

Research Article

Naziha Sultan Ahmed, Faten Hammed Kamil, Adnan Ali Hasso, Ahmed Nazar Abduljawaad, Thekra Fadel Saleh, and Saffanah Khuder Mahmood*

Calcium carbonate nanoparticles of quail's egg shells: Synthesis and characterizations

<https://doi.org/10.1515/jmbm-2022-0001>

Received Apr 29, 2021; accepted Nov 14, 2021

Abstract: Avian eggshell is a natural biomaterial that has been used as an alternative natural source of CaCO_3 and is accessible in big amounts from egg manufacturing. This study was planned to estimate CaCO_3 in quail's eggshell because it has a probable use in the progress of a novel choice of many applications. Physical properties: mineralogical documentation of the natural eggshell nanoparticles were approved using XRD and FTIR to explore the chemical bond or molecular structure of the materials. Micrographs were obtained using FESEM/EDX and TEM to identify the morphology and size of nanoparticles. The results showed that quail eggshell was soft, with white to light sand color, and a smooth texture which allows good deposition of different color spots, from black to brown spots. The resulted of eggshells signifies almost 8.4% w/w of the overall weight (12.2) gram of quail egg and 91.60% w/w of the micropowder to the full weight of (0.94) gram of quail eggshell. The results presented that calcium is the main element in an eggshell; frequently occurs in a formula of CaCO_3 and the crystal construction was almost pure calcite. FTIR spectra for quail eggshell demonstrated the existence of the out of plane bending, the asymmetric stretching, and the plane bending styles of the carbonate groups, specific of normal dolomite, situated at 873 cm^{-1} , 1405 cm^{-1} , and 710 cm^{-1} , respectively. The FESEM and TEM for nanoparticles were shown calcite CaCO_3 nanoparticles with an ordinary size of $\leq 100\text{ nm}$ for FESEM and with a variety size of $\leq 50\text{ nm}$ for TEM. Unfortunately, eggshell is an egg product manufac-

turing deposit. These incomes will let fast developments in proportional studies of the organic elements of avian eggshell and their purposeful consequences by usages of eggshell in nourishment and medicine which can be applied for many resolutions that diminish their consequence on environmental contamination.

Keywords: CaCO_3 nanopowder, descriptions, eggshell, production, quail

1 Introduction

The egg and egg derived intake harvest a big quantity of remaining shells which carriage an ecological contamination as an outcome of microbial accomplishment as it is hard to be despoiled by soil microorganisms [1–3]. Eggshell are discarded materials from hatcheries, homes and fast-food manufacturing and can be freely composed in sufficiently [4, 5]. Most of the discarded is generally predisposed of in a landfill without any pretreatment, producing smells from biodegradation, terminating microbial action, and exchanging the worth of soil [6]. Challenges related to eggshell discarding need cost, obtainability of discarding sites, odor, flies and insensitivity [5]. Calcium carbonate (CaCO_3) itself can be initiate in big amounts in nature, with submissions frequently as fresh material for the ceramic calcium carbonate progress in quickly growing technology and research, for example hydroxyl apatite (HA) material production as a substitute to the teeth and bones of human [7, 8]. Quick technological expansions have mandatory research in all fields of science and technology is no exclusion to remain to renovate in the technology of discarded exploitation and waste [9]. It is chiefly motivating to study the discarded bin of numerous types of poultry eggshells, with a statement that a lot of eggshells have calcium carbonate. Application of discarded eggshells as a source material for manufacturing other combinations those are able for association to harvest a new compound. Eggshells are a well basis of calcium for calcium oxide (CaO), calcium carbonate (CaCO_3), calcium hydroxide (Ca(OH)_2), calcium

*Corresponding Author: **Saffanah Khuder Mahmood:** Department of Anatomy, College of Veterinary Medicine, University of Mosul, Mosul, Iraq; Email: saffanhjeber@uomosul.edu.iq; saffanh.jeber@gmail.com

Naziha Sultan Ahmed, Adnan Ali Hasso, Ahmed Nazar Abduljawaad, Thekra Fadel Saleh: Department of Anatomy, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Faten Hammed Kamil: Chemical and Petrochemical Research Center, Corporation of Research and Industrial Development, Ministry of Industry and Mineral, Baghdad, Iraq

phosphate, or hydroxyl apatite (HA), in comparison to other sources for example carbonaceous rock, occasioned soil, teeth and bone, and crab or shrimp shells and the structure of an eggshell is actually like to that of our bones and teeth [6, 10, 11].

Chicken eggs are the maximum normally spent eggs; they are also a cheap protein source [12–14]. Paralleled to chicken egg duck egg is 30% larger and its nutritive profile is actually high [15]. Ingesting of quail eggs has increased in current years, due to their dietary qualities, as a good source of proteins, antioxidants, lysozyme, vitamins, and minerals, further than their dietetic importance which is 4-5 times greater than that of chicken eggs [16].

The benefits of eggshell remaining applications are to provide the normal sources of calcium (CaCO_3 , CaO , Ca(OH)_2), to decrease the discarded problematic in household, to preserve ordinary resources from rock and soil, to moderate the global environment warming, and to improve originality of green ceramic materials and products: dielectrics, catalysts, biomaterials, fuel cell, and fillers [6, 17–22]; and these incomes will permit quick improvements in proportional studies of the organic elements of quail eggshells and their serviceable effects [23, 24].

This research aims to product of CaCO_3 nanopowder from uncooked quail eggshells and examinations of the nanostructure and particle size, chemical composition, organics (C/H/N) substances and practical groups, and provide the endorsements for uses of this type of nanoparticles in the future.

2 Materials and methods

Fifteen quail eggs were collected from animal house in the College of Veterinary Medicine which took from adult quail (*Coturnix coturnix*) aged 12 weeks that were fed on a standard ration. Quail eggs transported to the laboratory of Anatomy in the College of Veterinary Medicine, University of Mosul. In the laboratory, egg sample was weighed for whole on a weighing balance (Sartorius AG Gottikåen, GP5202, $d = 0.01$ g, Germany), and detached the eatable part of eggs. Natural and raw eggshells were carefully washed numerous times only with warm water and a piece of gauze till lost their natural spots pattern, and eggshells white was totally removed and skin off all of the shell's membranes from inside of the shells. The shells were washed again with deionized water then rap into a toilet paper to dry the water contented totally. The remainder of eggshells were weighed and crushed into small pieces in a porcelain mortar and grinded into a fine powder using blender (Good and Well[®],

Taiwan)²⁵, then weighed, sieved through $\leq 75 \mu\text{m}$ sieve (Endecotts Ltd, London, England). The micropowder was more dried in the oven (Memmert, UM 500, Germany) at 50°C for 5 days and converted into nanoparticles using a mechanical method in the existence of Ball mill (Wisd[®] Ball Mill, Korea) for 7 days (Figure 1) then kept at 50°C in a sterile container before use [26, 27].

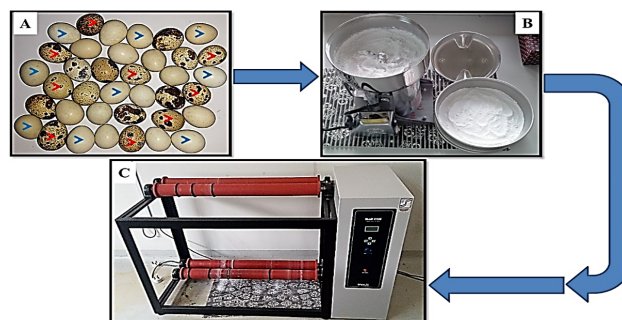


Figure 1: Photographs show the quail eggshells (A) before cleaning (red arrowhead) and after cleaning (blue arrowhead), (B) the powder after grinding by using the Crasher, and (C) converted into nanoparticles using a mechanical method in the existence of Ball mill instrument.

Physical properties of the samples like color, texture and hardness were considered. The mineralogical documentation of the natural eggshells was approved out using Powder X-ray diffraction (PXRD) on a diffractometer (Angstrom Advanced Inc. ADX-2700, X-Ray Powder Diffraction Instrument) in a tube $\text{CuK}\alpha$ operating at a voltage of 40kV and a current 40mA to identify the crystalline phases and the crystallite size of eggshells [19, 28, 29]. To complete the analysis of the powders were located in the cavity of a support used as a sample container. The crystalline phases achieved matched by Joint Committee on Powder Diffraction Standards (JCPDS). Fourier Transform Infrared (FTIR) which was run in the variety of $4000\text{--}400$ wavenumber/ cm^{-1} to explore the chemical bond or molecular structure of the materials [19]. Micrographs were gotten using a Field Emission Scanning Electron Microscope, (FESEM) tracked by image analysis using their particular software's correspondingly and a Transmission Electron Microscope (TEM) (Hitachi H-7100, Japan) to identify the morphology and size of nanoparticles. The preparation of SEM and TEM samples of the raw materials were conceded out on a grid. The samples were firstly enclosed with a thin layer of gold (10 nm) using a sputter coater and detected using (FESEM/EDX) (20 kV) under a vacuum of 1.33×10^{-6} mbar (Joel, Japan) [19, 30, 31].

3 Results

The results involved physical description data showed that raw quail eggshells had smooth texture, soft shell and white to light color with different black to brown spots. The proportional analysis of calcium carbonate (CaCO_3) content in them, which include percentage method of analysis by using 15 eggs presented in Table 1.

The ultrastructure and crystal construction of quail eggshells was considered in laboratory. The eggshell crystal construction was distinguished using an X-ray diffraction spectra (XRD) of normal (not boiling or exposure to any treatment) eggshells sample which achieved with $\text{CuK}\alpha$ radiation ($\lambda = 0.15406 \text{ nm}$) at 30 kV, 16 mA, scan speed of 8.0 θ/min and scan range 5–60 θ . Figure 2 appearances a X-ray diffraction spectrum of normal eggshells. Four obvious peaks were start individually in CaCO_3 calcite nanoparticles powder bands. The 1st conventional peaks were $2\theta = 23.057^\circ$ to 29.408° , while the 2nd conventional peaks were $2\theta = 31.46^\circ$ to 39.456° and the 3rd conventional peaks were $2\theta = 43.209^\circ$ to 48.601° and the 4th conventional peaks were $2\theta = 56.755^\circ$ to 58.148° . Chief peak performed at 2θ

= 29.4. Equaling the XRD peak data of (Figure 2 and Table 2) with the classic CaCO_3 diffractogram from the shell powders seen to match systematically to calcite phase of a typical CaCO_3 structure ICDD-card number 01-083-1762. The deflection peaks were sharp and deep, signifying that the nanoparticles are extremely crystalline and equal very

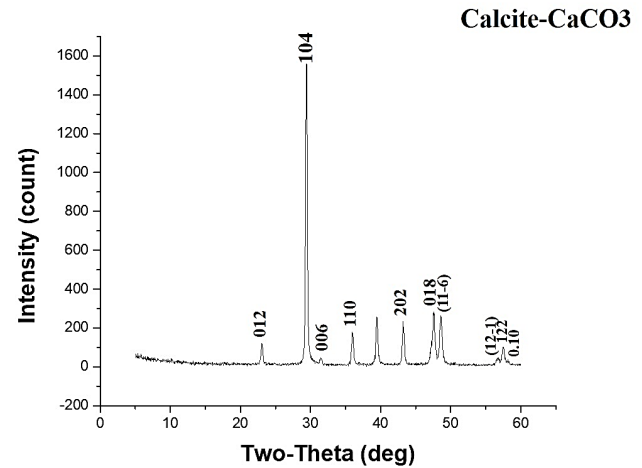


Figure 2: PXRD analysis of quail eggshell.

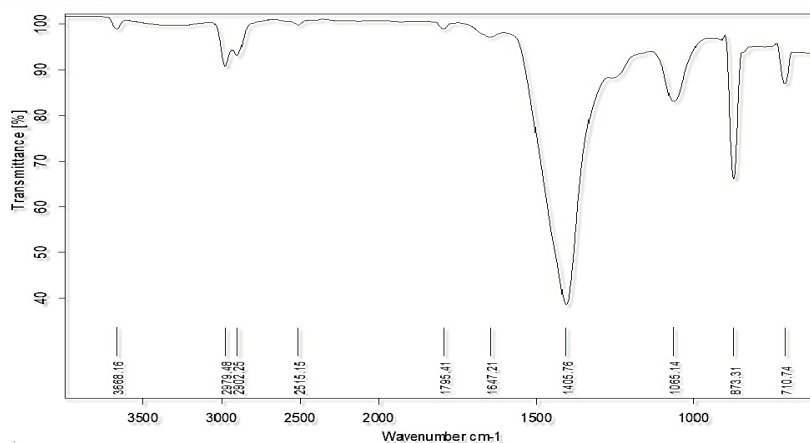
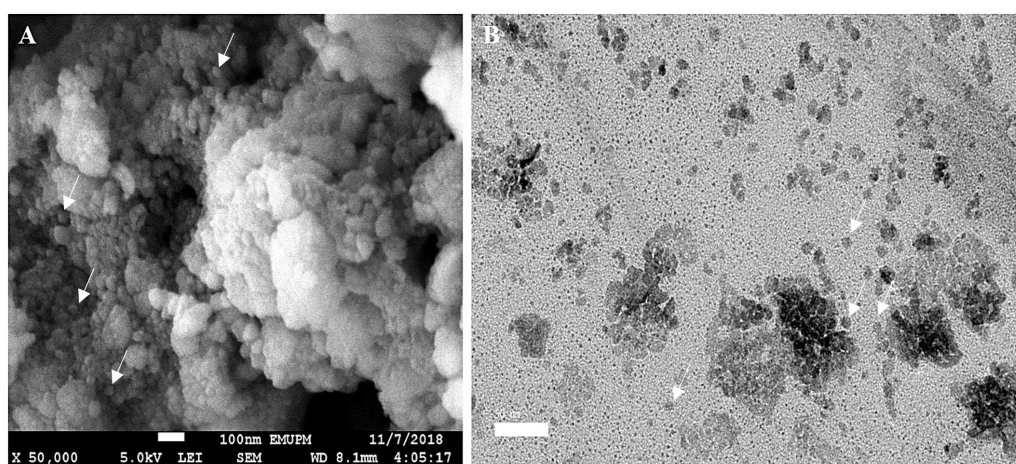
Table 1: Percentage analysis of micropowder of 15 quail eggshells.

No. of sample	whole weight of quail egg (g)	whole weight of quail eggshell (g)	Percentage of whole weight of quail eggshell (g) / whole weight of quail egg (g)	whole weight of quail eggshell micropowder (g) / whole weight (g)	Percentage of whole weight of quail eggshell micropowder (g) / whole weight of quail egg (g)
1	12.36	1.01	8.17	0.95	94.06
2	11.21	0.94	8.39	0.88	93.60
3	10.83	0.94	8.68	0.88	93.60
4	12.55	1.03	8.21	0.92	89.30
5	11.44	0.94	8.22	0.87	92.60
6	12.70	1.07	8.43	1.01	94.40
7	12.81	1.15	8.98	1.05	91.30
8	12.66	1.06	8.37	1.01	95.30
9	12.06	0.99	8.21	0.85	85.90
10	13.04	1.17	8.97	1.11	94.90
11	12.52	1.02	8.15	0.93	91.10
12	12.09	1.00	8.27	0.92	92.00
13	11.97	0.96	8.02	0.80	83.30
14	12.07	1.00	8.29	0.93	93.00
15	12.97	1.16	8.94	1.05	90.50
Average (two numbers after decimal point)	12.22	1.03	8.42	0.94	91.66

Table 2: PXRD analysis of nanopowder of 15 quail eggshells.

2-Theta	d (nm)	(h k l)	BG	Height	I%	Area	I%	FWHM	XS (nm)
23.057	0.38541	(0 1 2)	8	104	6.8	692	6.7	0.283	28
29.408	0.30347	(1 0 4)	24	1535	100	10274	100	0.284	29
31.46	0.28412	(0 0 6)	15	28	1.8	147	1.4	0.223	37
35.963	0.24952	(1 1 0)	12	162	10.6	1077	10.5	0.283	29
39.456	0.2282	(1 1 3)	11	244	15.9	1897	18.5	0.33	25
43.209	0.2092	(2 0 2)	10	219	14.3	1492	14.5	0.29	29
47.559	0.19103	(0 1 8)	13	265	17.3	2640	25.7	0.423	20
48.601	0.18718	(1 1 6)	11	245	16	2304	22.4	0.4	21
56.755	0.16207	(1 2 1)	4	36	2.3	318	3.1	0.375	24
57.5	0.16014	(1 2 2)	8	88	5.7	873	8.5	0.422	21
58.148	0.15851	(1 0 10)	6	23	1.5	151	1.5	0.279	32

2 theta: the diffracted angle, h,k,l: Miller indices of plane, BG: Back Ground, I: Intensity, Area: Area under the peak, FWHM: Full Width of Half Maximum, XS (nm): crystal size (nm).

**Figure 3:** FTIR analysis of quail eggshells.**Figure 4:** SEM (A) & TEM (B) micrographs of quail eggshells show that quail eggshells has construction of spherical shape of particle size (white arrows), (A) magnification of (50000X), (B) magnification of (50 nm).

well with the typical data card. There is no signal of any contamination phase current in the sample.

The results presented that calcium is the main element in an eggshell; frequently occurs in a formula of CaCO_3 and the crystal construction was almost pure calcite.

The quail eggshells expressions a particle size of ≤ 50 nm (CaCO_3). The reduction of the crystal size can be recognized to the mechanical method using roller mill method. The lower strength peaks for quail eggshells could be associated to the decrease in the crystallite size.

Figure 3 displays the FTIR spectra for mixed of all samples of quail eggshells. The spectrum of quail eggshells in Figure 3 demonstrations the existence of the out of plane bending, the asymmetric stretching, and the in plane bending styles of the carbonate groups, specific of normal dolomite, situated at 873 cm^{-1} , 1405 cm^{-1} , and 710 cm^{-1} , respectively. In addition, the internal modes, the grouping of the prior bending modes has also been detected at 1795 cm^{-1} and 2515 cm^{-1} . Lastly, the band positioned at 3668 cm^{-1} has been accredited to H-bonded water of the humidity. Moreover, a band about 2902 cm^{-1} performs apportioned to alkyl C-H stretch because of the organic substance of the quail eggshells.

The FESEM and TEM for nanoparticles shown calcite calcium carbonate nanoparticles with an ordinary size of ≤ 100 nm for FESEM and with variety size of ≤ 50 nm for TEM (Figures 4(A) and (B)). There was no conversion in the elemental structures of the gotten CaCO_3 calcite nanoparticles powder after production using of roller mill method. This reveals the roll milling in the breakdown of the larger sized calcite rods into very smaller spherical ones.

Figure 4(A) appearances the SEM micrographs of the organized eggshells NPs. The particles were spherical in shape with constant size spreading. The eggshell ultrastructure was detected using SEM gotten from standard eggshells under high magnification (50000X). TEM images of the raw quail eggshells samples are presented in Figure 4(B) at a magnification of 50 nm. The raw quail eggshells had a normally regular crystal construction.

4 Discussion

The results showed that quail eggshells were soft, with color between white to light sand color, and a smooth texture which allows good deposition of color spots, with different color levels from black to brown spots. These results agreed with the studies by Sezer & Tekelioglu [32], Duval *et al.* [33], Stolić *et al.* [34] and Drabik *et al.* [35].

The resulted of eggshells signifies almost 8.4% w/w of the overall weight (12.2) gram of quail egg and 91.60% w/w of the micropowder to the full weight of (0.94) gram of quail eggshell. The study of the quail eggshells exposed a high quantity of calcium in formula CaCO_3 . These results confirm the results of studies by Thapon & Bourgeois [1]; Romanoff & Romanoff [36] and Nys *et al.* [37].

The eggshells contain chiefly of CaCO_3 , therefore calcium shows an essential part in the eggshells construction [38]. The previous studies determined that the chief structure material of the shell represented by CaCO_3 (96%), and the residual modules are magnesium, phosphorus, but also copper, zinc, iron [39] and numerous trace elements, amongst them lithium, strontium and bar [40]. They described that all eggshells had parallel chemical substances which mostly composed of CaCO_3 and a little of other elements, *i.e.* S, Mg, P, Al, K and Sr.

The quail eggshells comprise more calcium levels [24, 41, 42]. They are spent often, but till now the shells are unused and static infrequently used particularly for HA material [43, 44].

The ultrastructure and crystal construction of quail eggshells was considered in laboratory [45]. The eggshell crystal construction was distinguished using an X-ray diffractometer. Results presented that the eggshells crystal construction was almost pure calcite (CaCO_3) in quail eggs. These results agreed with the results of studies by Cahya & Marfuah [7] and Murakami *et al.* [28].

The FTIR spectra for quail eggshells displayed in Figure 3 demonstrates the existence of the outer plane bending, the asymmetric stretching and the in plane bending styles of the carbonate groups, specific of normal dolomite, situated at 873 cm^{-1} , 1405 cm^{-1} , and 710 cm^{-1} , respectively. In addition, the internal modes, the grouping of the prior bending modes has also been detected at 1795 cm^{-1} and 2515 cm^{-1} . Lastly, the band positioned at 3668 cm^{-1} has been accredited to H-bonded water of the humidity. Moreover, a band about 2902 cm^{-1} performs apportioned to alkyl C-H stretch because of the organic substance of the quail eggshells. These results confirm the results of studies by Cahya & Marfuah [7]; Wei *et al.* [45]; Viriya-empikul *et al.* [46] and Correia *et al.* [47].

The eggshells ultrastructure was detected using SEM presented in Figure 4, gotten from standard eggs [42]. The quail eggshells expressions a particle size of ≤ 50 nm (CaCO_3). The reduction of the crystal size can be recognized to the exothermic circumstances of the mechanical method using roll mill machine. The lower strength peaks for quail eggshells could be associated with decrease in the crystallite size. These results agreed with the results of studies by Park *et al.* [10]; Marques *et al.* [25] and Mahmood *et al.* [28].

The persistence of SEM and TEM description is to conclude the morphology of the quail shell nanopowder which are presented in Figure 4. This image indicates that the typical nanoparticles of quail eggshells nanopowder is the spherical nanoparticles with diameter about 50 nm. The image also designates the existence of agglomeration which occurred because of the existence of other phosphate compounds [48]. This remark is agreed with the results described by Park *et al.* [10]; Marques *et al.* [25]; Mahmood *et al.* [28] and Kalita & Verma [49].

Consequently, quail eggshell is a rich basis of mineral salts, principally calcium carbonate that is possibly the superlative normal source of Ca^{+2} .

5 Conclusion

Shells of quail eggs are a remarkable alternate to the presently used products in supplementation of additional normal sources of Ca^{+2} to humans and animals. Greater solubility of CaCO_3 from the shells of quail eggs, matched to synthesized CaCO_3 mark them a brilliant biomaterial for the manufacture of new nutritional supplements. Furthermore, the alteration of CaCO_3 micropowder, consequences in Ca^{+2} salt nanoparticles with enhanced properties matched to CaCO_3 . The CaCO_3 got from quail eggshells is categorized by an appropriate purity and comprises valued minerals in its structure. These incomes will let fast developments in proportional studies of the organic elements of avian eggshells and their purposeful consequences by using of eggshells in nourishment and medicine which can be applied for many resolutions that diminish their consequence on environmental contamination.

Acknowledgement: The authors thank the Department of Anatomy in College of Veterinary Medicine, University of Mosul for supporting this study.

Funding information: The authors state no funding involved.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest: The authors state no conflict of interest.

References

- [1] Thapon JL, Bourgeois CM. L'Oeuf et les ovoproducts. Paris: Lavousier; 1994.
- [2] Djaja W. The Right Steps to Make Compost from Animal Manure & Garbage. AgroMedia Pustaka; 2008.
- [3] Owuamanam S, Cree D. Progress of Bio-Calcium Carbonate Waste Eggshell and Seashell Fillers in Polymer Composites: A Review. J Compos Sci. 2020;4(2):70.
- [4] Amu OO, Fajobi AB, Oke BO. Effect of eggshell powder on the stabilizing potential of lime on an expansive clay soil. Res J Agric Biol Sci. 2005;1(1):80–4.
- [5] Glatz P, Miao Z. High value products from hatchery waste. Australia: Rural Industries Research and Development Corporation; 2009 p. 1-2.
- [6] Tangboriboon N, Kunanuruksapong R, Sirivat A. Preparation and properties of calcium oxide from eggshells via calcination. Mater Sci Pol. 2012;30(4):313–22.
- [7] Cahya M, Marfuah N. Identification of calcium carbonate (CaCO_3) characteristics from different kinds of poultry eggshells using X-Ray Diffraction (XRD) and Fourier Transformation Infra-Red (FTIR). 2014 International Conference on Physics and its Applications (ICOPIA-14); 2014 Sep 16-17; Solo, Indonesia. Atlantis Press; 2015 p. 138-142. <https://www.atlantispress.com/proceedings/icopia-14/16759>
- [8] Syafaat FY, Yusuf Y. Effect of Ca: P concentration and calcination temperature on hydroxyapatite (HAp) powders from quail eggshell (*Coturnix Coturnix*). Int J Nanoelectron Mater. 2018;11:51–8.
- [9] Beckhoff B, Kanngießner B, Langhoff N, Wedell R, Wolff H. Handbook of practical X-ray fluorescence analysis. Berlin: Springer; 2007.
- [10] Park HJ, Jeong SW, Yang JK, Kim BG, Lee SM. Removal of heavy metals using waste eggshell. J Environ Sci (China). 2007;19(12):1436–41.
- [11] King'ori AM; King'ori AM. A review of the uses of poultry eggshells and shell membranes. Int J Poult Sci. 2011;10(11):908–12.
- [12] Adenowo JA, Awe FA, Adebambo OA, Ikeobi CO. Species variations in chemical composition of local poultry eggs. 26th Annual NSAP Conference; 1999 March 21-25; Ilorin, Nigeria. 1999 p. 278-80.
- [13] Curabay B, Sevim B, Cufadar Y, Ayasan T. Effects of adding spirulina platensis to laying hen rations on performance, egg quality, and some blood parameters. J Hell Vet Med Soc. 2021;72(2):2945–52.
- [14] Tufarelli V, Baghban-Kanani P, Azimi-Youvalari S, Hosseintabar-Ghasemabad B, Slozhenkina M, Gorlov I, et al. Effects of Horsetail (*Equisetum arvense*) and Spirulina (*Spirulina platensis*) dietary supplementation on laying hens productivity and oxidative status. Animals (Basel). 2021 Jan;11(2):335.
- [15] John LK, Loewenstein G, Prelec D. Measuring the prevalence of questionable research practices with incentives for truth telling. Psychol Sci. 2012 May;23(5):524–32.
- [16] Tunsaringkarn T, Tungjaroenchai W, Siriwong W. Nutrient benefits of quail (*Coturnix coturnix japonica*) eggs. Int J Sci Res Publica. 2013;3(5):1–8.
- [17] Gongruttananun N. Effects of using ground eggshells as a dietary calcium source on egg production traits, hatching performance

- and eggshell ultrastructure in laying hens. *Agri Natural Reso.* 2011;30(45):209–20.
- [18] Dolińska B, Jelińska M, Szulc-Musiola B, Ryszka F. Use of eggshells as a raw material for production of calcium preparations. *Czech J Food Sci.* 2016;34 (4): 313–17.
- [19] Mohadi R, Sueb A, Anggraini K, Lesbani A. Calcium oxide catalyst based on quail eggshell for biodiesel synthesis from waste palm oil. *J Pure Applied Chem Res.* 2018;7(2):130.
- [20] Vuong TT, Rønning SB, Ahmed TA, Brathagen K, Høst V, Hincke MT, et al. Processed eggshell membrane powder regulates cellular functions and increase MMP-activity important in early wound healing processes. *PLoS One.* 2018 Aug;13(8):e0201975.
- [21] Dizaj SM, Sharifi S, Ahmadian E, Eftekhari A, Adibkia K, Lotfipour F. An update on calcium carbonate nanoparticles as cancer drug/gene delivery system. *Expert Opin Drug Deliv.* 2019 Apr;16(4):331–45.
- [22] Awogbemi O, Inambao F, Onuh EI. Modification and characterization of chicken eggshell for possible catalytic applications. *Heliyon.* 2020 Oct;6(10):e05283.
- [23] Hincke MT, Nys Y, Gautron J, Mann K, Rodriguez-Navarro AB, Mc-Kee MD. The eggshell: structure, composition and mineralization. *Front Biosci.* 2012 Jan;17(1):1266–80.
- [24] Shwetha A, Shravana Kumara SM. Comparative study on calcium content in egg shells of different birds. *Int J Zoology Stud.* 2018;3(4):31–3.
- [25] Marques CL, Cecilia JA, Rodríguez-Castellón E, Cavalcante CL, Vieira RS. Relevance of the physicochemical properties of calcined quail eggshell (CaO) as a catalyst for biodiesel production. *J Chem.* 2017;2017.
- [26] Dahiru M, Jimoh WL. Evaluation of aphrodisiac efficacy of quail egg shell concoction in albino rat. 6th International Conference on Environmental Science and Technology (IPCBE); 2015 May 23–25; Singapore. 2015;84:19.
- [27] Mahmood SK, Zakaria MZ, Razak IS, Yusof LM, Jaji AZ, Tijani I, et al. Preparation and characterization of cockle shell aragonite nanocomposite porous 3D scaffolds for bone repair. *Biochem Biophys Rep.* 2017 Apr;10:237–51.
- [28] Murakami FS, Rodrigues PO, Campos CM, Silva MA. Physicochemical study of CaCO₃ from egg shells. *Food Sci Technol (Campinas).* 2007;27(3):658–62.
- [29] Venkateswarlu K, Sreekanth D, Sandhyarani M, Muthupandi V, Bose AC, Rameshbabu N. X-ray peak profile analysis of nanostructured hydroxyapatite and fluorapatite. *Int J Biosci Biochem Bioinform.* 2012;2(6):389–93.
- [30] Islam KN, Bakar MZ, Ali ME, Hussein MZ, Noordin MM, Loqman MY, et al. A novel method for the synthesis of calcium carbonate (aragonite) nanoparticles from cockle shells. *Powder Technol.* 2013;235:70–5.
- [31] Isa T, Zakaria ZA, Rukayadi Y, Mohd Hezmee MN, Jaji AZ, Imam MU, et al. Antibacterial activity of ciprofloxacin-encapsulated cockle shells calcium carbonate (Aragonite) nanoparticles and its biocompatibility in macrophage J774A. *Int J Mol Sci.* 2016 May;17(5):713.
- [32] Sezer M, Tekelioglu O. Quantification of Japanese quail eggshell colour by image analysis. *Biol Res.* 2009;42(1):99–105.
- [33] Duval C, Cassey P, Miksík I, Reynolds SJ, Spencer KA. Condition-dependent strategies of eggshell pigmentation: an experimental study of Japanese quail (*Coturnix coturnix japonica*). *J Exp Biol.* 2013 Feb;216(Pt 4):700–8.
- [34] Stolić I, Jurak M, Kujundžić M, Popović M, Mršić G, et al. Visualization of latent fingerprints on the surface of quail eggshells. *Vet Stanica.* 2019;50(4):337–44.
- [35] Drabik K, Batkowska J, Vasiukov K, Pluta A. The impact of eggshell colour on the quality of table and hatching eggs derived from Japanese quail. *Animals (Basel).* 2020 Feb;10(2):264.
- [36] Romanoff AL, Romanoff AJ. *The avian egg.* New York (NY): John Wiley & Sons, Inc.; 1949.
- [37] Nys Y, Gautron J, Garcia-Ruiz JM, Hincke MT. Avian eggshell mineralization: biochemical and functional characterization of matrix proteins. *C R Palevol.* 2004;3(6-7):549–62.
- [38] Shen TF, Chen WL. The role of magnesium and calcium in eggshell formation in Tsaiya ducks and Leghorn hens. *Asian-Australas J Anim Sci.* 2003;16(2):290–6.
- [39] Nys Y, Hincke MT, Arias JL, Garcia-Ruiz JM, Solomon SE. Avian eggshell mineralization. *Poult Avian Biol Rev.* 1999;10(3):143–66.
- [40] Goran GV, Crivineanu V, Tudoreanu L, Udrea D. Dynamics of some mineral elements in hen eggs. *Bull Univ Agric Sci Vet Med Cluj-Napoca Vet Med.* 2010;67:88–95.
- [41] Genchev A. Quality and composition of Japanese quail eggs (*Coturnix japonica*). *Trakia J Sci.* 2012;10(2):91–101.
- [42] Agustini TW, Fahmi AS, Widowati I. Utilization of clam shell waste (*Amusium pleuronectes*) in the manufacture of calcium-rich cookies. *J Pengolah Has Perikan Indones.* 2013;16(1).
- [43] Neunzehn J, Szuwart T, Wiesmann HP. Eggshells as natural calcium carbonate source in combination with hyaluronan as beneficial additives for bone graft materials, an *in vitro* study. *Head Face Med.* 2015 Apr;11(1):12.
- [44] Chen W, Shen T. Comparisons of ultrastructure and crystal structure in eggshells between brown Tsaiya duck and white Leghorn hen. *J Chin Soc Anim Sci.* 2000;29:345–55.
- [45] Wei Z, Xu C, Li B. Application of waste eggshell as low-cost solid catalyst for biodiesel production. *Bioresour Technol.* 2009 Jun;100(11):2883–5.
- [46] Viriya-Empikul N, Krasae P, Puttasawat B, Yoosuk B, Hollacoop N, Faungnawakij K. Waste shells of mollusk and egg as biodiesel production catalysts. *Bioresour Technol.* 2010 May;101(10):3765–7.
- [47] Correia LM, Saboya RM, Campelo NS, Cecilia JA, Rodríguez-Castellón E, Cavalcante CL Jr, et al. Characterization of calcium oxide catalysts from natural sources and their application in the transesterification of sunflower oil. *Bioresour Technol.* 2014 Jan;151:207–13.
- [48] Sari M, Kristianto NA, Chotimah, Ana ID, Yusuf Y. Carbonated Hydroxyapatite-based honeycomb scaffold coatings on a Titanium Alloy for bone implant application-physicochemical and mechanical properties analysis. *Coatings.* 2021;941(11):1–18.
- [49] Kalita SJ, Verma S. Nanocrystalline hydroxyapatite bioceramic using microwave radiation: synthesis and characterization. *Mater Sci Eng C.* 2010 Jan;30(2):295–303.