



Development and analysis of bread fortified with calcium extracted from chicken eggshells of Pakistani market

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Abstract

Calcium fortification is usually achieved by employing wheat flour as the most frequently used vehicle. In this study, potential of calcium extraction from chicken eggshell and its utilization as a bread fortificant in straight grade flour (SGF) is investigated. In addition, the fortified and unfortified (control) flour samples were evaluated for nutrient composition and rheological properties. Results showed that addition of fortificant at different concentrations significantly ($p < 0.05$) enhanced calcium content of fortified flour and it was considerably increased from 115.80 mg/kg (T_0) to 1135.1 mg/kg (T_3). Nutrient composition data of fortified flour have shown a significant ($p < 0.05$) reduction in moisture content of flour from 12.7% (T_0) to 11.76% (T_3) and protein content from 9.89% (T_0) to 9.79% (T_3). However, a significant increase in ash content was observed by increasing fortificant level from 0.54% (T_0) to 1.04% (T_3). Rheological properties indicated a significant increase in water absorption from 63.60 to 67.527%, dough development time (DDT) from 7.38 to 11.37 min and dough stability from 13.79 to 17.37 min. Textural characteristics of fortified bread revealed a significant reduction for hardness values from 11.40 N (control) to 6.50 N (T_3) in response to fortificant addition. Quality assessment of bread indicated that bread quality was significantly affected with corresponding rises in fortificant addition levels.

Keywords: chicken eggshell; calcium; fortificant; physicochemical; rheology; texture; sensory attributes.

Practical Application: Chicken eggshell calcium can be exploited for bread preparation.

1 Introduction

Eggs are used in enormous quantities throughout the world and food manufacturers produce thousands of tons of wasted chicken eggshell after processing. This poses a serious threat to the environment by causing waste disposal issues and pollution proliferation. Hence, calcium fortified foods have been placed on the market shelf in order to render improvement in recommended calcium intake (almost 1000 mg/day) in humans on daily basis (National Institutes of Health, 2020). This purpose of fortification is served by employing calcium derived from wide range of sources, such as calcium phosphate, calcium carbonate, calcium from dairy products and cattle bone powder (Ray et al., 2017). Cereal flour is currently the most frequently used vehicle for calcium fortification. Unfortunately, calcium in cereal is poorly bioavailable due to presence of anti-nutritional factors like phytic acid that reduce its intestinal absorption which leads to calcium deficiency (Wimalawansa et al., 2018).

Chicken eggshell is chemically comprised of water (2%), solid material (98%), protein (5%) and ash (93%). Apart from calcium, it also provides small quantity of other mineral elements including iron, zinc, magnesium, copper, manganese, sulfur, silicon

(Ali et al., 2019). Calcium carbonate is the best source owing to its cost-effectiveness and high bioavailability. Dissoluble calcium extraction can be carried out by different techniques, such as enzymatic extraction or acidic and alkali hydrolyzed extraction (Li et al., 2013). Acidic method is generally considered as the best choice because of high extraction rate and without causing any injurious effect to human body (Lin et al., 2012). The most important acid for the extraction of dissoluble calcium is citric acid (Shin and Kim, 1997; Yingchun et al., 2005).

The most important source of calcium is chicken eggshells which as a nutritional supplement enjoys great commercial prospects. Traditionally, the chicken eggshell calcium carbonate has been utilized as a fertilizer, conditioner of soil and as feed additives for animal due to its high nutrient availability. In food industry, eggs are used in huge quantity to develop a range of food products all over the world. Therefore, thousands of tons post-processing chicken eggshell waste is produced, which heightens waste disposal issues. In one-way, chicken eggshell waste burdens and pollutes the environment. In other way, it can be utilized in food products to combat calcium deficiency,

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which has become a serious menace for human health especially in developing countries. In this matter, products fabricated by calcium fortification from chicken eggshell waste might contribute recommended dietary intake of calcium for human. Previous report by Naves et al. (2007) indicated that the meal fortified with powder of chicken eggshell significantly contributed to the adequate amount of calcium intake and helped in prevention of calcium deficiency. As a good source of dietary calcium, chicken eggshell may serve as an excellent replacer of shells of crustacean origin. Furthermore, enhanced and easier absorption of calcium derived from chicken eggshell has been reported as compared to commercially available CaCO_3 .

Bread fortified with micronutrients like vitamin and mineral supplement has shown high quality of stability during baking process and free of any kind of off flavor. Calcium used for bread fortification is usually obtained from different sources, such as calcium carbonate, calcium phosphate, calcium citrate and calcium lactate and used at different levels of 0.8, 1.2 and 1.6 g calcium/kg flour. Addition of calcium at different level has little effect on the farinograph characteristics of wheat flour.

It has been reported that amount of calcium absorption from chicken eggshell is higher when compared it to that of dried milk solution as stated previously by Oguido et al. (1995). The best possible way to utilize chicken eggshell as a dietary calcium source is to incorporate in bread, pizza, or spaghetti since minor changes were observed in texture and nearly no change in flavor (Brun et al., 2013). Dietary calcium deficiency has been regarded as one of the most common nutritional issues globally as well as in Pakistan. Keeping in view above all, present study was designed to elucidate the extraction of calcium from chicken eggshell and its utilization as a fortificant in wheat flour. Also, the effect of fortificant was investigated on nutrient composition and rheological properties. Moreover, this study also aimed on the utilization of extracted calcium for bread preparation and examine impact on sensory quality.

2 Materials and Methods

2.1 Procurement of raw materials

Wheat variety was procured from Ayub Agricultural Research Institute, Faisalabad. The chicken eggshell was purchased from local market of Faisalabad and washed from both inside and outside to get rid of dirt and other organic materials followed by sun-drying and crushing to small pieces. All the chemicals used in present study were of analytical grade and purchased from local market.

2.2 Wheat milling

Wheat grains free from dockage and foreign matter were subjected to tempering at 15.5% moisture level. Tempering of wheat was carried out in plastic bins at room temperature for 24 hours in order to equilibrate the moisture within grains. The amount of water for tempering was calculated as per method No. 26-95 given in American Association of Cereal Chemist (2000). Tempered wheat was milled through Brabender Quadromat Senior Mill (C.W. Brabender Instruments, Inc.) to obtain different milling fractions i.e. break flour, reduction flour,

bran and shorts. Then these fractions were weighed and their percentage was calculated on the basis of total material recovered according to American Association of Cereal Chemist (2000) method No. 26-21 A. Straight grade flour yield was determined by blending the break roll flour and reduction roll flour fractions.

2.3 Calcium extraction

Yield of calcium carbonate in terms of percentage from chicken eggshells was given in Table 1. Calcium was extracted from chicken eggshell by following method of Garnjanagoonchorn & Changpuak (2007) with some modifications. The shells were washed with deionized water and peeled off all of the membranes from inside of shell. Then, chicken eggshells were dried with a paper towel and placed in an air forced draft oven (Memmet Germany) for about 20 min to complete drying. Then, dried chicken eggshells were ground to powder by using grinder. The extraction method comprised following steps: soaking cleaned chicken eggshell in 4% sodium hydroxide (NaOH) for 3 h. After this, NaOH residual solution was drained out followed by dewatering using ethanol for soaking. Centrifugalizing at 2000 rpm for 25 min was performed. Same steps were repeated twice. Then, slurry mix was mixed with table salt followed by precipitate dilution with ethanol. After re-precipitating, supernatant was removed and precipitate was recycled. This procedure was repeated twice. Obtained precipitate was centrifuged. The supernatant was taken and subjected to hot-plate heating at 110-115 °C aided with continual stirring until drying. Crystals were obtained by removing solvent and again dried through lyophilizer. Final crystals were obtained in the form of calcium carbonate. The yield of extracted calcium carbonate from eggshells was calculated by the following formula given below.

$$\% \text{ Yield of } \text{CaCO}_3 = \frac{\text{Weight of crystals obtained from drying}}{\text{Weight of crushed eggshell used}} \times 100$$

2.4 Flour fortification

Wheat flour was fortified with different levels of dissolvable organic calcium in terms of CaCO_3 as mentioned in Table 2.

Table 1. % yield of calcium carbonate from chicken eggshells.

Calcium content	Yield (%)
1	87.87 ± 1.77
2	90.34 ± 0.64
3	91.24 ± 0.63

Table 2. Levels of calcium carbonate in SGF of wheat.

Treatments	Level of organic calcium
T ₀	0%
T ₁	1%
T ₂	2%
T ₃	3%

2.5 Chemical analysis

Fortified wheat flour samples were analyzed for proximate composition *i.e.*, moisture content (method No. 44-15A), ash content (method No. 08-01), fat content (method No. 30-25), fiber content (method No. 32-10) and protein content (method No. 46-10) as described in American Association of Cereal Chemist (2000).

2.6 Farinographic properties

Rheological properties of fortified wheat flour samples were determined by Brabender farinograph (Brabender D-4100, Germany) (Lei et al., 2008). Rheological parameters were included as water absorption, dough development time, dough stability, Mixing Tolerance Index (MTI) and softening of dough.

2.7 Determination of calcium content by atomic absorption spectrophotometer

Calcium content in fortified flour was determined by Atomic Absorption Spectrophotometer (Varian AA 240, Victoria, Australia) according to method described in Association of Official Analytical Chemists (2006). Sample (0.5 g) was firstly digested by wet digestion using 10 mL HNO₃ at medium temperature range (60-70 °C) for 20 min then it was digested with HClO₄ at high temperature 190 °C till the remaining 1-2 mL solution become clear and fumeless. Digested sample was transferred to 250 mL volumetric flask and further subjected to filtration according to method of Duhan et al. (2002). Filtered sample solution was loaded to the Atomic Absorption Spectrophotometer. Standard for calcium was prepared by running samples of known strength (American Association of Cereal Chemist, 2000).

2.8 Preparation of bread

Bread was prepared according to American Association of Cereal Chemist (2000) straight dough method No 10-10B. The ingredients were mixed for 5-10 min in a mixer to form dough and allowed to ferment at 30 °C and 75% R.H. for 180 min. First and second punches were made after 120 and 150 min, respectively. The dough was molded and panned into 100 g test pans, and final proofing was done for 45 min at 95 °F (35 °C) and 85% R.H. The bread was baked at 232 °C for 13 min.

2.9 Texture analysis of bread

Texture analysis of bread was performed according to Piga et al. (2005) with some modifications using Texture Analyzer (TA-XT, Plus, Stable Microsystems, Surrey, UK) interfaced with a computer. For the data analysis, the Texture Expert program (version 4.0.9.0) was used. Three repeated measurements were taken for every formulation and mean values were calculated.

2.10 Sensory evaluation of bread

The sensory scores for external characteristics (symmetry of form, evenness of bake, texture, taste, volume, color of crust and aroma) were determined for each loaf assigned by a panel of trained judges according to the bread score method

developed by the American Institute of Baking and reported by Meilgaard et al. (2007).

2.11 Statistical analysis

The obtained data for each parameter was subjected to statistical analysis using different statistical software like SPSS version (version 13, 2004) to determine the level of significance at $p < 0.05$ and comparison of means was also carried out according to method as described by Steel et al. (1997).

3 Results and discussion

3.1 Calcium content

The organic calcium was extracted from the chicken eggshell. The chicken eggshell is composed mainly of calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%) and organic calcium (4%). A good quality chicken eggshell will contain approximately 2.2 g of calcium in the form of calcium carbonate. Approximately 94% of a dry chicken eggshell is calcium carbonate and has a typical mass of 5.5 g. Calcium in the form of calcium carbonate was extracted from chicken eggshell and yield of calcium obtained has been presented in Table 1. The % yield of the present study was found in accordance to Garnjanagoonchorn & Changpuak (2007).

3.2 Effect of fortificant on nutrient composition

Mean values for nutrient composition of fortified flour have been plotted in Figure 1, which delved into significant ($p < 0.05$) differences among treatments. In present study, moisture content in unfortified flour was found to be higher (12.7%) in T₀ while, T₃ exhibited lowest moisture content (11.76%). A declining trend was observed for moisture content by increasing calcium concentration in flour. Moisture content of flour is very important in predicting shelf life; lower the flour moisture, the better is its storage stability.

Ash content of flour represents inorganic residue remaining after organic matter has been burnt away. It is an important parameter of wheat flour quality since, low ash content is associated with higher quality (Wei, 2002). From Figure 1, it could be observed that fortificant had a significant impact on ash content. Mean values for ash content were found to be varied from 0.54 to 1.04% in different treatments. Highest ash content (1.04%) was found in T₃ followed by T₂ (0.94%) while, lowest value (0.54%) was reported in T₀. Present observations are in conformity with Bradauskiene et al. (2017) as well as Ali et al. (2019), who have established an increase in moisture and ash levels of product by augmenting the chicken eggshell powder.

In present study, values regarding fat and fiber content were statistically similar and calcium fortification had no significant effect within treatments. Mean values ranged from 0.02 to 1.03% in fortified flour. In present work, protein content was found to be varying between 9.79 and 9.89% within different treatments of fortified flour. A significant reduction in protein content was observed with the addition of fortificant as from 9.89% (T₀) to 9.79% (T₃). It could be observed from data that nitrogen free

Proximate Composition

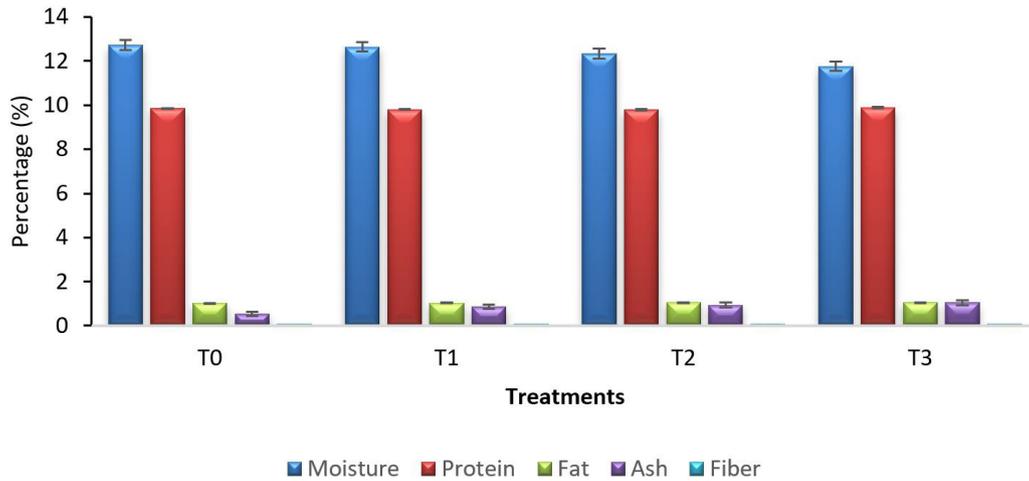


Figure 1. Showing the approximate % of composition of different parameters in calcium fortified wheat flour.

extract (NFE) content in different treatments of fortificant was found significantly different from unfortified flour (Figure 1). In this study, T₃ had significantly highest (76.23%) NFE content, whereas T₀ exhibited lowest (75.62%).

3.3 Effect of fortificants on calcium content of flour

Figure 2 illustrates the calcium content in fortified flour was found to be significantly different from straight grade flour. Addition of fortificant at different concentrations affected calcium content of fortified flour and it was considerably increased from 115.80 mg/kg (T₀) and 1135.1 mg/kg (T₃). In present study, calcium content was found to be significantly highest in T₃ (1135.1 mg/kg), whereas T₀ had the lowest (115.80 mg/kg). Present observations are in agreement with Ali et al. (2019) who have reported that addition of chicken eggshell powder resulted an increase in calcium content. These results are well justified by the work of Khan et al. (2017) who have found a significant ($p \leq 0.05$) improvement in calcium content of breads fortified with bone extract, chicken eggshell powder and CaCO₃. In comparison, leavened bread made by fortification with chicken eggshell powder at 1.5% level resulted higher calcium concentration as 1545.3 mg/100g. In another study, Romanchik-Cerpovicz & McKemie (2007), calcium content of fortified tortillas was found to be 8.6 times higher than control.

Furthermore, Ali & Badawy (2017) have compared various sources of chicken eggshells including white chicken, brown chicken, duck and quail and evaluated their supplementation effect on bread strips. Among various chicken eggshells, duck eggshell indicated maximum calcium concentration followed by quail, white chicken and brown chicken eggshells. In an earlier study, Hassan (2015) examined the impact of chicken eggshell addition at 3, 6 and 9% on quality parameters of biscuits. Research findings pointed out that biscuits supplemented with chicken eggshell powder resulted in a prominent increase in calcium levels i.e., 607.33 mg/100g (3%), 1378.11 mg/100g (6%) and 2175.23 mg/100g (9%).

NFE and Calcium content

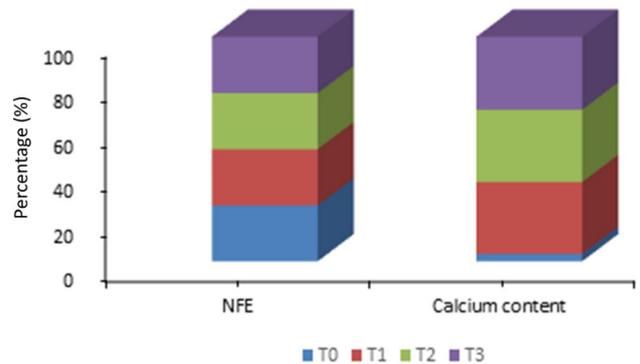


Figure 2. NFE and calcium content of fortified wheat flour.

3.4 Farinographic properties of calcium fortified flour

For cereal scientists, rheology is the most important regarding the evaluation of flour quality. Basically, rheological tests are employed to evaluate mechanical properties, and behavior of materials in processing and to evaluate end use quality throughout processing chain (Dobraszczyk & Morgenstern, 2003). In this matter, Brabender Farinograph is an eminent instrument for dough analysis and mostly used to determine dough physical characteristics (Hadnadev et al., 2011). Generally, it is used to reckon water absorption on flour and also gives information about mixing characteristics of flour by recording dough mechanical resistance in mixing and kneading (Mondal & Datta, 2008).

Water absorption in different treatments of fortified flour varied significantly in response of the addition of fortificant as obvious from Figure 3. In present investigation, water absorption was found in the range of 63.60-67.527%. Within treatments, highest water absorption (67.52%) was recorded in T₃ whereas, control exhibited

the lowest value (63.60%). Present results established an increasing trend for water absorption by increasing the level of fortificant in wheat flour. A more plausible explanation might be that chicken eggshell is a hydrophilic filler, which tend to absorb large amount of water as described by Shuhadah and Supri (2009). These results are consistent with Ali & Badawy (2017) who have found a considerable increase in water absorption ratio of fortified bread strips. Present results can also be justified by Salem et al. (2013), who studied the impact of chicken eggshell addition on dough rheology of cake. According to research findings, water absorption was found to be increased by augmenting the level of chicken eggshell addition.

Figure 3 demonstrates statistical data regarding Dough Development Time (DDT) shows a highly significant effect of fortificant on treatments. In current study, a significant increase in DDT was reported from 7.38 min (control) to 11.37 min (T_3). Mean values for dough stability varied from 13.79 min to 17.37 min. Among treatments, T_3 revealed significantly highest dough stability (17.37 min) while, control had the lowest (13.79 min) A similar increasing trend was noticed for dough stability by increasing the level of fortificant in wheat flour. These results can be justified by a recent work of Alsuhaibani (2018), who reported that bread supplemented with egg and oyster shells indicated higher values of water absorption, DDT and dough stability in comparison to control. However, in contrast to present findings, Salem et al. (2013) have designated a reduction in dough development time and dough stability with chicken eggshell addition in wheat flour. Statistical data regarding MTI and degree of softening showed that fortificant addition did not cause a significant effect on different treatments. Mean values regarding MTI and degree of softening in fortified wheat flour were recorded in the range of 19.75-22.0 BU and 19.89-20.47 BU, respectively.

3.5 Textural characteristics of calcium fortified bread

Bakery products have a characteristic shape and definite texture that is accepted by the consumers. Any significant deviation from optimal texture characteristics of product can be

considered as a reduction in the quality. Texture has a significant influence on the consumer's perception of a good bread quality. One of most imperative attributes of bread include hardness, which is defined as the force required for biting bread samples.

Hardness values for bread fortified at various levels presented in Table 3, divulged a significant influence of fortificant on different treatments. Mean values have shown a significant reduction in hardness values from 11.40 N (control) to 6.50 N (T_3) by increasing the level of calcium fortificant in wheat flour. Moreover, the impact of fortificant on bread chewiness was highly significant however, springiness was observed to be statistically similar.

3.6 Organoleptic properties of bread

Data regarding bread volume show that, all the treatments were statistically similar as compared to control and addition of fortificant did not influence volume of bread (Figure 4). However, addition of fortificant at different levels significantly reduced aroma of bread from 6.93 (control) to 3.96 (T_3). Statistical results regarding quality assessment of bread fortified at different levels showed that fortificant caused a highly significant impact on crumb color, crust color, of bread. A decreasing trend was noted for all the quality parameters of bread by increasing the addition of fortificant in bread except crumb color. Moreover, taste and texture was also significantly different from unfortified bread

Table 3. Textural analysis of calcium fortified bread.

Treatments	Chewiness	Hardness	Springiness
T_0	3.94 a	11.40 a	0.72 a
T_1	6.12 c	8.70 b	0.94 b
T_2	6.93 b	7.20 c	1.17 c
T_3	7.11 a	6.50 d	1.23 d

Any two means not sharing same letter (a-d) differ significantly from each other
 T_0 = Control; T_1 = 1% $CaCO_3$; T_2 =2% $CaCO_3$; T_3 =3% $CaCO_3$

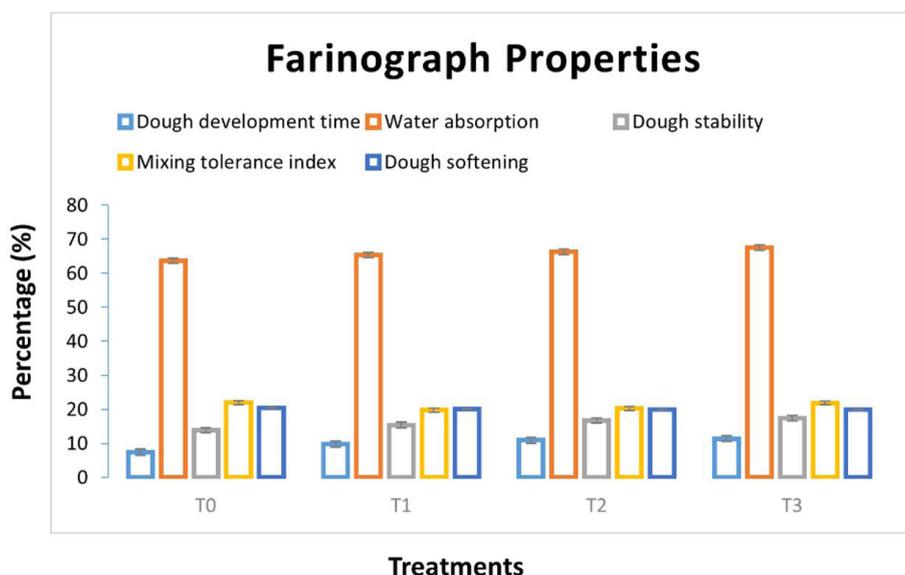


Figure 3. Farinograph properties of calcium fortified wheat flour.

Sensory Attributes

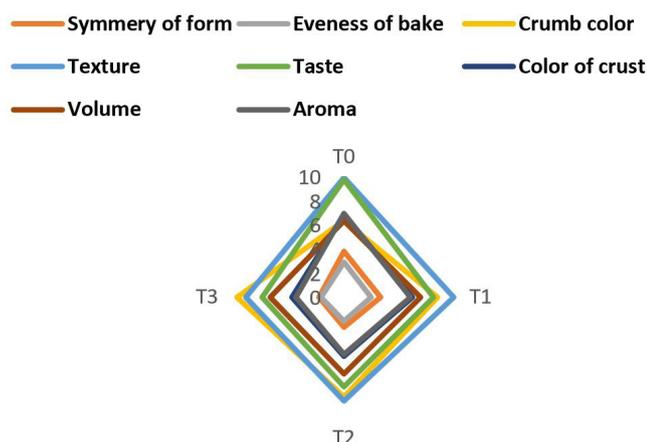


Figure 4. Sensory attributes of calcium fortified wheat bread.

and a significant reduction was observed for taste from 9.73 (control) to 6.80 (T₃) and texture of bread from 9.90 (control) to 8.13 (T₃).

In another study, Bradauskiene et al. (2017) studied the possibility for the enrichment of chicken eggshell powder (ESP) at 0, 2.5, 5.0, 7.5, 10.0, 12.5 g in bread making. Sensory assessment of bread exhibited improved overall acceptability than control although, taste and flavor was alike or became worse. Among various concentrations studied, bread baked by adding 5 g ESP was regarded best. In a recent work, Ali et al. (2019) reported that optimum level of chicken eggshell addition in wheat flour is 8% with respect to calcium concentration and bread quality.

Khan et al. (2017) evaluated the effect of fortification of calcium on leavened and unleavened bread by adding chicken eggshell powder, chicken bone extract and CaCO₃ in whole wheat flour at 0.5, 1.0 and 1.5% levels. According to their findings, bread made by fortification with bone extract, chicken eggshell powder and CaCO₃ at different levels had a significant ($p < 0.05$) impact on overall acceptability value. On the whole, sensory quality was declined by increasing the level of all fortificants especially with bone extract.

In addition to bread, chicken eggshell powder has been widely utilized in other bakery products. In this perspective, Jumma & Ali (2017) studied the impact of wheat flour substitution with chicken eggshell on quality attributes of biscuit and suggested that by increasing the substitution of wheat flour with chicken eggshell considerably increased calcium content of fortified biscuits. Moreover, Ray et al. (2017) explored the application of chicken eggshell powder in chocolate cake as a dietary calcium source at 3, 6 and 9%. Their findings revealed that supplementation of cakes with chicken eggshell powder remarkably heighten the calcium content of cakes as 504.5 mg/100g (3%), 816.8 mg/100g (6%) and 1364.5 mg/100g (9%). It was established that chicken eggshell could be exploited to supplement cake formulations up to 6% level in order to achieve desirable calcium level, texture and sensory quality.

4 Conclusion

In this study, potential of calcium extraction from chicken eggshell and its utilization as a bread fortificant in straight grade flour (SGF) is investigated. In addition, the fortified and unfortified (control) flour samples were evaluated for nutrient composition and rheological properties. Results showed that addition of fortificant at different concentrations significantly ($p < 0.05$) enhanced calcium content of fortified flour and it was considerably increased from 115.80 mg/kg (T₀) to 1135.1 mg/kg (T₃). Nutrient composition data of fortified flour have shown a significant ($p < 0.05$) reduction in moisture content of flour from 12.7% (T₀) to 11.76% (T₃) and protein content from 9.89% (T₀) to 9.79% (T₃). However, a significant increase in ash content was observed by increasing fortificant level from 0.54% (T₀) to 1.04% (T₃). Similarly, rheological properties indicated a significant increase in water absorption, DDT and dough stability, which is advantageous for bread making that require high water absorption values. Textural analysis of fortified bread revealed that unfortified bread was harder in comparison to fortified bread. Quality evaluation of bread pointed out that fortified bread was acceptable at all three levels.

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