Journal of Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2018-057

Research Article

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Textural, Bioactive and Sensory Attributes of Breadsticks Containing Germinated and Non-Germinated Legumes

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Received: May 06, 2018
Accepted: July 23, 2018
Published: July 25, 2018

Citation: Sattar DS, Ali TM, Abbas T, Hasnain A. 2018. Textural, Bioactive and Sensory Attributes of Breadsticks Containing Germinated and Non-Germinated Legumes. *J Food Chem Nanotechnol* 4(3): 51-56.

Published by United Scientific Group

Abstract

Breadsticks, a pre-meal wheat-based appetizer is a pencil-sized stick of dry bread. The novel approach of this research is to incorporate germinated and non-germinated legumes in the recipe of breadstick in order to enhance its nutraceutical properties. Contents of total phenols, flavonoids, flavonols and antioxidant activity were evaluated in breadsticks before and after incorporation of germinated legumes. Breadsticks containing germinated black gram demonstrated the highest antioxidant activity i.e. 92.9% followed by breadsticks containing germinated green gram 87.4% and germinated lentils 86.23% with improved bioactive compounds. Moreover, hardness value for breadsticks decreased on addition of germinated legumes because of its higher moisture content and showed acceptable firmness. In terms of organoleptic characteristics, higher scores were given to the breadsticks containing germinated legumes. Results clearly suggested that germinated legume containing breadstick appeared as a promising functional product to prevent chronic diseases.

Keywords

Germination, Legumes, Breadsticks, Sensory attributes, Textural attributes, Bioactive compounds, Antioxidant activity

Introduction

Food technologists are showing interest in increasing the nutritional value of foods by incorporating healthier ingredients like dietary fibers, vitamins, minerals and essential oils. Intake of bioactive compounds such as antioxidants, total phenols, flavonoids and flavonol helps in maintenance of health and protection from diseases such as cancer, cardiovascular diseases and many other degenerative diseases [1]. Recently, germinated legumes have received great attention among food scientists due to their functional components and health-promoting effects [2-6]. Many studies showed that phenolic contents of sprouts could be correlated with their antioxidant activities and nutraceutical properties [7].

Germination is the process in which seed reserve compounds are degraded and used for respiration and synthesis of new cell of the developing embryo, thus causing significant changes in the biochemical, nutritional and sensory characteristics of these legumes. It also improves the nutritional quality of legumes by reducing anti-nutritive compounds with increasing levels of free amino acids, available carbohydrates, dietary fiber and bioactive compounds due to activation of enzymes [8].

Bakery products are consumed widely all over the world. Breadstick is one of the most popular bakery product extensively eaten because of its stability (long shelf life) and taste [9]. Breadstick is a pencil-shaped stick of bread, consumed as a pre-meal appetizer that is rolled and baked to a crispy texture [10]. Nutraceutical food products are largely consumed for their health promoting properties. The incorporation of germinated legumes in breadsticks could be a novel way to increase consumption of germinated products. Moreover, it is one of the main strategy to adopt for the improvement of the nutritional quality of people in rural areas of developing countries as it is cheap and could be easily made at home.

Therefore, the goal of this work was to formulate germinated legume flour based breadsticks which could be consumed for improving nutritional and health status in children. Moisture content, storage stability, textural, levels of bioactive compounds and sensory parameters were evaluated in breadsticks containing germinated and non-germinated lentils, green gram and black gram.

Material and Methods

Raw materials

Green gram (*Vigna radiata*), black gram (*Vigna mungo*) and lentil (*Lens culinaris*) were obtained from Pakistan Agriculture Research Council (Karachi, Pakistan). Whole wheat flour, honey, yeast, all-purpose wheat flour, olive oil and salt were purchased from local market.

Germination

Hundred grams of seeds were washed with sodium hypochlorite solution (0.07%, w/v) to remove surface microflora of seeds. Seeds were then soaked in distilled water for 12 hrs and then spread on a wet jute bag for 24 hrs to germinate. The seeds were rinsed after 6 hrs to reduce fungal contamination. Germinated seeds were then subsequently dried in an oven dryer at 40 °C for 3 days. Dried sprouts were ground to fine powder (particle size 0.5 mm) using hammer mill [11].

Preparation of legume breadsticks

To prepare breadsticks 100g whole wheat flour was mixed with 150 g water, 4.5 g honey and 1.5 g yeast. Dough was stirred and was allowed to rest for 10 mins followed by addition of 150 g all-purpose wheat flour, 10.5 g olive oil, 3.5 g salt and 12.5 g germinated or non-germinated legume flour. Dough was mixed, rolled and coated with oil and kept for one hour. Dough was then shaped like a long irregular snake like stick. Breadsticks dough was allowed to rest for 15 mins followed by baking for 60 mins at 120 °C. Breadsticks without legume was considered as control.

Determination of moisture content and storage stability

Moisture content of breadsticks (control, non-germinated and germinated) was determined using the method reported by Williams [12]. It was measured by taking the difference between moisture recorded before and after baking. Storage stability was determined by storing breadsticks for 120 days at room temperature in a sealed polyethylene bag on the basis of moisture content. Moisture content was assessed at 0, 30, 60, 90 and 120 days of storage [13].

Determination of textural properties

The texture of legume breadsticks was determined using 1 mm diameter pointed probe by Universal Testing Machine (Zwick/Roell, GmbH and Co, D-89079 Ulm). The force F_{max} required to break the breadsticks was measured [13].

Preparation of breadsticks extract

Crushed breadsticks (0.5 g) was suspended in 20 mL of mixture of methanol and water in a ratio of 60:40 (v/v). This mixture was centrifuged at 2000 g for 10 mins Breadstick extract was obtained from supernatant and was used to analyze bioactive properties [14].

Determination of total phenolic content

The extracted sample (0.5 mL) was added in 0.5 mL of Folin–Ciocalteau (FC) reagent. The sample was mixed thoroughly followed by addition of 0.5 mL of an aqueous 7.5% (w/v) solution of sodium carbonate. The mixture was stirred and allowed to stand for 30 mins. The sample absorbance was measured at 765 nm using UV-visible spectrophotometer (JASCO model V670, JASCO Corporation, Tokyo, Japan). A blank sample consisting of water and reagents was used as a reference. Quantification was performed with respect to the standard curve of gallic acid. The results were expressed as milligram of gallic acid equivalent per gram of legumes [14].

Determination of flavonols

Breadstick extract (200 μL) was reacted with 2000 μL of $AlCl_3$ (2%, w/v) solution, followed by addition of 3000 μL (50 g/L) sodium acetic acid solute. The blend was shaken and kept for 2.5 hrs at 20 °C. Absorbance was measured against a prepared blank at 440 nm. Total flavonols were expressed as mg of quercetin equivalents (QE) per gram of dry weight (mg QE/g extract) using the calibration curve made with quercetin [15].

Determination of flavonoids

Total flavonoid content was determined according to the method described by Gould & Lister [15]. Extract (250 μL) was diluted with 1250 μL water and was then reacted with 75 μL of 5% (w/v) sodium nitrite solution, followed by reaction with 150 μL of 10% (w/v) aluminum chloride to form a flavonoid-aluminum complex. Subsequently, 500 μL of 1 M sodium hydroxide solution was added to the mixture. Volume was then made up to 2.5 mL with distilled water. Absorbance was measured against a prepared blank at 510 nm. The flavonoid content was determined by a (+)-catechin standard curve and expressed as the mean of mg (+)-catechin equivalents/100 g dry weight.

Determination of antioxidant activity

The 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay was used to determine free radical scavenging activity according to the method of Ashoush & Gadallah [14]. The extract (200 µL) was mixed with 1.0 mL of 0.1 mM DPPH in methanol. The reaction mixture was shaken well and allowed to stand for 20 mins at room temperature. Absorbance was determined at 517 nm using UV-visible spectrophotometer JASCO, Model V670 (JASCO Corporation, Tokyo, Japan) against methanolic blank.

Sensory analysis

The tests were conducted in a sensory laboratory. A laboratory with necessary facilities, viz., separate booths, provisions for adequate diffused light and air-conditioned odor-free environment, was employed for product evaluation. Sensory evaluation of breadsticks was performed by eleven trained panelists using nine-point hedonic scale (1 = extremely dislike and 9 = extremely like). Sensory assessment was made in terms of color, taste, appearance, crispness and overall acceptability.

Statistical analysis

Analyses were performed in triplicate. The data was analyzed by analysis of variance (ANOVA) using SPSS (Version 17.0. Inc, Chicago, USA) statistical program. Duncan's multiple range tests were carried out to test any significant differences among the treatments employed. Significant levels were defined at $p \leq 0.05$.

Results and Discussion

Moisture content

Water plays a major role in staling of baked products [16]. Staling is a complex process in which multiple mechanisms are involved. It is described as the time-dependent loss in quality of flavor and texture of product [17]. Percent moisture loss was observed in figure 1. It can be observed that breadsticks containing non-germinated black gram had the highest moisture loss compared to other samples. Product with low moisture gives firmer texture [18]. Breadsticks with germinated legumes had comparatively lower loss of moisture content after baking. Moisture pick up is the most significant change observed during breadstick staling. During prolonged storage, amylopectin crystalizes and water mobility changes due to re-association of polymers which could increase staling [19]. Figure 2 shows that moisture content increased over this period. Control breadstick showed the highest increase of moisture content after 120 days followed by non-germinated legumes containing breadsticks. Whereas, breadsticks containing germinated legume gained less moisture content. The breadsticks could increase heterogeneity by the changes in the components such as proteins, starches and fibers, which might contribute to the change in moisture retention during baking. This result could be due to the increased amount of fibers by adding germinated legumes [6], resulting in increased moisture retention in breadsticks [20]. Similar results were reported by Artz [21]. Activated enzymes during germination induced the degradation of starch and protein into smaller sugars and peptides, respectively [22]. The degradation to the smaller molecules raised the osmotic pressure, and thus made cookies retain relatively higher amounts of water. Therefore, it could be suggested that the incorporation of germinated legumes prevent sogginess of bread sticks.

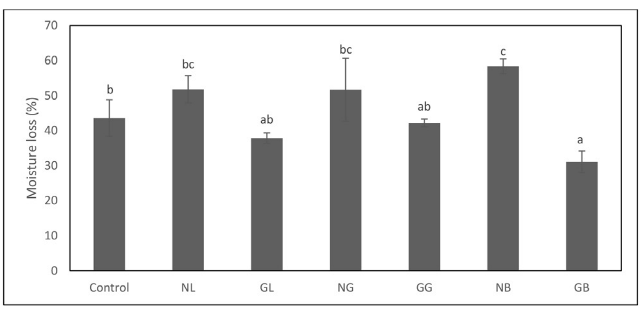

Figure 1: Effect of non-germinated and germinated legumes on moisture loss of breadsticks. Different lowercase letters on each bar represent significant difference at p<0.05. Control: Breadsticks without legumes; NL: non-germinated lentil breadsticks; GL: germinated lentil breadsticks; NG: non germinated green gram breadsticks; GG: germinated green gram breadsticks; NB: non germinated black gram breadsticks; GB: germinated black gram breadsticks.

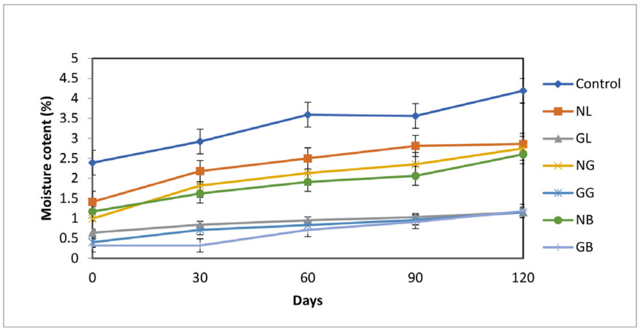

Figure 2: Effect of non-germinated and germinated legumes on storage stability based on moisture content of breadsticks. Control: Breadsticks without legumes, NL: non-germinated lentil breadsticks; GL: germinated lentil breadsticks; NG: non-germinated green gram breadsticks; GG: germinated green gram breadsticks; NB: non-germinated black gram breadsticks; GB: germinated black gram breadsticks.

Hardness

For a novel baked food product, it is important to evaluate the impact of germinated legume flour on food quality attributes. Hardness is an important factor for the quality of breadsticks [23]. According to table 1, control breadsticks had the highest value of hardness (66.86N) whereas breadsticks containing non-germinated legumes had significantly higher hardness than their germinated counterparts. Significant difference in hardness could be due to the altered starch properties caused by germination [24]. It depends on moisture and protein content. It was also reported that hardness depends on the structure of composite matrix of protein aggregates, lipids, and sugars, which are embedded in some of ungelatinized starch granules. The continuous protein network is mainly formed by gluten during kneading and baking [25]. Harder texture was obtained in control breadsticks compared to the germinated and non-germinated legume breadsticks as legume do not have gluten which could form gluten network.

Bioactive compounds

Effect of non-germinated and germinated legumes on total phenols, flavonoids and flavonols of breadsticks are shown in table 2. According to observations, breadsticks containing germinated black gram showed the highest bioactive compounds content followed by breadsticks containing germinated green gram. Even though processing caused losses of phenolic compounds, breadsticks containing germinated legumes showed higher content of total phenols, flavonoids and flavonols than those containing non-germinated legumes. Flavonoids reached higher levels in breadsticks containing germinated black gram followed by addition of germinated green gram and germinated lentils; 254, 222 and 204 mg catechin/100 g, respectively compared to the non-germinated and control. Similarly, germinated legume breadsticks showed higher total flavonol content than their non-germinated counterparts and control. Therefore, it was clear from the analysis of the product that the breadsticks containing germinated legumes increased the content of bioactive compounds, thus increasing the nutraceutical properties of the product.

Table 1: Effect of non-germinated and germinated legume incorporation on breadstick hardness (F_{max}).

Samples	F_{max} (N)
Control	66.86 ± 10.5[d]
NL	52.69 ± 3.39[c]
GL	18.67 ± 0.96[a]
NG	51.90 ± 4.40[c]
GG	15.99 ± 0.71[a]
NB	41.96 ± 10.1[bc]
GB	33.88 ± 2.51[b]

[a]All values are mean of triplicate determinations. Means within a column with different superscripts are significantly different at p<0.05.
Control: Breadsticks with no legumes, NL: non-germinated lentil breadsticks; GL: germinated lentil breadsticks; NG: non-germinated green gram breadsticks; GG: germinated green gram breadsticks; NB: non-germinated black gram breadsticks; GB: germinated black gram breadsticks.

Table 2: Effect of non-germinated and germinated legume incorporation on bioactive compounds of breadsticks.

Samples	Total phenols (mg Gallic acid/g)	Flavonoids (mg Catechin/100 g)	Flavonol (mg Catechin/100 g)
Control	1.14 ± 0.04[a]	142.86 ± 7.0[a]	161.86 ± 8.0[a]
NL	1.55 ± 0.18[b]	165.40 ± 6.5[bc]	342.33 ± 5.0[b]
GL	3.61 ± 0.05[c]	204.46 ± 6.7[d]	391.20 ± 12.1[cd]
NG	1.17 ± 0.05[a]	155.13 ± 13.2[ab]	354.46 ± 4.2[bc]
GG	3.90 ± 0.07[d]	222.40 ± 2.4[d]	419.80 ± 14.8[de]
NB	1.15 ± 0.10[a]	176.93 ± 12.1[c]	369.93 ± 48.0[bc]
GB	3.84 ± 0.06[d]	254.40 ± 21.6[e]	445.60 ± 29.8[e]

[a]All values are mean of triplicate determinations. Means within a column with different superscripts are significantly different at p<0.05.
Control: Breadsticks with no legumes, NL: non-germinated lentil breadsticks; GL: germinated lentil breadsticks; NG: non-germinated green gram breadsticks; GG: germinated green gram breadsticks; NB: non-germinated black gram breadsticks; GB: germinated black gram breadsticks.

Antioxidant activity

Scavenging the stable DPPH radical model was used to evaluate antioxidant activity. DPPH is a table free radical with characteristic absorption at 517 nm. Antioxidant react with DPPH and convert it to 2,2-diphenyl-1-picrylhydrazine. The extent of decrease in absorbance at 517 nm indicates the scavenging potential of the antioxidant extract, which could be due to the hydrogen donating ability [26]. The DPPH radical scavenging activity of the breadsticks is shown in table 3. Antioxidant activity of control breadsticks (without legumes) was 78.90%. While the addition of non-germinated lentils, green gram and black gram flour in breadsticks resulted in 80.08%, 82.65% and 83.81% antioxidant capacity, respectively. Breadsticks containing germinated legumes showed significantly ($p \leq 0.05$) higher antioxidant activity than those containing non-germinated legumes. The highest percent antioxidant activity was observed when formulating germinated black grams in breadsticks i.e. 92.92% followed by the addition of germinated green gram 87.35% and germinated lentils 86.23%. Thus, incorporation of germinated legume flour into breadsticks increases health benefits by increasing antioxidant properties.

Table 3: Effect of non-germinated and germinated legume incorporation on breadsticks antioxidant activity (%).

Samples	Percent antioxidant activity (%)
Control	78.90 ± 0.27[a]
NL	80.08 ± 1.53[a]
GL	86.23 ± 1.62[c]
NG	82.65 ± 0.55[b]
GG	87.35 ± 0.90[c]
NB	83.81 ± 0.64[b]
GB	92.92 ± 1.53[d]

[a]All values are mean of triplicate determinations. Means within a column with different superscripts are significantly different at p<0.05.
Control: Breadsticks with no legumes, NL: non-germinated lentil breadsticks; GL: germinated lentil breadsticks; NG: non-germinated green gram breadsticks; GG: germinated green gram breadsticks; NB: non-germinated black gram breadsticks; GB: germinated black gram breadsticks.

Sensory analysis

When making a nutraceutical bakery product, it is important to evaluate physical and sensory characteristics to assess consumer acceptability. The samples tested by the sensory panel in this study were the control (with no legumes) and breadsticks with germinated and non-germinated legumes (Figure 3). All the panelists showed similar visual attraction for breadsticks color that irrespective of whether it contained germinated or non-germinated legumes. In contrast, the taste of breadsticks improved on incorporation of germinated legumes and the highest score was given to the breadsticks containing germinated black gram. The results of appearance revealed no significant difference among NL, GL, NG, GG and NB and the breadsticks containing germinated black gram was given the highest score. The highest score of the crispiness was given to GL, NG, GG, NG and GB. However, control breadsticks showed least scores in organoleptic properties. Whereas, the overall acceptability appreciation was given to the breadsticks containing germinated legumes. Thus, addition of germinated legumes in breadsticks would be more beneficial because of having higher antioxidant capacity with improved sensory properties compared with non-germinated and control breadsticks.

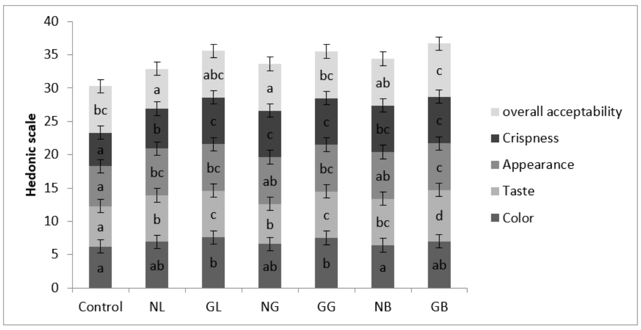

Figure 3: Effect of non-germinated and germinated legumes on storage stability based on moisture content of breadsticks. Control: Breadsticks without legumes, NL: non-germinated lentil breadsticks; GL: germinated lentil breadsticks; NG: non-germinated green gram breadsticks; GG: germinated green gram breadsticks; NB: non-germinated black gram breadsticks; GB: germinated black gram breadsticks.

Conclusion

Healthier snacks have captured a huge market due to the growing consumer demands for healthy foods. The present study also made an attempt to make healthy breadsticks through incorporation of germinated and non-germinated legumes. Addition of legumes led to increase in flavonol, phenol and flavonoid content of breadsticks. Snacks elaborated with germinated legumes exhibited higher antioxidant activity and also acceptable texture, color and taste. Sogginess, a common defect of breadsticks was reduced by incorporating germinated legumes in breadstick formulation.

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