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Determination of Organic and Inorganic Components of Commercial Chicken Eggshell Obtained From Sebha Local Market-Libya

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Abstract An experimental study has been carried out aiming to identify the composition of eggshells of chicken eggs obtained from Sebha markets during March 2016. The selected chicken eggshells were washed, dried, and grinded to a powder. The results obtained showed that the tested chicken eggshells samples contain [0.6±0.24%] moisture, [99.75±0.02%] dry matter, [2.57±0.10%] protein, [4.7±0.1%] fiber, [1.97±0.24%] Carbohydrate and [90.75±0.21%] ash. The mineral elements contained in the selected chicken eggshells samples have been identified and the results obtained showed different amounts as [mg / 100g] of dry weight [Ca 2+ =2442.6, Mg 2+ =134.8, Cu 2+ =12.12, Na + =4.97, K + =5.21, Fe 2+ =5.61, Mn2+ =0.36, Cd2+ =0.74 and Zn2+ =1.69], Pb2+ was not detected. As a result, the amounts of minerals detected showed that this type of tested chicken eggshells are of good quality and can be further utilized for different purposes.
Keywords: Chicken eggs, eggshell, moisture, ash and mineral elements.

تحديد المكونات العضوية وغير العضوية لقشرة بيض الدجاج التجاري المتحصل عليه من السوق المحلي سبها – ليبيا

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الملخص تقدم هذه الورقة دراسة عملية أُجريت لغرض التعرف على مكونات قشرة بيض الدجاج المأخوذ من أسواق مدينة سبها خلال شهر مارس 2016، قشور بيض الدجاج المختارة غُسلت وجُففت ثم وُضعت في وعاء محكم الإغلاق، أوضحت النتائج المتحصل عليها أن عينة قشور بيض الدجاج المُختبرة تحتوي على [0.6±0.24%] رطوبة، [99.75±0.02%] مادة جافة، [2.57±0.10%] بروتين، [4.7±0.1%] ألياف، [1.97±0.24%] كربوهيدرات، و [90.75±0.21%] رماد. العناصر المعدنية التي تحتويها عينة قشور بيض الدجاج تم التعرف عليها وأظهرت النتائج كميات مختلفة لها مثل [Ca 2+ =2442.6، Mg 2+ =134.8، Na + =4.97، K + =5.21، Fe 2+ =5.61، Cd2+ =0.74 ، و Zn2+ =1.69]، عنصر الرصاص [Pb2+] لم يعثر عليه، و كنتيجة المقادير المعدنية المكتشفة بينت أن قشور بيض الدجاج المختبرة من نوعية جيدة ويمكن استخدامها والاستفادة منها في أغراض مختلفة، مستقبلا نوصي بدراسة مكونات قشرة البيض المستعمل في هذه الدراسة على فترات متفاوتة، خلال العام لمعرفة التأثير البيئي عليها.

الكلمات المفتاحية: بيض الدجاج ، قشر البيض ، الرطوبة ، الرماد والعناصر المعدنية.

1. Introduction

Birds lay eggs of different shape, volume, weight and the amount of whole contained. The shape of eggs laid down by birds can mainly be divided into oval shape of one rounded end and another conical shape of one end more pointed[1]. The bird eggshell provides mechanical protection and controlled gas exchange medium. Also, it forms an embryonic chamber for the developing chick and a container that provides protection to the contents of a unique package of a valuable food. Moreover, the bird eggshell comprises of calcified shell and shell membranes which make up 10.2% of the whole egg[2]. It is constructed mainly from calcium carbonate and some traces of magnesium carbonate and tri calcium phosphate. Bird eggshell forms a rich source of inorganic salts[3].
Generally, minerals are classified to macro and micro elements. The macro elements are calcium, phosphorus, magnesium and sodium, and they are required in amounts greater than 100 mg/dL. Whilst, the micro elements are iron, copper, cobalt, potassium, Iodide, zinc, manganese, molybdenum, fluoride, chromium, selenium and sulfur. They are required in amounts less than 100mg/dL[4, 5]. Most of the bird eggshells of good quality contain about [95-97%] calcium carbonate, [29-35%] water, [89.9-91.1%] ash, [35.1-35.4%] calcium, [0.15-0.17%] sodium, [0.37-0.40%] magnesium, [0.10-0.13%] potassium, [0.09-0.19%] sulphur and some traces of iron, zinc and copper[6, 7]. The traces of micro minerals elements like zinc, copper, manganese have shown contribution to the eggshell quality. They have an influence on both calcite crystal growth during the eggshell formation and also the mechanical properties of the eggshell[8].The

minerals elements contained in the bird eggshells can be used in formulating various feeds for birds, other animals and also for human nutrition. Mineral substances are usually required in small amounts in food, from less than 1 to 2500 mg per day. Also, their presence in all body tissues and fluids is necessary to maintain the normal physicochemical processes that essential for every living matter[4]. In addition to this, bird eggshells contain useful substances which in the form of eggshell powder [ESP] can be used for treatment, to reduce bone pain and increase its strength by increasing bone mineral density [BMD][9].

In Sebha city chicken eggs are consumed daily and the chicken eggshells are usually disposed as general rubbish without any further utilization. It is of great importance to study the composition of eggshells of chicken eggs available in Sebha market, with the object of recommending them for further utilization. The aim of this study is to estimate the amounts of organic and inorganic substances contained in the eggshells of chicken eggs obtained from Sebha market-Libya, during March 2016.

2. Materials and methods

Three chicken eggs were collected from a well trusted farm in Sebha city-Libya during March 2016 and used in this study. The farm is working as egg whole supplier to Sebha local market.

The selected chicken eggshells were washed with distilled water. After that, the eggshells were dried and stored in a well tight container.

The macro elements contained in the ash of the chicken eggshells [sodium, calcium and magnesium] as well as the micro elements [potassium, iron, manganese, cobalt, zinc, cadmium and lead] were determined according to the method described by [A.O.A.C. 1997][10]

Three different instruments have been used to analyze the ash content of the chosen chicken eggshell. For instance, automatic flame photometer [JENWAY PFP7 9523] has been used to determine the concentrations of [K+ and Na+]. In addition to the above instruments used, ultra violet spectrophotometer [JENWAY 6305] used to determine mineral element like [Cu2+, Mn2+ Fe2+ and Co2+]. Furthermore, atomic absorption technique and thermo elemental spectrophotometer [SOLAAR-54] used to identify other micro mineral elements such as [Cd2+, Pb2+and Zn2+]. Finally, mineral elements such as [Ca2+ and Mg2+] have been determined by titration.

2.1. Proximate analysis

Firstly, a sample of chicken eggshells was washed with distilled water and dried. Then the amount of moisture contained in the sample was determined by drying it at 105 oC for three hours, in accordance with the method described by [A.O.A.C. 1997][10]. After that, the chicken eggshells sample was grinded to a fine powder. The ash content of the chicken eggshells was determined after ashing the sample in a muffle furnace oven at 550 oC for overnight, and then cooled in a desiccator and weighed.

The results obtained showed that the eggshell and the membranes form about [10.49 ± 0.16%] of the whole chosen chicken egg. Also, they showed that the chicken eggshell contained [0.24 ± 0.6%] moisture, [99.75 ± 0.02%] dry matter and [90.75 ± 0.21%] ash.

The organic matter of the chicken eggshell was evaluated as following:

Organic matter [%] = [100- Moisture % - Ash %].

The organic matter content of the studied chicken eggshells was [9.00 ± 0.21%].

The amount of crude lipid contained in the chicken eggshell was determined using the procedure described by AOAC [1980]. A known weight of dried sample of chicken eggshell [2g] was placed inside a thimble. After that, the thimble was placed in the soxhlet apparatus and petroleum ether [98%] was added to it, used as a solvent. The apparatus then heated and allowed to reflux for [6 hrs]. Consequently, the solvent evaporated and the flask weighed, no lipid was found deposited on the flask.

The crude nitrogen contained in the chicken eggshells was determined by means of Kjeldahl method [9]. A [0.5g] from the chicken eggshells has been digested by placing it in a digestion flask. Then [0.25g] of copper sulphate [97%], [0.25g] of sodium sulphate anhydrous [99%] and [8to10 ml] of concentrated sulphuric acid [93.6-95.6%] were added to it. The mixture in the digestion flask was slowly heated until the sample frothed. When frothing subsided, the color of solution changed from black to green indicating a complete digestion. Moreover, the digest was cooled and diluted with distilled water. After that [10 ml] of sodium hydroxide [97%] was carefully added to the resulted diluted digest before distilled. Furthermore, the distillate from the diluted digest was added to [5ml] of boric acid [99.8%] in a new receiving flask. Finally, the mixture obtained was titrated against [0.01M] of hydrochloric acid [37%]; the titre value was recorded and used to calculate the amount of crude nitrogen. Consequently, the amount of crude protein in the chicken eggshells was calculated by multiplying the value of crude nitrogen obtained by a conversion factor equal to [6.25] [7].

The amount of fiber contained in the chicken eggshells was determined by the same method described in [A.O.A.C. 1980]. Firstly, a milled sample [2.00 g Wsmple] of chicken eggshells was dried and decarbohydrated-defatted using ethanol [99.8%], acetone [99.5%] and petroleum ether [98%], respectively. Secondly, a certain amount of decarbohydrated-defatted sample of chicken eggshell has been taken and extracted using acid and alkali. The process has been carried out on amount of decarbohydrated-defatted sample by boiling it with [1.25%] of sulphuric acid [93.6-95.6%], filtered and washed with boiling water until the washing was no longer acidic. Then the residue from the extraction process was boiled in a flask with [1.25%] of sodium hydroxide [97%], filtered and washed with boiling water until the washing was no longer alkaline. After that, the residue was dried at 100°C, left in a desiccators to

cool and then weighed [W1]. The cooled and weighed residue was thereafter incinerated in a muffle furnace at about 600°C, left in a desiccator to cool and then the resulted ash weighed [W2].
The amount of protein was estimated from the remaining decarbohydrated-defatted sample of chicken eggshell [W3], after digesting it using the previously described method [9]
The amount of crude fiber was calculated as:
Crude fiber [%] = [[W1-W2-W3]/Wsample]*100
The amount of carbohydrate contained in the chicken eggshells was estimated as follows:
C.H.O = 100 – [% Crude ash + % Crude lipid + % Crude protein + % Crude fiber][7, 11].

Grams of ash were taken from the resulted ash of the chosen chicken eggshells and digested in 7 mL of concentrated Hydrochloric acid [37%] and then evaporated on a hot plate. After that some drops of hydrogen peroxide solution [34.5-36%] were added to the evaporated digested ash. The digested ash samples were diluted using 100 mL of distilled water. The mineral elements [macro and micro elements], in mg/100g of dry weight were determined based on the analysis made to the digested ash sample the materials in table 1 show that the percentage of materials.

2.2. Mineral analysis

Table 1: Percentage of materials

Materials		petroleum ether	sulphuric acid	hydrochloric acid	ethanol	acetone	hydrogen peroxide [sol.]
percentage	Liquid	[98%]	[93.6-95.6%]	[37%]	[99.8%]	[99.5%]	[34.5-36%]
Materials	Solid	copper sulphate	boric acid	sodium hydroxide		sodium sulphate anhydrous	
percentage	Solid	[97%]	[99.8%]	[97%]		[99%]	

3. Results and discussion

The results in table 2 show that the amount of moisture contained in the chicken eggshells was low. The presence of high moisture in the eggshell causes microbial activities inside the egg during storage. Accordingly, this type of chicken eggs can be stored for a long period of time with no risk of any microbial activities to happened [7, 12]. Moreover, the results show also the presence of organic matter, which can bind with calcium and yields further strength to the eggshell [7].
Furthermore, the results in table 2 demonstrate that the amounts of fiber and protein were high compared to those of previous studies. The presence of fiber in considerable amounts helps to reduce the absorption of cholesterol from the gut. It delays the digestion and the conversion of starch to simple sugar. In human nutrition, the fiber is an important factor in the management of diabetes and also helps to prevent cancer[13, 14]
The results in table 2 show also the presence of a reasonable amount of protein in the chicken eggshell. Protein serves as enzymatic catalyst. It is necessary to control growth and cell differentiation. It is essential to support various body activities. Protein is unlike energy, it is not stored inside the body and the daily protein intake should match the daily protein metabolism to satisfy the body daily needs. So, this type of chicken eggshell can be considered as source of protein.[15]
Moreover, the results obtained show the presence of carbohydrates in the chicken eggshell. The carbohydrates are a major source of energy necessary for growth, metabolism and other functions. In foods, carbohydrates can be classified to simple and complex carbohydrates. The simple carbohydrates [sugar] can be digested and absorbed; whilst complex carbohydrates [starch and fiber] are non-digestible.
More than that, the results obtained show that the percentage of ash contained in chicken eggshells was in the recommended ratio of good quality chicken eggshells [6, 7]. This good quality type of chicken eggshells can be considered as a valuable source of inorganic substances which could be used for different purposes like feed formation, treatments, plant fertilizers, soil enrichment and soil stabilizers for construction materials[16].

Table 2: Proximate content [dry weight]

Composition	Chicken eggshell [%weight]	
	Present study	Previous studies
Moisture	0.24 ± 0.6%	[0.5± 0.03 %][7]
Dry matter	99.75±0.02%	--
Organic matter	9.00 ±0.21%	[12%][17]
Crude protein	2.57±0.10	[1.35 ± 0.400 %][7]
Crude fiber	4.7±0.1	[3.0 ± 0.300%][7] [-%][17]
Carbohydrate	1.97±0.24	[51.7 ± 0.440%][7] [1%][17]
Ash	90.75±0.21%	[89.9 – 91.1%][6, 7]

The results in table 3 show the amounts of sodium [Na] and potassium [K] elements contained in the tested chicken eggshell. Sodium is necessary for improving the eggshell quality, by supplying the bicarbonate ions for the process of calcium carbonate formation in the blood [18, 19]. On the other hand, potassium is used to regulate neuromuscular excitability, muscles contraction and used also for the activation of variety of important enzymes[9]. The amounts of these

detected elements sodium and potassium are comparable to the those reported in available literature [7]. It shows also that the amounts of calcium [Ca] and magnesium [Mg] detected in the chicken eggshells of this study were low compared to the results of previous studies [5]. The presence of both calcium and magnesium elements can affect egg production as well as eggshell strength[20]. For instance, the presence of calcium [in high or low amounts] in feed has an adverse effect on egg production. It must be supplied in right amounts to maintain good quantity levels of egg production, magnesium is important for egg weight and shell thickness. On the other side, magnesium in large amounts when combined with low amounts of calcium would increase eggshell deformity, both they must be supplied in right proportions [21].

Moreover, the results in table2 show the presence of other useful minerals like iron and zinc. The presence of these minerals types is essential as they can perform and improve different functions. For example copper [Cu] has a positive effect on bone metabolism. [22]. It is a major component of oxygen carrying part of blood cells. Along with vitamin C it is important for keeping blood vessels and skin elastic and flexible [23] Iron [Fe] is a good supplement in the body for oxygen transport [by hemoglobin and enzymatic oxidation reactions][24]. Zinc [Zn] is associated with higher activity of carbonic anhydrase. It contributes well in improving the quality of eggshell[5]. Manganese [Mn] has an activating effect on alkaline phosphatase. It is important for the right formation of bone tissue and eggshell. It increases egg weight and eggshell thickness. Also, it works as an enzyme Co-factor which is important for controlling synthesis of mucopolysaccharides [19]. Cobalt [Co] is an essential trace element and forms part of the active site of Vitamin B12. Cobalt salts are effective and used in small amounts to correct mineral deficiencies, large amounts of cobalt salts are carcinogenic [25].

Furthermore, the results obtained in table 2 show low traces of cadmium [Cd] found in the tested samples of chicken eggshells, no traces of lead [Pd] element were detected. The low amounts of these traces give an advantage for this type of chicken eggshells to be used to form a powder. The eggshell powder [ESP] was considered as valuable source of calcium over natural sources of calcium which may be found polluted with greater amounts of cadmium and lead [26]. In addition to that, the amounts of lead and cadmium detected in chicken eggshell can be used as an indication to the presence of these undesirable elements in the surrounding environment and feeds [9].

Table 3: Mineral Composition [mg/100g in dry weight]

Specification		Chicken eggshell[mg/100g]		Recommended nutrient intakes mg/day[27]
		Present study	Previous studies	
Macro elements	Na	4.97	[4.75][7]	[3000]
	Ca	2442.6	[2534.4±10.6][25]	[1250]
	Mg	134.8	[356][9] , [247.7][5]	[4]
Microelements	K	5.21	[9.0] [7]	[7000]
	Cu	12.12	[1.016] [9]	[1.2]
	Mn	0.36	[0.93±0.01] [19]	[4.00]
	Fe	5.61	[2.266][9]	[10 –18]
	Zn	1.69	[2.00][7]	[4.2-15]
	Cd	0.74	[15.^{x}10-4][9]	[3]
	Pb	-	[1.36±0.1][19]	[100]
	N	403.12	[397.6][26]	-

4. Summary

In summary, our results showed that the percentage of moisture contained in the chicken eggshells samples was low [0.24 ± 0.6%], compare with another study in [Nigeria], [0.5± 0.03 %]. Moreover, found high moisture causes microbial activities in chicken eggs during storage, organic matter contains [the crude fiber, crude protein, carbohydrates, Lipid]. Furthermore, in this studying is low percentage [9.00 ± 0.21%]. And was found the structure of eggshell is Organic matter content is high percentage [12%], [Inorganic] ash contained in chicken eggshells samples was high [90.75 ± 0.21%]. So, our present study was the first studied in Libya.

5. Conclusion

The study presented in this paper provides a clear idea into the composition of chicken eggshells obtained from Sebha city of Libya during March 2016. It showed that the tested chicken eggshells are composed of different substances, organic and inorganic. It illustrated also that they contained various useful minerals elements. Therefore, this type of chicken eggshells is of a good quality and can be exploited in different applications. For future work, the composition of the chicken eggshell used in this study should be determined for longer intervals during the year, to understand the environmental effects.

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