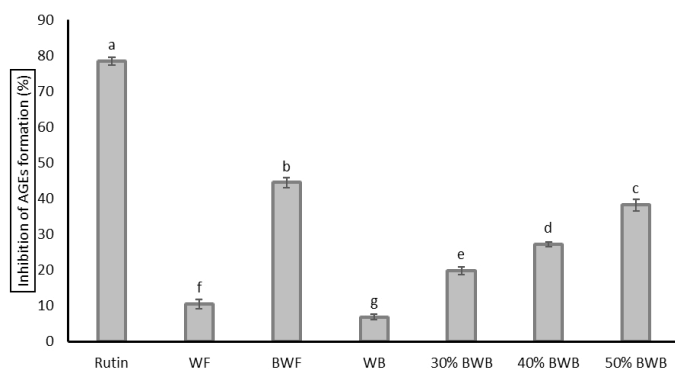


Figure 1: Inhibitory effects of raw materials and bread samples on the formation of AGEs in BSA-glucose model. Values are means \pm SD of triplicate analyses.

Inhibitory effect on advanced glycation end products

The assessment of the buckwheat enhanced wheat bread extracts inhibitory effects on the development of AGEs was conducted using the glucose/bovin serum albumin (BSA) method (Figure 1). This study offers a helpful instrument for assessing the glycation inhibitory potential of wheat bread enriched with buckwheat. The concentration of 50 mg/ml of bread samples was chosen based on the data obtained in order to investigate the inhibitory effects of buckwheat enriched wheat bread against the development of AGEs.

The present study examined the effect of incorporating buckwheat bread samples at different levels into bread on polyphenol content, antioxidant activity, and glycation effects. The hypothesis was that the incorporation of buckwheat flour into bread would improve its polyphenol content and antioxidant activity. Moreover, it would also reduce glycation.

Total phenolic, flavonoid and rutin content of raw materials and bread samples significantly increased after the addition of buckwheat flour. This is because of the higher content of phenolics, flavonoids and rutin in buckwheat flour. Buckwheat contains 2-5 times more phenolic compounds as compared to oats and barely Holasova (2002). This finding is in line with previous studies that buckwheat flour and samples had an increase in total phenolic and flavonoids content than in wheat flour (Beitane et al., 2018). The phenolic concentration increased in loaf, crust and crumb of buckwheat bread Verado et al. (2018). Buckwheat flour and dough also contain a greater concentration of phenolic content Vogrincic et al. (2010).

The flavonoid content in the raw material of buckwheat flour was significantly higher ($p < 0.05$) as compared to wheat flour. The total flavonoid content of Buckwheat containing bread samples was significantly higher ($P < 0.05$) in comparison to control bread. The results of our study were supported by Chlopicka et al. (2012), who studied that the TFC in buckwheat flour was 2-4 folds higher as compared to wheat flour. The results also specified that TFC in bread made with incorporation showed significant difference. The lowest flavonoid levels were presented in control bread. Buckwheat was also recognized as a good source of flavonoid which alleviates the risk of cardiovascular disease and has the potential to inhibit lipoprotein oxidation (Jang et al., 2021). Sprouts of common buckwheat are good sources of phenolic compounds. Sprouts are typically eaten as fresh veggies with noodles made of flour and as ready to eat salad-vegetables Nam et al. (2015). Sprouts of common buckwheat are essential to human diets because of their bioactive compounds. Flavonoids, phenolic acids, stilbenes and tennins are the four categories of phenolic chemicals, according to Jung et al. (2021). Phenolic chemicals are mostly found in the leaf, stem, and root sections during sprouting (Abdel-Aty et al., 2021; Lim et al., 2021).

Alton *et al.* (2009), Sharma and Gujral (2011) state that changes in the chemical structure of phenolic compounds, possibly polymerization, which results in extractability and oxidation, may be the cause of phenolic content. The composition and concentration of phenolic compounds have differed in different parts of grain within, as well as between buckwheat species (Lan-Sook *et al.*, 2015). Verify that soaking common buckwheat achenes in tap water for one hour prior to dehulling resulted in a considerable decrease in the amount of all identified chemicals in buckwheat hulls, with the exception of rutin. The increase in rutin level could be caused by the release of rutin from another compound. Sensory (2006) looked into how processing affected the bioactive chemicals in buckwheat. Buckwheat flours total phenolic content remained unchanged after processing. On the other hand, extrusion at 170 °C had no effect on the antioxidant activity, but roasting at (200 °C, 10 min) did.

Comparing buckwheat plants to amaranth, wheat, and quinoa, Alvarez-Jubete *et al.* (2010) found that the former had greater phenolic and antioxidant activity. Antioxidant level of wheat bread was lower than that of buckwheat bread. Pashikanti (2010) have found that rutin and its metabolite suppress AGEs. With the exception of buckwheat as a excellent source of dietary rutin. All cereals and pseudo cereals have been shown to be devoid of rutin, with the exception of buckwheat, which is a major source of rutin in the diet (Jiang *et al.*, 2007).

Due to a higher amount of polyphenols than wheat flour, buckwheat flour had higher antioxidant activity. In comparison to raw flour, baking increased the scavenging activity of radicals (Sedej, 2011). Processing like baking can increase antioxidant activity (Sharma and Gujral, 2014). This may be because baking causes the production of brown colours called melanoidins, which are the results of millard reaction (Manzocco, 2000).

In another study, Sakac (2011) reported similar results in which the total antioxidant activity of white bread increased with increased incorporation levels of buckwheat flour. Selimovic (2014) revealed that the addition of buckwheat flour to bread significantly increased antioxidant activity; the study also demonstrated that when buckwheat content increased from 15% to 40%, the antioxidant activity

of the bread also increased from 43.11% to 64.19% compared to wheat bread, respectively. According to Zmijewski (2015) showed that bread had a higher antioxidant capacity (131.6 mmol TE/100g dw). When compared to buckwheat flour (53.8 mmol TE/100g dw) buckwheat bran had the highest antioxidant activity (197.5 mmol TE/100g dw).

Conclusions and Recommendations

In conclusion, the bread samples showed a significant ($P < 0.05$) enhancement in antioxidant, total phenolic, total flavonoid, rutin content compared to control bread. Baking of bread led to reduction in phenolic compounds and antioxidants activity of composite bread samples. However, polyphenolic content and antioxidant activity of treatment bread samples were still higher than CB samples. The inhibition of glycation was also higher 50% BWB as compared to CB. In conclusion, incorporation of buckwheat flours into wheat bread enhanced the polyphenol content and antioxidant activity, inhibit glycation reduced starch digestibility without affecting consumer acceptability.

Novelty Statement

The potential benefits of buckwheat in improving cardiometabolic biomarkers due to its nutritional composition and bioactive compounds, and offering individuals a tasty and nutritious dietary option to support their overall health and well-being.

Author's Contribution

Khoulia Begum: Principal author and PhD Scholar, performed research work, collection, and analyzed data, interpreted the outcomes. Finally wrote draft of the manuscript.

Imran Khan: Major Supervisor, who designed the research also helped in proofreading and improved quality of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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