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Chemical composition and microbial evaluation of egg shell powder and bones extract powder fortified cookies as a source of Ca boom

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Abstract

Ca is an important part of skeletal system of body. Its deficiency is occurring mostly in adolescent boys, causing weak bones. No doubt, the synthetic source of Ca is already available in market, but there is need of natural yet inexpensive Ca rich sources to be utilized to make daily consumable food products. For this purpose control, Egg shell powder (ESP), Bones extract powder (BEP) and $CaCO_3$ fortified cookies were developed and studied regarding proximate composition and microbial properties. The obtained results indicated that the addition of ESP, BEP and $CaCO_3$ impacted non-significantly in case of proximate composition and microbial analyses, while significantly impacted the ash, Ca, and P content of cookies. Composition of ESP and BEP indicated that the highest amount of Ca (38.2g/100g) was in ESP, P (14.6g/100g) in BEP and TPC of control treatment was 1.51 ± 0.339^{A} Log 10 (cfu/g), while for ESP, BEP and $CaCO_3$ was in acceptable range. It was concluded that naturally fortified cookies with BEP yielded 1979.2 ± 13.1mg Ca/100 g so with respect to the calcium content, if BEP fortified cookies developed at commercial level it could be a wealthy and healthy substitute nutraceutics in comparison to synthetic choices.

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Introduction

As we know calcium (Ca) as a nutrient is most necessary part of body for development and metabolism of bone. Above 99 % of total body Ca is present as Calcium hydroxyapatite (Ca_{10} $[PO_4]_6[OH]_2$) in bones and teeth (Del *et al*., 2011).Ca intake discovered in the children of Pakistan who consuming an average of 607.5 mg of Ca/day. When the Ca consumption determines on the provincial basis it discover that the consumption of Ca in Punjab are 637 mg/day (National Nutrition Survey Report, 2011).

The major structure of bones is organic (30 %) and inorganic material (70 %). Mineral portion of bones provide toughness and appropriate mechanical properties (Orlovskii *et al*., 2002). Mostly leak out of Ca from the body ensue over and ended with urine, feces as well as oblivious loss. (Kerstetter *et al*., 1989). For teenage skeletal deposit is 300 to 400 mg / day, urinary loss is about 100 mg and unresponsive loss is about 40 mg per day (Abrams and Stuff, 1994). In Pakistan nearby 51.1 % of the women are hypocalcaemic out of which 51.9 % live in urban areas and 50.8 % are resident of rural areas.

In Punjab-Pakistan 52.3 % women suffer from Ca deficiency (National Nutrition Survey Report, 2011).The high level of osteoporosis found 97 % in women aged 75-84 and 55 % women 45-54 years in Pakistan predisposed to osteoporosis (Habiba *et al*., 2002; Shahid *et al*., 2019). Almost, 1.66 million hip fractures occur every year world-wide, that the incidence is set to increase 4-fold by the year 2050 because of the growing numbers of older people, and that age-adjusted incidence rates are many times higher in Western countries than in Asia and sub-Saharan Africa (Melton, 1995).Calcium and vitamin D supplements are extremely cost-effective, and expanded use could substantially reduce fractures and related costs Weaver (2019).

In Pakistan eggs consumption is high. As a major supplier to the nation‘s food supply, 28 % of eggs consumption is being consumed in processing plants manufacturing products such as cakes, bakery etc. This results in huge amount of egg shell as a waste. Almost, 90 % of this waste volume is Calcium carbonate, (Bowero, 1992). Reid (2015) reported that no connection of Ca dietary and rate of loss.

The eggshell calcium 300 mg/d was given to patients. At the early stage bone mass (Speed of Sound (SOS)) was checked, at the center stage (6th month) and the last stage (12th month) by the single blind process. Significantly enlarged SOS of the eggshell group at 12 Mo ($p<0.05$) and which also was higher significantly than that of the placebo and Ca-Carbonate groups at 12 mo ($p<0.05$). Sakai *et al*., 2017). Consumption of waste egg shells by industries will be beneficial step for growth rate. (Waheed, 2019).

Materials and methods

Product development

Commercial straight grade flour (CGSF), sugar, butter, egg, chicken bones, egg shells were purchased from market.

Extraction of Ca from chicken bones

Chicken bones was de-flashed after boiled then for 10 mints dried in hot air oven, then boiled with NaOH (3: 1 w/v) 3 % solution, filtered, again washed with 1 % HCl (1:1 w/v) then neutralized with deionized water, dried at 100 °C for 2 hours in Hot Air Oven at the end make fine powder (adapted from Chavasity, 1996).

Extraction of Ca from egg shells (ES)

ES were washed through water, boiled in water at 100 °C for 10 minutes. Dehydrated in Hot Air Oven at 70 °C for 1hourthen prepared fine powder. Furthermore, before consuming powder in product made the sterilization in an Oven at 134 °C for 15 minutes (adapted from Schaafsma*et al*., 2000).

Preparation of calcium fortified cookies

4treatments of cookies were prepared as given in Table 2 with variations in recipe (table 1) given by AACC (2000). For homogeneous mass Ca fortificant (975mg) were mixed in treatments T_1, T_2, T_3whereas

T_0 is control (without added Ca powder). The cookies were baking at 175±5°C for 25min.

The cookies were cool, sealed in bio-oriented polypropylene (BOPP) wrappers, packed and stored at ambient temperature for additional analyses.

Analytical method

Proximate analysis

Moisture content

After dehydrating the sample in hot air oven (Model: ED 115, Binder, Germany) moisture was measured similar to method number 44–15a described in AACC (2000).

The moisture of cookies was firm by measuring a sample of 5 g in a china dish and drying at a temperature of 105 ± 5 °Ctill a continuous weight of the dry object was achieved.

Crude fat

The raw fat content was composed with a Soxhlet device (Model: 0503011, Extraction Unit, Barcelona, Spain) according to the method No. 30-10 described in AACC (2000).

Crude protein

The nitrogen content of cookies was measured through the Kjeltech instrument (model: 4061412, S1, Behr Labor Technik, GmbH, Germany) concerning to method number 46–10 given in AACC (2000).

Total ash

It is a non- inorganic remainder after being totally removed organic materials at a temperature of 550 ° C in a muffle furnace (model, SNOL 8, 2/1100, Lithuania).

Cookie samples were surveyed for ash content, following the method steps in AACC (2000) method number 08-01.

Nitrogen free extract (NFE)

The NFE was calculated at the interval prescribed for 60 days of storage according to the following expression.

NFE % = 100 - (crude protein % + crude fat % + crude fiber % + total ash %)

Mineral analysis

Ca Content

The sample for CA analysis was organized by wet digestion method.

The filtered digested sample solutions were run by an atomic absorption spectrophotometer (model: AA-6300, Shimadzu Corporation Japan). Samples of power were first passed to gain classic curves. The CA content of the samples was determined using the corresponding standard curve (AACC, 2000).

Phosphorus contents

Phosphorus was determined by gravimetric method and by precipitation with the ammonium molybdate and nitric acid know as Precipitating reagent. The yellow ammonium phosphormolybdate as aprecipitate was used in the measurements of P content. (kangsadalampai and sung puage, 1984).

Microbial analysis

Total plate count was determined by using AACC method No. 42-11 (8).

Results and discussion

Chemical composition of egg shell powder (ESP) and bone extract powder (BEP).

[a]produced by alkaline treatment method. [1]Kettawan, A., Sungpuag, P., Sirichakwal, P., & Chavasit, V. 2002.

Chicken bone calcium extraction and its application as a food fortificant. Warasan Samnakngan Khan akam makan Wichai Haeng Chat.

Table 1. Cookies recipe.

Ingredients	Quantity
CGSF	500 g
Shortening	333 g
Sugar	175 g
Eggs	01 no.
BakingPowder	07 g

Microbial count

The amount of microorganism was below log_{10}Cfu/g. TPC values 1.51, 1.51, 1.52 and 1.52. All values for T_0, T_1, T_2 and T_3 Ca fortified cookies. All values are in safe range. (APHA, 2001).

Chemical analysis of the calcium fortified product

At the beginning of storage (0 days) and then every two weeks, the cookies were analyzed up to 60 days approximately.

The following table values were measured by Kirk and Sawyer (1999) and reported in terms of dry weight.

197 As observed from Table 4, the moisture content of T_0, T_1, T_2 and T_3 Ca fortified cookies is2.62, 2.45, 2.54 and 2.53 respectively. Fat content was estimated to be 22.61 %, 22.34 %, 22.38 % and 22.51 %, for T_0, T_1, T_2 and T_3 Ca fortified cookies respectively.

Protein content 6.62 %, 6.58 %, 6.81 % and 6.61 % for T_0, T_1, T_2 and T_3 Ca fortified cookies. Ash content 1.05 %, 2.34 %, 2.43 % and 3.87 % for T_0, T_1, T_2 and T_3 respectively. NFE content 66.77 %, 65.92 %, 65.5 % and 64.12 %. Ca content 31.26 mg, 1969.9 mg, 1960.3 mg and 1979.2 mg. P content 136.12 mg, 136.36 mg, 141.5 mg and 1114.5 mg.

Table 2. Different treatments of fortified cookies (RDA, 75 %).

Treatments	$CaCO_3$ (g)	Egg Shell Powder (g)	Chicken Bones Powder (g)
T_0	-	-	-
T_1	2.43	-	-
T_2	-	2.55	-
T_3	-	-	3.24

The output of the basic chemical quality factors of ESP, BEP, $CaCO_3$ as described in Table 4 exposed that the ESP as a mineral elevating component e.g. calcium and Pact an important role for showing health benefits.

Moisture content (%)

Non-significant changes in the moisture of cookies was noted due to treatment and storage (Table 4). The phenomenon of the insertion of moisture during the rest timeline is also preserved by Wade (1998), who discovered that when cookies were packed in a moisture free packaging, some moisture also present in air during packaging quickly increased.

Come into evenness with the product, causing ingrowth of moisture. Moisture of Cookies in red palm oil was found of same trend of moisture in added cookies, rice bran oil cookies and biscuits during storage (Butt *et al.*, 2004).

Table 3. Chemical composition of ESP and BEP per 100g[a1].

Components	ESP	BEP
Moisture	0.52 g	0.49 g
Protein	2.13 g	0.96 g
Calcium	38.7-40.2 g	30.94 g
Potassium	41.65 mg	---
Sodium	87.07 mg	---
Phosphorus	99.36 mg	14.61 g
Ferrum	0.54 mg	---
Magnesium	375.01 mg	---
Ash	96.9 g	85.36 g
Fat	---	6.45 g
Energy (Kcal)	---	85.51
Carbohydrate	---	5.32 g

Crude fat (%)

Storage and treatment has affected the cookies fat content non-significantly as in Table 4. In the recent study, cookies have been packaged into bio refined poly-propylene films, thus preserving composition of the final product while act as anobstacle during storage against atmospheric variables. The results of the latestfinding are in accordance with the results of Mahmud *et al.* (2008), who explained non-significant changes during storages in the fat content of cookies.

Crude fiber (%)

No crude fiber content was found in cookies, might be due to no addition of any source of fiber.

Crude protein (%)

Non-significant deviation in protein content of cookies was noted due to treatment and storage (Table 4). Non-significant variation of crude protein may be due to non-addition of the protein source in the treatments.

The results are in close agreement about changes in crude protein with the findings of Rahman *et al.* (2009), who looked at the amount of that raw protein non-significantly among apricot-dates treatments.

Ash content (%)

The treatments affected the cookies ash content significant however storage as non-significant as in Table 4. Significant variations in treatments ash content might be due to that gradual increase in mineral source.

Results regarding changes in raw ash are similar with the judgments of Rahman *et al.* (2009), who noted that the quantity of crude ash in the treatment of apricot palm bark was quite high.

Table 4. Chemical analysis of the calcium fortified cookies per 100 g.

Treatment	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	NFE (%)	Ca (mg)	P (mg)	TPC Log 10 (cfu/g)
T_0	2.62±0.24^{A}	22.61±0.97^{A}	6.62±0.09^{A}	1.05±0.011^{C}	66.77±1.19^{A}	31.26±0.84^{C}	136.12±0.85^{CD}	1.51±0.339^{A}
T_1	2.45±0.19^{A}	22.34±0.97^{A}	6.58±0.18^{A}	2.34±0.042^{BC}	65.92±1.04^{A}	1969.9±19.7^{BC}	136.36±0.86^{C}	1.51±0.336^{A}
T_2	2.54±0.21^{A}	22.38±0.94^{A}	6.81±0.12^{A}	2.43±0.043^{B}	65.5±1.22^{A}	1960.3±34.3^{A}	141.05±0.84^{B}	1.52±0.331^{A}
T_3	2.53±0.16^{A}	22.51±0.99^{A}	6.61±0.20^{A}	3.87±0.047^{A}	64.12±1.19^{A}	1979.2±13.1^{B}	1114.5±0.94^{A}	1.52±0.328^{A}

Nitrogen free extracts (NFE) (%)

The treatments and storage affected the NFE content of cookies non-significantly (Table 4). Variation in NFE content depends on change in other proximate because there is non-significant verification in most of the proximate that might be reflect in non-significant change in NFE content of cookies. Similar finding were detected by Kenny *et al.* (2000).

Calcium (Ca) Content (mg)

The treatments affected the Calcium content of cookies significant while storage affected the Ca content of cookies non-significant (Table 4) Weaver, 2014 reported that whereas some studies indicate that calcium plus vitamin D supplementation reduces the risk of fractures.

Phosphorus (P) content (mg)

Significant variation was observed in P content of cookies due to treatments and storage (Table 4). Pilon *et al.* (2006) reported non-significant variations in minerals during a storage study.

TPC Log 10

Non-Significant variation was observed in TPC log 10(cfu/mg) content of cookies due to treatments variation due to storage (Table 4). All values within the threshold limit (1.3979 to 2.477 cfu/mg) (APHA, 2001). Yusuf (2016) reported that cookies formed from merged flour of maize, AYB and plantain are microbiologically safe during 2 months of storage but which can cause health hazard after 3 months.

By the standard of WHO (1994) the highest acceptable limits in baked products (cake, bread and biscuits) for total plate count (TPC) is 2.0x105 cfu g-1.

Conclusion

It can be concluded that eggshell powder and bones extract powder is an appropriate richest and cheapest natural source of Ca for human nutrition. Eggshell powder and bones extract powder fortified cookies with wheat flour in 975mg Ca per day was considered best in this study. It should be focused for commercial replication.

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