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Enhancing Nutritional and Antioxidant Properties of Breadsticks through germinated and Non-germinated Cowpea flours

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Abstract

This study evaluates the impact of incorporation of whole wheat flour with germinated and non-germinated cowpea flours (12 % addition) on textural, sensorial, and physicochemical properties of breadsticks. Incorporation of germinated cowpea flour in breadsticks exhibited significantly highest total phenolic content (5.61 mg Gallic acid/g) and antioxidant activity (93 %), compared to non-germinated cowpea (78 %) and whole wheat flour (76.9 %). The inclusion of germinated cowpea flour significantly enhanced protein content while reducing carbohydrates and fat content. Hardness and the crispness were also significantly influenced by incorporation of cowpea flours. Moreover, germinated breadstick achieved the highest hedonic scores in terms of appearance, overall acceptability, and taste by semi-trained panelists. During 120 days of storage, germinated cowpea breadsticks retained better colour, taste and texture due to reduced moisture uptake. Therefore, germinated cowpea flour in breadsticks could be an antioxidant enriched alternative, without causing substantial changes in product quality and acceptability, by 12% addition of whole wheat flour.

Keywords: *Cowpea, germination, bioactive properties, antioxidant activity, sensory evaluation*

1.INTRODUCTION

The raising demand for nutritionally enhanced foods has driven the search of versatile and cost-effective protein sources to address the dietary proteins deficiency. However, with increasing concerns over sustainability of animal-based proteins, plant-based alternatives have gained attention due to their affordability, accessibility, and environmental benefits (Bryant, 2022). Thus, many researchers are showing attention to legume proteins as substitute to animal proteins by reason of environmental sustainability and lower risk of diseases. Legume seeds are not only considered as the source of proteins but also dietary fibers and antioxidants (Barman et al., 2018). Bioactive compounds such as antioxidant, total phenolic contents, flavanols and flavonoids play an important role in human health. It not only maintains the health but also protect from various life-threatening diseases like cancer, diabetes, cardiovascular diseases etc (Sattar et al., 2015). Among legumes, cowpea (*Vigna unguiculata* L.) is a neglected and forgotten, cheep and easily available crop. It’s nutritional richness, affordability and adaptability to diverse climate, makes it an excellent ingredient for food fortification (Desire et al., 2021).

Germination alters the biochemical, metabolic and sensory characteristics of legume seeds significantly because of the degradation of reserved compounds. Studies have shown that germinated legume flours exhibit improved antioxidant properties, phenolic content, improved enzymatic activities, contributing to functional and health promoting attributes (Onwuka et al., 2024). Nutritional quality is improved with the reduction or elimination of anti-nutritional compounds such as tannin, oligosaccharides etc. with the help of enzymes (Sattar et al., 2015).

Ready to eat foods have been the most popular food commodity among the consumers. They have been more focused towards food industry for development of products that would be enriched with nutrition, innovation, functionality, convenience of consumption, ease of preparation and storage (AlOudat et al., 2021). Dipping stick, also known as breadstick, is the bakery product that is consumed widely because of its crispness and long shelf life. It is in the category of bread shaped like pencil consumed as a pre-meal appetizer with soup or any sauce. It is basically made of whole wheat flour or all-purpose flour (Sattar et al., 2018). This work was an attempt to introduce germinated cowpea flour in the recipe of bread sticks to enhance nutritional profile. The impact of addition whole wheat flour with germinated and non-germinated cowpea flours (12 %) on the physicochemical, sensory and antioxidant properties of breadsticks was studied. Thus, the aims and objectives of the study includes (i) evaluate the effect of germination on the proximate composition and hydrolytic enzyme (amylase, protease) activity of cowpea flour; (ii) compare the impact of incorporating germinated versus non-germinated cowpea flour, at a fixed 12% addition level, on the bioactive compound content (total phenolics, flavonoids, flavanols), antioxidant activity, textural properties, and sensory acceptability of breadsticks relative to a whole-wheat flour control; and (iii) assess the storage stability of these breadsticks over 120 days in terms of moisture content and sensory quality retention.

2. MATERIAL and METHODS

2.1 Raw materials

Whole wheat flour (moisture 11.8 %, ash 1.45 %, wet gluten 28.5 %) and all-purpose wheat flour (moisture 13.2 %, ash 0.52 %, wet gluten 26.0 %) were purchased from Metro Cash & Carry (Multan, Pakistan). Seeds of cowpea (*Vigna unguiculata* L. Walp, cv. 'Sadabahar') were obtained from the local seed market of Multan, Pakistan, and authenticated at the Department of Botany, Bahauddin Zakariya University, Multan, Pakistan. Commercial ingredients including olive oil (Pietro Coricelli, Spoleto, Italy), honey (Salman’s, Karachi, Pakistan), active dry yeast (Saccharomyces cerevisiae; Saf-Instant, Lesaffre, France), and food-grade table salt (National Foods Ltd., Karachi, Pakistan) were purchased from the same retail source. All chemicals and reagents used for biochemical and antioxidant analyses were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck KGaA (Darmstadt, Germany).

2.2 Germination of cowpea

Cowpea (100 g) was washed with (0.07%, w/v) solution of sodium hypochlorite (NaOCl) to remove surface microflora of seeds. Then, cowpea seeds were soaked in distilled water for 12 h and subsequently spread on a wet jute cloth for 24 h to germinate. After every 6 h, the seeds were rinsed with distilled water to reduce fungal contamination during the 24 h germination period. An average germination rate of 89.9% was observed while the average radicle elongation length was 1.5 cm. The germinated cowpea seeds were dried in a lab oven at 40 °C for 3 days. The drying process was continued for approximately 72 h until the sprouted seeds reached a constant weight (yielding a final moisture content of 10.63 as confirmed in Table 1). The dried sprouts were subsequently milled into a fine powder using a laboratory hammer mill, passed through an 80-mesh sieve pore size 0.177mm, and stored in airtight, moisture-proof Ziploc bags under refrigeration 4 degree celsius prior to analyses and baking process. (Sattar et al., 2015).

Table 1. *Compositional analysis of non-germinated and germinated cowpea[a]*

Composition	NGCP	GCP
Moisture Content (%)	5.42±0.74[a]	10.63±0.48[b]
Ash (%)	4.49±0.98[b]	1.22±0.98[a]
Fat (%)	5.91±0.007[b]	2.91±0.007[a]
Protein (%)	6.8±0.98[a]	9.0±0.14[b]
Carbohydrate (%)	77.3±1.62[ab]	76.2±0.51[a]
Amylase (U/mL)	0.39±0.03[a]	5.69±0.08[b]
Protease (U/mg)	0.96±0.03[a]	2.89±0.11[b]

[a]All values are mean ± standard deviation of triplicate determinations. Means within a row with different superscripts are significantly different at $p < 0.05$.
NGCP: non germinated cowpea; GCP: germinated cowpea

2.3 Determination of proximate composition of cowpea flours

Percent ash, protein, crude fiber and lipid contents were determined according to the methods of Latimer, (2019). Protein content was calculated by multiplying N with the factor of 6.25 whereas, the total carbohydrate content was calculated by difference methodology.

% Carbohydrate content = [100- (%protein + %lipid + %ash+ % crude fiber)].

2.4 Determination of amylase and protease activity

The ground cowpea samples (2 g) were mixed with 10 mL double distilled water and kept for 1 h at room temperature. Afterwards, samples were centrifuged by using AllergaTM X-22 centrifuge (Beackman Coulter, Inc. USA) for 10 min at 10,000 g. The supernatant was collected and used as crude extract while the sediment was discarded. Amylase activity assay was carried out by the method of Rehman et al., (2008). One unit of α-amylase is defined as the amount of enzyme which released one μmol of reducing sugar, per minute using glucose as the standard. A standard curve of tyrosine activity based upon casein was prepared and used to estimate the protease activity of the extracts.

2.5 Preparation of cowpea breadsticks

Breadsticks were prepared by mixing whole wheat flour (100 g) with water (150 g), honey (4.5 g) and yeast (1.5 g) and kneading it into a dough. After 10 mins, all-purpose wheat flour (150 g), olive oil (10.5 g), salt (3.5 g) and germinated or non-germinated cowpea flour (12.5 g) was added into the dough. Then, the mixture was kneaded, rolled, and coated with olive oil and left for an hour at ambient temperature. Afterwards, dough was shaped into long irregular sticks and allowed to rest for 15 min followed by baking for 60 min at 120 °C in oven. Breadsticks with germinated flour, non-germinated flour and without cowpea flour were named as GCP, NGCP and control (WF), respectively.

2.6 Estimation of bioactive compounds

2.6.1 Total phenolic content (TPC)

Quantification of total phenolic content in breadsticks was performed by using the method of Aryal et al., (2019). The results were expressed as milligram of gallic acid equivalent per gram of cowpea.

2.6.2 Flavonols

The protocol mentioned by Aryal et al., (2019) was used to determine flavonols. Total flavonol content were expressed as mg of quercetin equivalents (QE) per gram of dry weight (mg QE/g) using the calibration curve made with quercetin.

2.6.3 Total flavonoid content (TFC)

The method described by Aryal et al., (2019) was used to estimate total flavonoids present in breadsticks. Flavonoid content was determined by a (+)-catechin standard curve and expressed as the mean of mg (+)-catechin equivalents/100 g dry weight.

2.7 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

The 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay was used to determine free radical scavenging activity according to the method of Ahsan et al., (2023) with slight modification. The extract of breadsticks sample (200 µL) was placed in a glass test tube followed by addition of 1.0 mL of 0.1 mM DPPH solution and was stored in a dark cabinet for 30 min. Absorbance was noted at 517 nm using UV–visible spectrophotometer (Model V670, JASCO Corporation, Tokyo, Japan) against diluted blank. The results were calculated from calibration curves, prepared with ascorbic acid (AEAC—ascorbic acid equivalent antioxidant capacity). The radical scavenging activity was calculated as a percentage of DPPH discoloration using the below mentioned equation:

$$\text{DPPH radical scavenging (\%)} = [(A0 - A1)/A0] \times 100$$

Where, A0 is the absorbance of the DPPH solution and A1 is the absorbance of the sample.

2.8 Textural properties of breadsticks

The texture of cowpea breadsticks was studied in terms of hardness by using 1 mm diameter pointed prob Universal Testing Machine (Zwick/Roell, GmbH and Co, D-89079 Ulm). The force (measured in N) required to break the breadsticks (Fmax) was noted (Chung et al., 2014).

2.9 Storage stability based on moisture content and sensory evaluation

Storage stability was determined by storing breadsticks in sealed plastic bags at ambient laboratory conditions (approximately 25 ± 2°C) for upto 120 days. Moisture content (%) and organoleptic properties were assessed at 0, 30, 60, 90 and 120 days of storage through moisture analyzer and semi-trained panelist, respectively (Chung et al., 2014).

Sensory evaluation was conducted with 15 semi-trained panelists (including both male and female, aged 21–40 years). The methodology of Moin et al., (2019) was followed. The assessment was conducted in a well lit laboratory with proper ventilation. Clean drinking water was given to clean the pallet between different breadsticks samples. The 9-point hedonic scale (1=extremely dislike and 9= extremely like) was employed. Color, taste, appearance, crispness, and overall acceptability of breadsticks was assessed.

2.10 Statistical analysis

The triplicate data was analyzed by (analysis of variance) ANOVA using SPSS (Version 17.0. Inc, Chicago, USA) statistical program. Duncan's multiple range tests and T test were carried out to test any significant differences among the treatments employed. Significant levels were defined at $p \leq 0.05$.

3. RESULT and DISCUSSION

3.1 Nutritional Composition of breadsticks

Evaluation of proximate composition of any product is important in developing nutritional value and overall acceptance of the consumer. The findings indicated that incorporation of germinated cowpea flour significantly changes the macronutrients composition (Table 1). Ash content decreased significantly in germinated breadsticks as compared with its non-germinated counterpart. This reduction is attributed to the loss of minerals from the rootlet and washing of cowpea during germination. Loss in total mineral content was also reported for white sorghum (Afify et al., 2012) and corn varieties (Sokrab et al., 2012). Fat and carbohydrate contents decreased significantly in germinated cowpea. During sprouting process, fat is oxidized to carbon dioxide and water to generate energy for growth while carbohydrate hydrolyzes into smaller molecules such as glucose and maltose by β-amylase (Keawkim et al., 2021). These are used for seed and embryonic growth. However, percentage of protein increased in germinated legumes. The increment is because of the utilization of carbohydrate and fat as a source of energy, which alters overall composition of sprouted legume. According to Sattar et al., (2017), increased in protein content is because of the synthesis of new proteins by germinating seeds and compositional changes after degradation of other constituents. The findings suggest that germinated legume flour could be used for the formulation of high protein variants. Similar results were also observed for germinated cowpea, lentils, chickpea (Ghavidel & Prakash, 2007), and soyabean (Kaushik et al., 2010).

3.2 Enzyme activities of germinated and non-germinated cowpea

Enzyme activities were studied in term of amylase and protease activity of non-germinated and germinated cowpea, shown in Table 1. It was observed that germinated cowpea showed a significant increase amylase and protease activity, which play a crucial role in starch and protein metabolism, respectively. The activation of hydrolytic enzymes breakdown macromolecules into more digestible and bioavailable components (Guzmán-Ortiz et al., 2019). It is also evident from the significantly change composition (Table 1) and enhanced phenolic, flavonoid and antioxidant activity of germinated cowpea (Table 2). Such enzymatic modifications can contribute to improved textural and digestibility of food products. Therefore, germination of seeds is considered as a natural biological treatment and has been extensively employed to enhance the nutritional and processing attributes (Wang et al., 2022). Notably, in both germinated and non-germinated sample amylase activity was higher than protease activity.

Table 2. *Effect of non-germinated and germinated cowpea incorporation on bioactive compounds of breadsticks*[a] .

Samples	Total phenols (mg Gallic acid/g)	Flavonoids (mg Catechin/100g)	Flavonol (mg Catechin/100g)	Antioxidant activity (%)
Control	1.16±0.04[a]	144.86±7.0[a]	168.86±8.0[a]	76.90±0.27[a]
NGCP	2.15±0.18[b]	157.40±6.5[b]	322.33±5.0[b]	78.08±1.53[a]
GCP	5.61±0.05[c]	210.46±6.7[c]	399.20±12.1[c]	93.23±1.62[b]

[a]All values are mean ± standard deviation of triplicate determinations. Means within a column with different superscripts are significantly different at $p < 0.05$.

Control: Breadstick with no cowpea; NGCP: non germinated cowpea; GCP: germinated cowpea.

3.3 Phenolics, flavonoids, flavanols and antioxidant activity

Effect of non-germinated and germinated cowpea on total phenols, flavonoids, flavanols and antioxidant activity of breadsticks are shown in Table 2. Breadsticks containing germinated cowpea flour exhibited highest level of bioactive compounds, particularly total phenolics (5.61 mg Gallic acid/g), flavonoids (210.46 mg Catechin/100g) and flavanols (399.20 mg Catechin/100g). Percent antioxidant activity was increased significantly when germinated cowpea was added in breadstick (93 %) as compared to the breadstick with non-germinated cowpea (78%). It is attributed to bioactive compounds release during sprouting (Naz et al., 2023). Post germination enhanced antioxidant activity and phenolic contents in 19 legumes cultivated in Taiwan was also reported (Lin & Lai, 2006). Oat grains also showed significant increase in total phenolic content after germination (Jian et al., 2009). Similarly, increased phenolic content and antioxidant properties were reported after germination of maize (Hiran et al., 2016). Our findings confirm that germinated cowpea behaved as a functional ingredient which improved the content of bioactive compounds, thus increasing the nutraceutical properties of the product. Enhanced antioxidant and bioactive properties were also observed in bread prepared with germinated brown rice (Cornejo et al., 2015).

3.4 Texture

Texture is the most important factor for the baked products because it is concerned with the quality parameters and sensory attributes. Hardness is a primary textural feature and it is defined as force required to attain a given deformation (Ahsan et al., 2024). As shown in Table 3, the highest hardness was observed in control breadstick while there was no significant difference in the hardness of GCP and NGCP breadsticks. The reduced hardness in cowpea incorporated breadsticks can be attributed to the lack of gluten in legumes which weakens the protein matrix (Ma et al., 2025). Texture of a bakery product depends on the structure of composite matrix of protein aggregates, lipids, and sugars, which are embedded in some of gelatinized starch granules. The continuous protein matrix could be achieved mainly by gluten during baking (Sharanagat et al., 2022). However, cowpea flour does not provide this strengthen matrix leading to softer texture of breadsticks. Moreover, lower hardness value of germinated cowpea breadsticks could be associated with enzyme induced modifications in macromolecules (Table 1), further affecting textural properties. In accordance with our results, softer breads were observed after germination of long grain brown rice (Wunthunyarat et al., 2020).

3.5 Sensory attributes and storage stability

Sensory analyses were performed by trained assessors and the results for fresh breadsticks are shown in Fig. 1 while Fig. 2 (a, b, and c) show the sensory evaluation of breadsticks during storage period of 120 days after every 30 days interval along with percent moisture loss. Taste, texture, and appearance of a food product are considered as three major factors that affect consumer acceptability (Moin, Ali, et al., 2024).

3.5.1 Colour

The colour of a food is considered as a primary quality attribute and a significant marketing factor (Moin et al., 2025). Colour of the freshly prepared wheat flour breadstick was slightly darker than those which were supplemented with germinated and non-germinated cowpea flour. The fresh control breadsticks were slightly liked (hedonic score 6) by the assessors while after storage for 120 days, the change in colour was unacceptable (hedonic score 4). Whereas, the fresh breadstick with non-germinated cowpea was liked by the panelists (hedonic score 7) however, the color scores also reduced to unacceptable on 120th day of storage. Significantly highest hedonic scores were achieved by breadstick with germinated cowpea (9) as compared to control and NGCP. During the storage at room temperature of GCP breadstick, there was no significant differences observed in colour for ninety days. However, on the 120th day of storage the color scores for GCP reduced to 7 hedonic point which is still in the liking range and significantly higher than the control one.

3.5.2 Taste

Taste hedonic scores showed similar trend with germinated cowpea breadsticks maintaining acceptability throughout the storage while control and NGCP declined to unacceptable level (hedonic score 3) (Fig. 1 and Fig. 2). The superior retention of taste in GCP could be due to the presence of higher antioxidants that delayed lipid oxidation and the off-flavour development (Table 3). These findings suggest the exploitation of germinated cowpea as a natural color and flavour stabilizer in bakery products. Moreover, cross-modal studies have linked the colour of food commodities with their taste (Spence et al., 2015). The highly acceptable taste of GCP could be a consequence of its highly preferred colour.

Table 3. *Hardness of breadsticks[a]*

Samples	Hardness (N)
Control	56.24±0.02^{b}
NGCP	43.34±0.1^{a}
GCP	42.44±0.06^{a}

aAll values are mean ± standard deviation of triplicate determinations. Means within a column with different superscripts are significantly different at $p < 0.05$. Control: Breadstick with no cowpea; NGCP: non germinated cowpea; GCP: germinated cowpea

3.5.3 Appearance and overall acceptability

Appearance is the parameter for acceptability to the consumer so they could pick up and taste any product (Moin, Ali, et al., 2024). Thus, the acceptance of food determines the quality of food that the consumer prefers. The GCP scores were the highest in both parameters (hedonic score 9) which is significantly higher from the control and NGCP. Bright color was observed after addition of germinated cowpea in breadstick consequently, it was preferred in terms of appearance and overall acceptability over the rest of the breadsticks (Fig. 1). Significant correlation of colour of biscuits with their overall acceptability is also reported (Moin, Zaid, et al., 2024). It must be noted that the overall acceptability of GCP reduced by 120th day but it was significantly higher than the control and NGCP samples (Fig. 2).

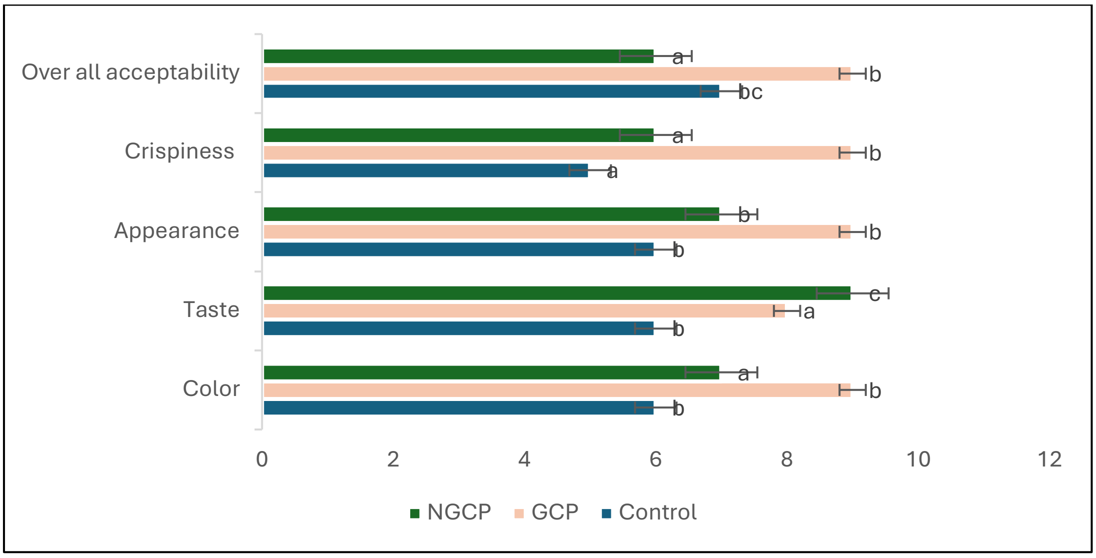

Figure 1. Effect of non-germinated and germinated cowpea on organoleptic properties of breadsticks.

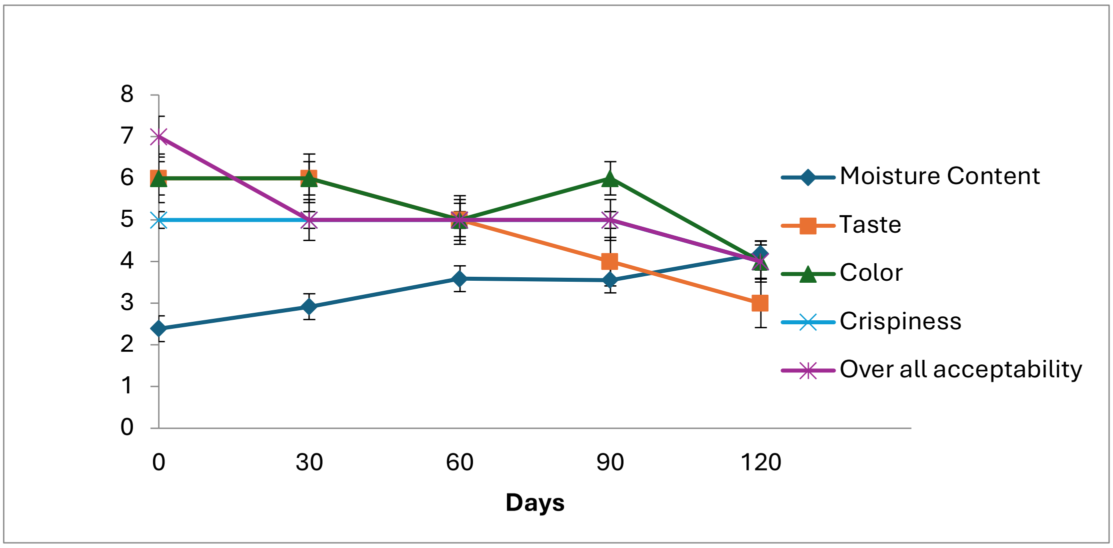

Figure 2a. Changes in moisture content and sensory attributes of control Breadsticks.

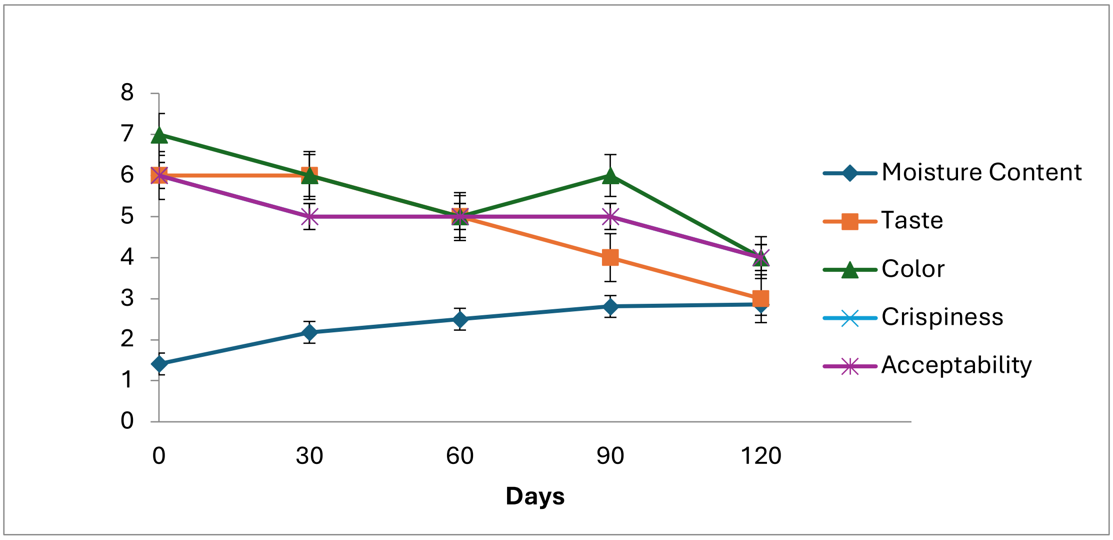

Figure 2b. *Changes in moisture content and sensory attributes of breadsticks incorporating non germinated cowpea.*

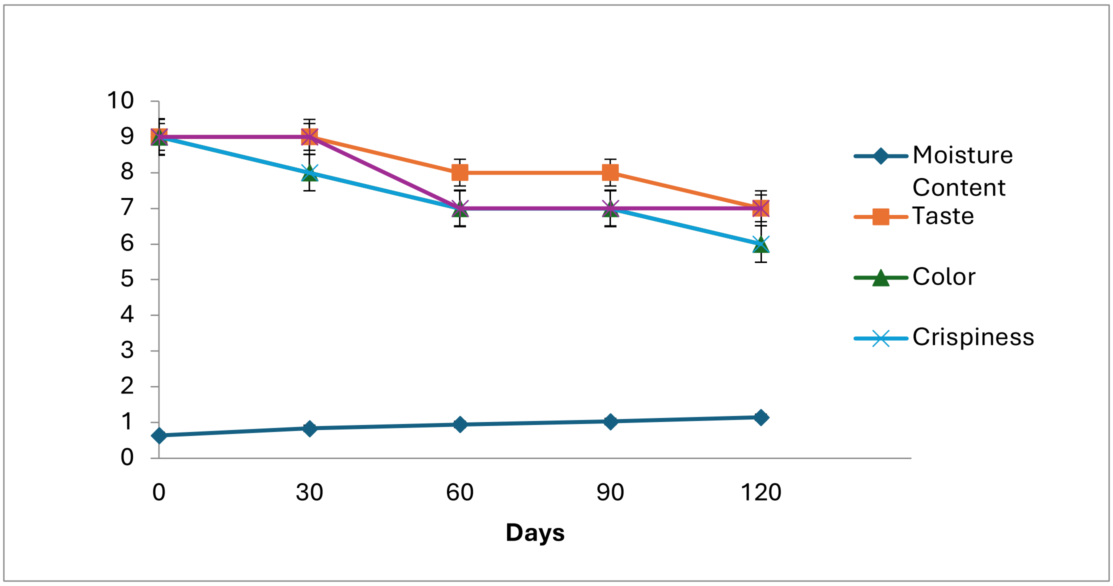

Figure 2c. *Changes in moisture content and sensory attributes of breadsticks incorporating germinated cowpea.*

3.5.4 Crispness and Moisture uptake

Crispiness of bakery snacks is an important sensory attribute. Significant correlation of crispiness with overall sensory experience and consumer acceptability is reported (Jaworska & Hoffmann, 2008). Crispiness was significantly affected by the incorporation of cowpea flour in breadsticks formulation. As shown in Fig. 1, Fig. 2a, 2b, and 2c, GCP were significantly crispier than control and NGCP. The inverse relationship between crispiness and moisture uptake is well established (Zhao et al., 2026). During storage of breadsticks, significantly higher moisture absorption was observed in control and NGCP and it increased till 120th day, resulting loss in crispness of breadsticks over time. Nevertheless, Breadstick with germinated cowpea (GCP) showed least moisture uptake thus maintaining the crisp texture till 120th day of storage. During baking, water vapor carries fats/oils emulsified with it toward the external layer. The quality decline in control and NGCP breadsticks during storage is caused by different deteriorative events, among which lipid oxidation and loss of crispness could be the main phenomena. The lower moisture retention in GCP could be due to changes in water binding capacity during germination process, which alters structural integrity of starch and proteins matrices.

4. CONCLUSION

The results of the present study highlighted the significant impact of germinated cowpea in the texture and colour preservation of breadstick. The germinated cowpea was found to have highest percentage of antioxidant and total phenolic content with better hardness, crispiness, and overall acceptability. Thus, germinated cowpea flour is concluded as a nutritious and sustainable addition in whole wheat flour in the formulation of breadsticks. The strong quality and nutritional enhancement potential of germinated cowpea flour could be exploited in the formulation of other bakery delicacies.

DECLARATION OF CONFLICTING INTERESTS

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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None.

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