Calcium Extract from Chicken Eggshells as Dietary Calcium Source in some Juices

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Abstract

The aim of this study to extract calcium from organic chicken eggshell and white chicken eggshell, Calcium determination in each, then fortifying fresh natural juices. Calcium was extracted from eggshells by using fresh lemon juice and lemon salt, and fortifying fresh natural juices (with extracted calcium at three levels of supplementation as follows 5, 10 and 15%). Study the effect of extracted calcium supplementation on each of calcium content, pH value and sensorial properties of juices were studied. The results showed that the higher calcium content was in the organic eggshell (30706.97mg/100gm) compared with white eggshell (28543.365mg/100gm), and calcium extract by lemon juice (973.6mg/100ml) higher than extract calcium by lemon salt was (965mg/100ml). In conclusion, addition calcium extract of eggshell led to a pronounced increase calcium content in the supplemented of juices compared to control. And a decrease in the pH values of juices during fortification in all treated samples compared to the control. There were, no significant differences (p<0.05) in all sensory characteristics of juices with 5%, 10% of extract calcium as compared to control sample. There were, significant differences (p<0.05) in odor, taste and flavor of juices fortified with 15% as compared to control sample.

Keywords: Functional foods, Fortification, Deficiency of calcium ,pH value and Calcium citrate

Introduction

Functional foods were receiving much interest from consumers across the world because they have beneficial actions in the body such as reduction of disorder risks, increasing some enzymatic activities and effect on many targets at the same time **(Guimaraes *et al.,* 2018)**. In addition to the traditional nutritional effects, "Functional foods" exert beneficial health effects on the human body. Well recognized examples of functional foods are those containing bioactive compounds like dietary fibers, oligosaccharides, vitamins, minerals and active "friendly" bacteria, called probiotics that promote the equilibrium of intestinal microflora **(Shah and Prajapati, 2013)**.

Fortification is achieved by adding one or more essential nutrients to foodstuff, usually to prevent or correct deficiencies and enhance the nutritional value **(Tomic *et al.,* 2017)**.

The deficiency of calcium (Ca) in the diet is a common problem. Ca intake from dairy products is an appropriate way to fulfill Ca requirements. However, people do not usually consume them in the amounts established by clinical guidelines. Calcium is an important micronutrient required for the growth and maintenance of teeth and bone and has an essential role in muscle contraction, blood clotting, and nerve conductivity **(Mann and Truswell, 2012)**. Furthermore, calcium is an important factor in regulating the cardiovascular system and an adequate intake is recommended to prevent hypertension **(van Mierlo et al., 2006)** and preeclampsia. A recent meta-analysis of 13 randomized controlled trials showed that calcium supplementation during pregnancy in populations at risk of low calcium intake reduces the incidence of preeclampsia by 55% and is associated with a reduced risk of preterm birth and increased birth weight **(Hofmeyr et al., 2014)**. Daily calcium supplementation (1.5–2.0 g oral elemental calcium) of pregnant women in populations with a low dietary intake is a current recommendation of the World Health Organization (WHO), to reduce the risk of preeclampsia **(World Health Organization (WHO), 2016)**. However, supplementation with tablets is costly and sometimes involves difficulties of in adherence to treatment. Chicken eggshell is a source of Ca, which is available at home that can be used as Ca supplementation **(Geovana et al., 2011)**.

Chicken eggshell is a waste material from domestic sources such as hatcheries, poultry farms, egg product factories, homes and restaurants **(Amu et al., 2005)**. Eggshell is a non-edible by-product with little saleable value but they may contain biologically active compounds **(Nakano *et al*., 2003)**. Calcium is the major component of an eggshell. Eggshell calcium is the best natural source of calcium and it is about 90% absorbable, then limestone or coral sources and was absorbed easier than commercial CaC03 in the rat small intestine **(Swiatkiewicz et al., 2015)**. Eggshells contain calcium and trace amounts of other microelements, i.e. magnesium, boron, copper, Iron, manganese, molybdenum, sulfur, silicon and zinc. One whole medium-sized eggshell makes about one teaspoon of powder, which yields about 750- 800 mg of elemental calcium **(Nys, 2004) (Nakano *et al*., 2003 and Kingori, 2011)**. The composition of an eggshell is very similar to that of our bones and teeth. It is recommended that people with osteoporosis take 400-500 mg calcium per day to supplement dietary sources. A risk factor for human health is the infection with Salmonella enteritidis at the consumption of undercooked eggs (**Guard, 2001**), as eggs can become contaminated internally and on the outer shell surface. However, it was proved that the salmonella groups were not detectable after 6 hours at pH 4.0. Despite the fact that pasteurization was predicted to be effective for reducing S. enteritidis. Therefore, it is important to emphasize the necessity to avoid contamination with *Salmonella*. By-products from processed foods are promising as good natural calcium sources such as egg-shell **(Geovana et al.,2011)**. Many researchers have been looking for ways to utilize the eggshell waste as a source of Calcium in human nutrition **(Schaafsma et al., 2000)**. These ways of utilizing egg shell made the egg producers could avoid the cost of waste disposal, moreover and reduce environmental pollution. **(Gaonkar and Chakraborty, 2016)**. This study aim to extracting calcium from organic chicken eggshell and white chicken eggshell, then utilize this extract to fortifying fresh natural juices to increasing dietary calcium, health benefits and improving their functionality.

Materials and Methods

1. Materials

- Egg, fresh lemon, citric acid, calyces of Roselle, *Guava Fruits*, sugar, milk, were purchased from a local market in Assiut, Egypt.

2. Methods

2.1 Preparation of Chicken eggshell Powder:

Chicken eggshells were washed with tap water both inside and outside to get rid of dirt and other organic materials, and boiled for 30 minutes. The shells were drained out. Then they were spread out on stainless steel baking sheet and dried for 12 hours. Decontaminated eggshells were oven dried at 200°C for 10 minutes. These were then processed into powder using the commercially available blending machine (Phillips, HR2118/01). To obtain the fine eggshell powder (ESP), it was then sieved through 2 layered fine net (sieved size 50 µm).

2.2 Preparation of solution of calcium extract from eggshells by two methods for treatments:

2.2.1- The treatment by using fresh lemon juice according to the method of Margie Joiner. (1997) with some modifications:

Fresh Lemon Juice Preparation:

Fresh lemons are cut into halves. These halves are then squeezed to extract the juice. An automatic juicer can be used, as well as a small hand juicer. The lemon juice is used the same day that it is squeezed.

Calcium Extraction Method:

The prepared lemon juice is poured into large contain and the eggs are then placed into and the mixture is stirred until all of the eggs are completely covered with the lemon juice. The mixture is stirred slowly, every half hour for the first 2-4 hours in order to ensure coverage of all of the eggshells. When the eggshells are placed into the lemon juice, a reaction will begin to take place almost immediately. Bubbles of carbon dioxide begin floating to the surface of the liquid and a foam will appear at the air/liquid/ interface. This reaction is allowed to continue for 22 to 24 hours at room temperature (70-75°F). After 22 to 24 hours, the liquid is again stirred and the remaining eggs are carefully lifted out of the juice mixture. These eggs are placed on a tray and allowed to dry It can be used to prepare different foods. The final percent extraction of the eggshell is calculated. The pH is tested on the lemon juice/ eggshell mixture, in order to determine the acidity of the product. The mixture is then filtered to remove any large particles of eggshell that might have remained in the bottom of the mixture. The mixture is then immediately transferred to a large kettle, where it is heated to a temperature of 184 F-190° F., and held at that temperature for five minutes. This is done in order to pasteurize the product for health safety. The mixture is then transferred, while still hot, to sterile gallon containers. These containers are stored in refrigerated storage until the mixture is used in the liquid state.

2.2.2- The treatment by using lemon salt according to the method of Margie Joiner. (1997) with some modifications:

lemon salt solution Preparation:

The calcium in the eggshell was extracted in the form of calcium citrate using lemon salt Instead of fresh lemon juice. Solution of lemon salt prepared by using 12 gm. of lemon salt dissolves well in 300 ml of filtered water.

Calcium Extraction Method:

The prepared lemon salt solution is poured into large contain and the eggs are then placed into and the mixture is stirred until all of the eggs are completely covered with the lemon salt solution. The mixture is stirred slowly, every half hour for the first 2-4 hours in order to ensure coverage of all of the eggshells. When the eggshells are placed into the lemon salt solution, a reaction will begin to take place almost immediately. Bubbles of carbon dioxide begin floating to the surface of the liquid and a foam will appear at the air/liquid/ interface. This reaction is allowed to continue for 22 to 24 hours at room temperature (70-75°F). After 22 to 24 hours, the liquid is again stirred and the remaining eggs are carefully lifted out of the solution mixture. These eggs are placed on a tray and allowed to dry It can be used to prepare different foods. The final percent extraction of the eggshell is calculated. The pH is tested on the lemon salt solution / eggshell mixture, in order to determine the acidity of the product. The mixture is then filtered to remove any large particles of eggshell that might have remained in the bottom of the mixture. The mixture is then immediately transferred to a large kettle, where it is heated to a temperature of 184 F-190° F., and held at that temperature for five minutes. This is done in order to pasteurize the product for health safety. The mixture is then transferred, while still hot, to sterile gallon containers. These containers are stored in refrigerated storage until the mixture is used in the liquid state.

2.3 preparation of guava fruit juice according to the method of Youssef et al. (2017):

Preparation of guava puree by using 2 kg of mature and ripe fresh guava fruit. They were washed by tap water, cut, removed seeds and blended using a domestic blender. Then after that adding guava puree (12.5%), sucrose (9%). Supplemented the obtained juice with three proportions of calcium extract 5%, 10%, and 15%. Pasteurized at 80°C for 15 min and immediately cold at 4°C to prevent contamination . **(Vieira and Silva, 2014)**. The obtained juice was filled into an untransparent glass bottle then kept at refrigerator temp (2 - 6 °C).

2.4 preparation of juice extract from Roselle according to the method of Egbere et al. (2007):

One hundred grams of the dried calyces was sorted out and rinsed under tap water. The calyces were ground in blender to obtain a paste. Two liters of warm filter water (at 60°C) was added to the paste and left for 1 hour to enable extraction. The mixture was then filtered through a muslin cloth and then flavored with 200 g of sugar. Fortification of the obtained mixture with 5%, 10%, 15% of calcium extract. Pasteurized at 80°C for 15 min and immediately cold at 4°C to prevent contamination (**Vieira and Silva, 2014)**. Mixture filled into glass bottle then kept at refrigerator temp (2 - 6°C).

2.5 Chemical Composition of raw materials:

Moisture, protein, fat and ash were determined according to the methods of **AOAC (2010)**. These analyzes were determined in Central Laboratory for Chemical Analysis, Faculty of Agriculture, Assiut University.

2.6 Determination of calcium content:

Calcium content was determined using the ICP (Inductively Coupled Plasma Emission Spectrometer) (ICAP6200) according to **Isaac and Johnson (1985)**. This analysis was determined in Central Laboratory for Chemical Analysis, Faculty of Agriculture, Assiut University.

2.7 Determination of PH values

The PH value of all fruit juice samples was measured separately by a digital PH meter (model 3505- JENWAY - UK) at 25°C as described in **AOAC (2005)**.

2.7 Sensory Evaluation of studied juices:

The sensory attributes of juices including color, taste, odor, flavor, viscosity and, overall acceptability of the Juices samples were evaluated by twenty trained member panelists, using a 5 point hedonic scale **(Jones *et al.*, 1955)**.The scale ranges from 1 – 5 with 1 representing the least score (dislike extremely) and 5 highest score (like extremely). The twenty member panelists were drawn from the staff of the College of Faculty of Specific Education, Department of Home Economics and ordinary consumers. Samples of juices were poured into crystal Glasses under strong white lighting during evaluation. Water was provided to rinse the palate between two tasting sessions.

2.8 Statistical Analysis:

Data were presented as the mean of duplicate± standard deviation (mean ± SD). T-test was used to establish the significance of differences among mean values at ($p< 0.05$). Analysis of variance (ANOVA) was carried out using Proc Mixed of SAS package version 9.2 (SAS 2008) and means were compared by Duncan test at a 5% level of significance **(Steel and Torrie, 1981).**

Results and Discussion:

Chemical Composition of egg shell types:

Data presented in **Table 1**, showed the chemical composition of eggshell types (white eggshell and organic eggshell) as raw materials. There are the highest values in ash, protein and moisture (93, 4.10 and 0.51%, respectively) in organic eggshell powder, while the lowest value in fat was (0.02%) in organic eggshell powder compared with white eggshell powder (0.035 %). From the same table, it could be seen that, the organic eggshell powder showed higher than white eggshell powder in value of Ca (30706.97 and 28543.365 mg/100g in organic eggshell powder and white eggshell powder respectively). that clarified that the eggshell is good sources of nutrients especially calcium. This illustrates that the shells are useful in the treatment of Osteoporosis and improve general health to the high content of calcium. **Yasothai and Kavithaa, (2014**) studied the chemical composition of eggshells and reported that its content of water (2%) and dry matter (98%). The dry matter is composed of 5% crude protein and 93% ash. **Hunton, (2005)** reported that the chemical composition showed that, eggshell is composed of about 97% calcium carbonate.

Table (1): Chemical composition of organic egg shells and white egg shell (%) on dry weight basis

Types of egg shells powder	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Calcium value (mg/100g)
Organic egg shell powder	0.51±0.01	4.10±0.3	93.0±1.3	0.020±	30706.97±13.15
White egg shell powder	0.50±0.02	3.40±0.4	90.2±2.1	0.035±	28543.365±15.24
T- test	0.245	9.12**	0.124	11.2**	21.15**

Mean of three replicates ±Std . deviation. n.s = > 0.05 , *=P 0.05 , =P 0.01**

2.6 Determination of Calcium Content:

Data in **Table (2)**, showed, calcium value of calcium extracted using lemon juice was better than that extracted with lemon salt, as lemon juice led to the extract a higher of calcium (973.6 mg/100ml) than calcium extracted with lemon salt (965 mg/100ml), because the use of lemon juice led to the total decomposition of eggshell, which resulted in a very fine precipitate while The precipitate that was formed from the decomposition of the eggshell with lemon salt was a coarse precipitate. **Schaafsma et al.,(2000)** 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.,(2014)** stated that calcium was the highest mineral constituent (225.35) mg/g in eggshells.

Table (2): Calcium value of calcium extract solution from organic and white egg shell by two treatments (mg/L).

Types of treatments	Calcium value (mg/100ml)		T- test
	Organic egg shell	White egg shell	
Lemon juice	973.6±7.6	806±**8.0**	**21.36****
Lemon salt	965±9.2	801.1±11.2	17.89**

Table (3) shows calcium value of juices fortified with different proportions of calcium extract solution. The addition of calcium extract to guava juice and roselle juice in leads to the increase in calcium content of juices. The highest calcium content of juices with 15% was 816.04 and 164.14mg/100mlin roselle juice and guava juice respectively. Therefore, ESP fortified juices are nutritionally more acceptable than ordinary juices. Calcium in the body has important uses such as muscle contraction and neurotransmitter release. Low calcium intake in the long term can cause poor blood clotting especially in a menopausal woman, and it can lead to osteoporosis. On the other hand, continuous intake of calcium supplements can lead to hyper-calcemia, impaired kidney function, and decreased absorption of many minerals **(Ross et al., 2011)**. Therefore, the best healthy choice is to get calcium from food and not from supplements. This study has proven that calcium extracted from organic eggshell is described as functional dietary calcium as it is available in most homes and is cheap. The development of calcium Juices is possible, allowing the consumption of these beneficial by people who do not like dairy products or with intolerance or allergy to milk components. juices represent a suitable carrier for the delivery of probiotics. Since, fruits are naturally rich in essential macro- and microelements.

Table (3): Calcium value of juices fortified with different proportions of calcium extract solution (mg/100ml).

Samples	Guava Juice	Roselle juice
Control*	19.11D±0.6	280.0D±2.7
5% extract calcium solution	66.69C±2.3	718.68C±6.3
10% extract calcium solution	115.46B±4.5	767.36B±5.6
15% extract calcium solution	164.14A±6.3	816.04A±7.5
F-test	31.25**	42.15**

Control*: juices prepared without addition of extract calcium solution.

Values followed by the same superscript letters within the same column were not significantly different

2.2.1. PH values:

Table (4) shows pH value of calcium extract solution from different treatments of two types of egg shell. The result of changes in pH values of treated juices is tabulated in **Table (5)** shows, The decrease in the pH values of juices during fortification can be observed in all treated samples compared to the control in both types of fresh juice, and this ensures that the juices are free of salmonella. Whereas the salmonella groups did not exist in a highly acidic medium and were not detectable after 6 hours at pH 4.0 **(Raquel et al, 2014)**. The highest value of PH was noticed in the control samples were 4.10 in guava juice and 3.80 in roselle juice respectively and in 10% was 3.51and 3.20 in guava juice and roselle juice respectively, while the lowest value of PH was noticed in the samples treated with 15% was 3.80 and 3.00 respectively in guava juice and roselle juice. These results are in agreement with those of **Ertem and Çakmakçı, (2018)** they mentioned that beverage pH was also decreased gradually over the study period as a result of the production of lactic acid by L. acidophilus which is known as post- fermentation.These results are consistent with those reported by **Czyżowska *et al*., (2006)** who studied lactic acid fermentation of red beet juice using *L. plantarum* and *L. casei* strains (pH was decreased to 3.5– 4.0).

Table (4): pH value of calcium extract solution from different treatments of two types of egg shell.

Treatments	pH values		T- test
	Organic egg shell	White egg shell	
Lemon juice	**3.22±0.02**	**3.32±0.02**	**0.631**
Lemon salt	3.00±0.01	3.05±0.02	0.321

Table (5): pH value of juices fortified with different proportions of calcium extract solution.

Treatments	pH values	
	Guava Juice	**Roselle juicea**
control	4.10A±0.3	3.80A±0.2
5%	3.90AB±0.1	3.60AB±0.1
10%	3.51B±0.4	3.20B±0.4
15%	3.80AB±0.2	3.00BC±0.3
F-test	8.12*	9.15*

Values followed by the same superscript letters within the same column were not significantly different

Sensory Evaluation of juices:

Result of sensory analysis of guava juice and roselle juice samples containing different levels of calcium extract substitution as compared with the control is shown in Table (8 and 9). The statistical analysis (ANOVA) revealed that there is no significant difference ($p>0.05$) among all the guava juice samples in (color, viscosity and overall acceptability). However, a significant difference ($p<0.05$) was observed between guava juice samples with 15% inclusion of calcium and control in the terms of (odor, taste and flavor). Evaluation of sensory properties of roselle juice containing calcium extract solution with 5, 10 and 15% calcium extract inclusion. They observed that, there was no significant difference ($p>0.05$) between the treatments in relation to color, viscosity and overall acceptability , while significant ($p<0.05$) exist between the treatments in terms of their odor, taste and flavor in juice samples with 15% compared to control. It is clear that, the overall acceptability for all the treatments of guava juice and roselle juice was very good. Such organoleptic characteristics are reported by **Luckow and Delahunty, (2004)** in non-dairy juice drinks. They showed that, consumers prefer the sensory characteristics of probiotic orange juice in comparison to conventional orange juices because of their health benefits. **Ranadheera *et al*., (2014)** underlined that; some fruit juices could naturally mask the "medicinal" taste of probiotics. Also, these results are in agreement with **Rezaei *et al*., (2012)** who cleared that, total acceptability of frozen yoghurts containing inulin (0, 1 and 2%), yoghurt with 2% inulin had the most appealing sensory characteristics.

Table (6): Sensory Evaluation of roselle juice fortified with different proportions of calcium extract solution.

Samples	**Sensory properties**					
	Color	**odor**	**Taste**	**Flavor**	**viscosity**	**Overall acceptability**
Control*	4.75a	3.5a	4.25 a	4.25a	4.5a	4.5a
5%	4.5a	4.0a	4.0a	4.5a	4.25a	4.25a
10%	4.25a	4.25 a	4.25a	4.25a	4.5a	4.5a
15%	4.5a	3.0b	3.25b	4.0b	3.05b	4.0b

Note: Means in the same column with different superscripts are significantly different at $p<0.05$.

Table (7): Sensory Evaluation of guava juice fortified with different proportions of calcium extract solution.

Samples	Sensory properties					
	Color	odor	Taste	Flavor	viscosity	Overall acceptability
Control*	4.9a	4.8a	4.9a	4.8a	4.8a	4.25a
5%	4.9a	4.7a	4.8a	4.8a	4.8a	4.5a
10%	4.8a	4.3 b	4.7a	4.7a	4.7a	4.5a
15%	4.8a	4.0b	4.0b	4.4b	4.4b	4.25

Note: Means in the same column with different superscripts are significantly different at $p<0.05$.

Conclusion

It can be concluded that organic eggshells is an appropriate source of Ca could use to prepare juices. Also study shows that supplementation juices with extract calcium from organic chicken eggshell by 5% and 10% formulation shows better overall acceptability.

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