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Chicken Eggshell Powder as Dietary Calcium Source in Biscuits

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Abstract: Chicken eggshells can be utilized as a dietary calcium source. In this study the effect of eggshell powder supplementation on each of chemical composition, physical, sensorial properties and bioavailability of biscuit were studied. The biscuit was prepared from wheat flour supplemented with eggshell powder at three levels of supplementation as follows 3, 6 and 9%. The boiling process for 30 min of eggshell kills any microbial growth on the surface. The obtained results indicated that the addition of eggshell powder led to a pronounced increase calcium contents in the supplemented of biscuits 607.33, 1378.11 and 2175.23mg/100g at 3, 6 and 9%, respectively. It also, small change in texture and sensorial properties of biscuits.The calcium bioavailability of biscuits containing chicken eggshell powder at 3, 6 and 9% were 26.0, 35.4 and 41.43%, respectively. With respect to the calcium content, texture, sensory properties and calcium bioavailability, we can suggest that the best way to use chicken eggshell as dietary calcium supplement is powdered to biscuit until 6% eggshell supplementation.

Key words: Calcium · Biscuits · Eggshell · Supplementation · Bioavailability

INTRODUCTION

In many countries around the world, egg products companies and some food manufacturers that use egg in their products generate tons of waste eggshell, this possess a serious environmental problem. Therefore, to increase daily calcium intake, commercially available calcium fortified foods have been placed on the market. These foods are fortified with many calcium sources such as calcium carbonate, calcium phosphate, cattle bone powder and milk calcium [1]. Eggshell makes up from 9-12% of the total egg weight; it consists largely of calcium carbonate (94%) with some magnesium carbonate and calcium phosphate deposited on the organic matrix [2]. Eggshell calcium is a good source of dietary calcium and an excellent replacement material for important crustacean shells [3]. Moreover, calcium from crushed eggshell powder was absorbed easier than commercial $CaCO_3$ in the rat small intestine [4]. Chicken eggshell is a waste material from domestic sources such as hatcheries, poultry farms, egg product factories, homes and restaurants [5, 6]. Many researchers have been looking for ways to utilize the eggshell waste as a source of Calcium in human nutrition [7]. Eggshell and shell membranes are non-edible byproducts with little saleable value but they may contain biologically active compounds [8]. From the chemical point of view, the egg shell consists of water (2%) and dry matter (98%). The dry matter is composed of 5% crude protein and 93% ash. Average values of mineral contents in different parts of the egg and egg shell [9]. Eggshells contain calcium and trace amounts of other micro elements, i.e. magnesium, boron, copper, iron, manganese, molybdenum, sulphur, silicon and zinc [10]. A risk factor for human health is the infection with *Salmonella enteritidis* at consumption of undercooked eggs [11], as eggs can become contaminated internally and on the outer shell surface. Despite the fact that pasteurization was predicted to be effective for reducing *S. enteritidis*. Therefore, the way to avoid *Salmonella* contamination is to eliminate the infectious agent from eggs through several cooking methods. Another way to avoid *Salmonella* is to thoroughly wash eggshells under running tap water, scrub with a domestic sponge and then immerse eggshells in a solution of 10 drops of sodium hypochlorite (domestic bleaching agent) per liter of tap water. Rinse, dry with paper towels and use the eggshells [12]. Therefore, it is important to emphasize the necessity to avoid the contamination with *Salmonella*. By-products from processed foods are promising as good natural calcium sources such as egg-shell [13]. Deficiency of calcium (Ca) in the diet is a common problem. Ca intake from dairy products is an appropriate way to fulfill Ca

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requirements. However, people do not usually consume them in the amounts established by clinical guidelines. Supplementation with tablets is costly and sometimes involves difficulties of adherence to treatment. Chicken eggshell is a source of Ca, which is available at home that can be used as Ca supplementation [14]. Calcium is a mineral required to human body health for a variety of physiological functions and the maintenance of bone tissues throughout life [15]. As a structural component, calcium combines with phosphorus to comprise the mineral portion of bone and teeth, also it has function as a metabolic component, biochemical and physiological processes [16, 17]. Calcium plays a key role in the treatment and prevention of bone demineralization. A widely used calcium enrichment source is purified $CaCO_3$ with a high calcium content (about40%). Chicken eggshell powder, with a calcium content of about 38%, is a promising but little known source of calcium for human nutrition. The use of chicken eggshell powder might be beneficial, it could increase bone density and reduce pain in patients with osteoporosis. Eggshell calcium is probably the best natural source of calcium and it is about 90% absorbable [10]. Biscuits are flour-based bakery products which attract consumers owing to their various tastes, long shelf life and relatively low cost [18]. Due to competition in the market and increased demand for health-promoted natural products, attempts are being made to improve biscuits nutritional value as well as functionality by modifying their nutritive composition [19]. Therefore, the expanded application of eggshell in food industry was worthy to study further. The present work was conducted to study the effects of biscuit fortification 3%, 6% and 9% with eggshell powder on chemical composition, mineral content, physical characteristics, organoleptic properties and in vitro bio-available Ca of the prepared biscuit.

MATERIALS AND METHODS

Materials: Wheat flour (72% extraction) was obtained from El-Haram Milling Company, Faisal, Giza, Egypt. All other ingredients like sugar, butter, baking powder, eggs and vanilla were obtained from local market. Chicken eggshells were collected the waste factory of Food Technology Research.

Preparation of Eggshell Powder: Chicken eggshells was washed twice and processed using four different ways:

- The chicken eggshell was boiled in deionized water for 30 minutes.
- The chicken eggshell was dried in hot air oven at 80°C for 2h.
- The chicken eggshell was sterilized in an automatic sterilizer autoclave for 15 min at 134°C then dried in oven
- Chicken eggshell was sterilized in an automatic microwave (Microwave SL 9000, Buenos Aires, Argentina) for 5 min then dried in oven.
- Each treatment eggshell of dried was ground to powder by using household mill (Braun, Germany). The eggshell powder which passed 40 mm sieve was used in the study.

Preparation of Biscuits: Biscuit was made according to the standard procedure for sweet biscuit at Bisco Misr Co., Cairo with some modification. The blends and formula of control biscuit and other suggestion formula to made biscuits were made according to the method described by Wade [20] with some modification.

For making Biscuit: All dry ingredients were mixed together eggshell by rate 3%, 6% and 9% in a dough mixer for 3 minutes, then all liquid ingredients were added to the dry mixture and mixed at low speed for 3 minutes then water added as require to obtained suitable smooth dough and the dough was let to rest for 5 min. Then sheeted to 3mm. thickness. Circle pieces cut of dough were formed by using of templates. The biscuits were baked at 180 °C for 12 minutes. After baking, biscuits were allowed to cool at room temperature for 1 hr. before sensory evaluation.

Methods:

Chemical Composition: Moisture, crude protein, fat and ash content were determined according to the methods described in A.O.A.C. [21].

Determination of Minerals Content: Minerals content (magnesium (Mg), sodium (Na), zinc (Zn), manganese (Mn), iron (Fe), calcium (Ca), potassium (K) and copper (Cu)) were digestion as described by Kirleis *et al.,* [22] and determined by using the atomic absorption spectrophotometer (Perkin- Elmer 3300).

Microbial Evaluation of the Eggshell Powder: Microbial count was determined according to standard method described by Giwa *et al.,* [23].

Physical Evaluation of Biscuits: Biscuit were evaluated for thickness (mm), diameter (mm) and spread ratio. Five biscuits were used for the evaluations from the three studied biscuits and averages were recorded. The spread

ratios were evaluated according to Manohar and Rao [24]. Spread ratio was calculated by dividing the diameter by value of thickness of biscuits.

Texture Analysis of Biscuits: Texture of biscuit was determined at different storage intervals according to Piga *et al.,* [25] by using a texture analyzer CT3. The HDP 3-point Bending Rig of texture analyzer (TA-XT2i, Stable Micro Systems, Haslemere, UK) was used to determine biscuit fracture strength (hardness).

Sensory Evaluation: Biscuits were evaluated for their sensory characteristics by ten panelists from the staff of Bread and Pastry, Research Dept., Food Tech. Research Institute., Agricultural Res. Center, Giza, Egypt. The scoring scheme was established as mentioned by Meilgaard *et al.,* [26] as follows: color, taste, flavor, appearance and overall acceptability degrees.

***In vitro* Calcium Bioavailability**: The method used for the determination of calcium bioavailability was the *in vitro* equilibrium dialysis according to method of Miller *et al.* [27]. The acceptability of biscuits-supplemented products was also evaluated. This analysis is based on the simulation of a gastrointestinal digestion of food with pepsin-HCl during the gastric stage and pancreatin-biliary salts during the intestinal stage. The fraction of diffused element through a semi-permeable membrane during the intestinal stage is measured to predict the calcium dialyzability.

Statistical Analysis: The gathered results in present study were statistically analyzed using the SPSS version 10.0 windows program [28]. Data were expressed as means ± SEM. Statistical analysis was performed using one-way analysis of variance followed by Turkey post hoc test. Duncan's tests were done to compare a pair of group means.

RESULTS AND DISCUSSION

Chemical Composition of Raw Materials: Data presented in Table 1, showed the chemical composition of raw materials used in the preparation of biscuits. It could be demonstrated that chicken eggshell powder contained the highest values in ash (90.2%), whereas it was the lowest values in Protein and fat value (5.40 and 0.02%, respectively) than wheat flour (9.50 and 0.80%, respectively). From the same table, it could be seen that, the Eggshell powder (ESP) had the highest value of Ca, Mg, Fe, Zn, Cu and Mn (35080, 262, 13.06, 145.1, 4.1 and 149.9 mg/100g, respectively), whereas wheat flour showed less than ESP contents of the aforementioned mineral. Walton *et al.,* [29] reported that the chemical composition of eggshell was 91.1% ash, 7.56% protein, 0.24% lipid, 36.4% calcium, 0.002% iron, 0.097% potassium, 0.398% magnesium, 0.152% sodium and 0.116% phosphorus. Also, Burley and Vadhera [30] studied the chemical composition of eggshell and reported that its content of water, proteins, lipids, inorganic salts and total solids were 1.66, 6.40, 0.03, 91.1 and 97.4%, respectively. Schaafsma *et al.,* [7] found from 385 to 401 mg Ca/g eggshell depending on the eggshell origin and concluded it may be used as a Ca source in human nutrition. On the other hand, Siulapwa *et al.,* [31] stated that calcium was the highest mineral constituent (225.35) mg/g in eggshells. The least mineral constituent in egg shells was copper, manganese,zinc, iron, potassium, sodium and magnesium.

Table 1: Chemical composition of Wheat flour and Eggshell powder (% on dry weight basis)

Parameter (%)	Wheat flour (72% extraction)	Eggshell powder (ESP)
Crude protein	9.50	5.40
Fat	0.80	0.02
Ash	0.45	90.2
	Minerals (mg/100g)	
Ca	45.95	35080
P	121.2	150.2
Mg	30.2	262.0
Fe	1.05	13.06
Zn	0.55	145.1
K	111.21	50.00
Na	55.9	47.9
Cu	0.20	4.1
Mn	0.25	149.9

Table 2: Microbiological assay for eggshell powder

	Total bacterial counts (cfu/g)			
Treatments	----------			Yeast and Mold (cfu/g)
Untreated*	--------$200x10^3$---------		$90x10^5$	Negative
Treated	Boiling	Negative	Negative	Negative
	Autoclaved	Negative	Negative	
	Oven	Negative	Negative	
	Microwave	Negative	Negative	

*Eggshell without any treatment

Microbial Evaluation of the Eggshell Powder: The results presented in Table 2 showed that, the using of eggshell powder without using any treatment led to microbial growth. The total bacterial count was recorded 90 x 10^5 (cfu/g) and no growth for yeast and mold.After treatment, asboiling, autoclave, microwave and oven.All treatments processes remove any microbial growth on the eggshell powder. Savil *et al.,* [32] observed that the minimum time necessary to eliminate contamination through cooking procedures was 5 minutes after the water starts boiling

Table 3: Chemical composition of produced biscuits (% on dry weight basis)

		Biscuits samples		
Components (%)	Control (Wheat flour 72%)	3% ESP	6% ESP	9% ESP
Crude protein	9.32[a]	9.39[a]	9.21[a]	9.12[a]
Ash	1.26[d]	4.29[c]	7.34[b]	9.70[a]
Fat	17.65[s]	17.62[a]	17.60[a]	17.57[a]
		Minerals (mg/100g)		
Ca	43.9[d]	607.33[c]	1378.11[b]	2175.23[a]
P	78.7[d]	102.1[c]	115.1[b]	131.2[a]
Mg	18.51[c]	18.96[b]	19.42[a]	19.88[a]
Fe	1.11[c]	1.15[c]	1.18[b]	1.22[a]
Zn	0.08[b]	0.18[b]	0.19[a]	0.39[a]
K	116.73	116.64	116.6	116.2
Na	519.0	521.2	523.32	524.47
Cu	0.031[b]	0.033[b]	0.036[a]	0.039[a]
Mn	0.25[d]	0.62[c]	1.01[b]	1.45[a]

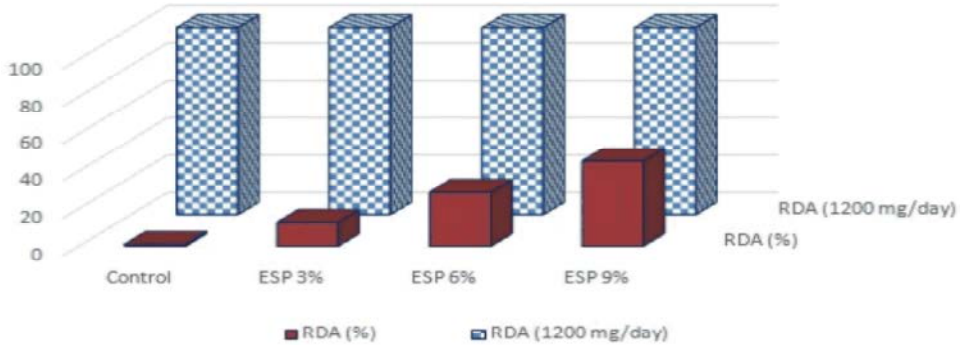

Fig. 1: Relationship between RDA (%) of calcium content in biscuits supplemented with ESP and Recommended Dietary Allowance (RDA) (1200 mg/day).

and also, cooking in the microwave and oven complete eliminates the microbial contamination. To make eggshell powder, boil eggshells in hot water for 5-10 min to kill pathogens, air dry them then grind into a fine powder [33]. The results in Table 2 cleared that, the boiling process removed the microbial contamination and the author decided supplementation of biscuits with eggshell boiling treatment because it easier than any process.

Chemical Composition of Produced Biscuits: The variations of mineral content of unfortified and fortified biscuits with 3, 6 and 9% eggshell powder were presented in Table 3. Data showed that, biscuit which containing ESP at level of 9% had the highest value of ash and lowest value of protein and fat compared with the control (wheat flour 72%). Also the results presented in Table 3, showed that samples of biscuits which containing ESP at level 9% had the highest values in minerals content i.e., Ca, Mg, Fe, Zn, Na, Cu and Mn compared with the biscuit control. The minerals contents for calcium, magnesium, sodium, iron and zinc contents of the supplemented biscuits with 3, 6 and 9% eggshell powder recorded increased than the control. The highest Ca contents by 607.33, 1378.11 and 2175.23 mg/100g of the supplemented biscuits with 3, 6 and 9% eggshell powder, respectively. Many researchers have been conducted to evaluate the possibility of eggshells utilization as a food ingredient as source of Ca [34]**.**This led to an increase in calcium to phosphorus ratio in the fortified biscuits which is in favor of the probable increase in calcium utilization in humans [35]**.** The most pronounced effect of biscuit was shown in calcium. The addition of eggshell powder led to a great increase in calcium, calcium to phosphorus ratio and iron contents in the fortified butter cake [36]. The data in Fig. 1 indicate the RDA (%) of Calcium content from biscuits supplemented with eggshell powder at levels 3, 6 and 9% which were 12.65, 28.71 and 45.4 % respectively. These reading were compared with the Recommended Dietary Allowance (RDA) (1200 mg/day) [37]. Moreover, these values attributed to biscuit produced by Bisco Luxe (Bisco Misr) which has weight 25g.

Table 4: Physical properties of the biscuits supplemented with eggshell powder

Biscuits	**Diameter (D) (mm)	**Thickness (T) (mm)	**Spread ratio (D/T)	Hardness (N)
*Control	46.0	5.93	7.76	34.50
3% ESP	45.8	5.97	7.67	35.00
6% ESP	45.3	5.97	7.59	36.50
9% ESP	45.0	6.01	7.49	39.00

*Control: wheat flour (72% extraction) **average of 10 biscuits

Table 5: Sensory Evaluation of biscuit supplemented with eggshell powder

Biscuits	Appearance (10)	Color (10)	Flavor (10)	Taste (10)	Overall acceptability (10)
*Control	9.5[a]	9.5[a]	9.7a	9.5[a]	9.55[a]
3% ESP	9.4[a]	9.4[a]	9.5[a]	9.5[a]	9.45[a]
6% ESP	9.3[a]	9.5[a]	9.5[a]	9.4[a]	9.38[a]
9% ESP	9.0[b]	9.4[a]	9.0[b]	9.0[b]	9.20[b]

*Control: wheat flour (72% extraction). Means followed by different superscripts within columns do not significantly different (P<0.05).

Physical Properties of Biscuit: The variations of physical properties of un supplemented biscuit and supplemented biscuit with 3, 6 and 9% eggshell powders are presented in Table 4. Diameter, thickness and spread ratio in unsupplemented biscuit (control) were 46.0 mm, 5.93 mm and 7.76 mm, respectively. Diameter and thickness increased but spread ratio decreased with increasing the level of eggshell powder. The fracture strength (hardness) of biscuits made from eggshell 9% was found to be the highest (39.0 N) whereas those made from wheat flour was the lowest (34.50N). The hardness of biscuit increased as the level of supplementation eggshell increased. However, there was no significant difference in pressing force used to break the crisp flour between the three levels of the same calcium source formulas. Two mechanisms, firstly, substitution of wheat flour by calcium sources reduces the amount of amylose and amylopectin which are the major components being puffed. Secondly, the particles of calcium sources depositing within the starch gel structure interfere with that structure. The higher levels of calcium sources added to the formulations lead to smaller size pores or air cells in the puffed crisp biscuit and by this means make the crisp wheat flour less crisp. However, there were no differences in crispiness between crisp flour formulated with the different levels of the same calcium source despite the differences found in expansion [38]. The increased hardness of biscuits after eggshell incorporation may be due to very high increase in ash content of the biscuits as compared to the control sample [39, 40].

Sensory Evaluation of Biscuits: The effect of eggshell powder supplementation on sensorial properties of biscuits was studied in Table 5. The biscuits were evaluated for organoleptic characteristics by a panel of trained judges. The mean scores for color, taste, flavor, appearance and overall acceptability of unfortified biscuit (control) were 9.5, 9.5, 9.7, 9.5 and 9.55, respectively. The results in this Table 5 also showed that, no statistically significant difference were observed between unfortified biscuit "control sample" and biscuit which supplemented with 3, 6 and 9% eggshell for color. Supplementation of biscuit with 9% eggshell resulted in significant (P<0.05) at taste and appearance. The sensory evaluation also indicated similarity of crispiness, flavor and taste profiles until 6%levels of the same calcium source. On the other hand, as higher levels of the calcium sources were added, also a stronger fishy smell was observed due to higher levels of calcium sources [4].This was confirmed with sensorial test showing that the firmness of eggshell increased as the level of supplementation increased. The acceptability of fortified eggshell calcium powder tended to until 6% egg shell fortification**.** Brun *et al.,* [14] found that the best way to use chicken eggshell as Ca dietary supplement is powdered to add to bread, pizza or spaghetti as there were small changes in texture and no changes in flavor. Calcium fortified foods should have similar physical and sensory characteristics as their non-fortified counterparts [41].

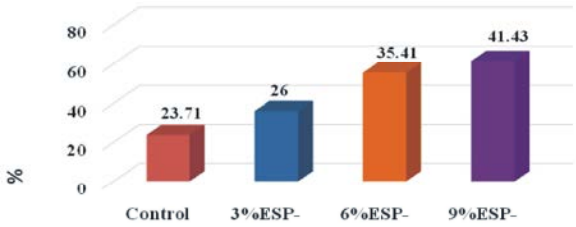

Fig. 2: The effect of fortified biscuits with eggshell powder on calcium bioavailability.

***In vitro* Calcium Bioavailability:** The *in vitro* equilibrium dialysis was used to evaluate the calcium bioavailability in all treatments. The results in Fig. 2 indicated that, the

calcium bioavailability from a biscuits containing chicken eggshell powder at 3, 6 and 9% were 26.0, 35.41 and 41.43%, respectively. There were few papers describing the use of chicken eggshells as calcium supplement in human beings. Schaafsma and Pakan [42] showed an increase in lumbar spine, total proximal femur and trochanter bone mineral density in osteoporotic postmenopausal women who received eggshell powder with vitamin D_3 and magnesium supplementation. Oguido *et al.,* [43] examined absorption of calcium from solution of dried milk and from eggshell were estimated in male. The percentage and rate of calcium absorption was greater for eggshell than for milk. The addition of vitamin D improves bone mineral density of the lumbar spine and the proximal tibia in rats treated with calcium carbonate ($CaCO_3$) or eggshell [44]**.** Several calcium sources are available for food fortification [45]. Calcium carbonate ($CaCO_3$) is the most widely used Ca salt because 40% of the compound is well absorbable. Natural Ca sources are of interests because they contain not only Ca but also other elements (e.g. Fe and P). Makai and Chudacek [35] in an elderly population study found that the use of ESP resulted in increased bone mineral density (BMD) and decreased pain in the tested subjects. These effects might be due to the presence of calcitonin which inhibits osteoclasts and reduces pain in subjects with a high bone turnover [46]**.** A piglet study of Schaafsma and Pakan [42] showed that Ca from ESP was as well absorbed as sources were given in combination with soya protein. Therefore, ESP is a good source of highly bioavailable Ca.

CONCLUSION

It can be concluded that eggshell powder is an appropriate and cheap source of Ca for human nutrition easily prepared at home. Eggshell powder supplementation with wheat flour did not produce important changes in flavor and texture in biscuit up to 6%.

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