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Estimation of Vitamin D3 Content in Table Eggs and its Stability during Cooking

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Abstract

Vitamin D3 has a pivotal role in bone development together with maintaining the normal level of both calcium and phosphorous inside the body. Dietary intake is of great importance as a good source of vitamin D in addition to sunlight. Eggs, for instance, are excellent sources for naturally occurring cholecalciferol (vitamin D_3) in daily meals. Hence, the aim of the current study was to measure the level of vitamin D_3 in two kinds of table eggs (Balady and farm eggs) in Assiut Governorate. 150 eggs were collected from supermarkets and outlet retails and then subjected to determine the level of vitamin D_3 using High-Performance Liquid Chromatography. Additionally, the stability of vitamin D_3 after cooking (boiling and frying methods) was estimated throughout the study. The obtained results showed that the level of vitamin D_3 was higher in farm eggs (0.13 to 8.5 mcg/100g) in comparison to Balady eggs (0.006 to 5.6 mcg/100g). After cooking, the reduction rate of vitamin D_3 content was lower after boiling (47.43 %) than frying (65.78 %). In conclusion, farm eggs are a good source for vitamin D_3, and the cooking methods may be implicated in adverse loss of vitamin D_3 content in eggs. Herein, it is suggested to use the boiling method for cooking eggs than frying method to minimize the possible loss in such vitamins.

KEYWORDS
Balady eggs, Farm eggs, Vitamin D3, HPLC, Boiling, Frying.

INTRODUCTION

Vitamin D is one of the most important fat-soluble vitamins in both human and animal diet. It could be existed in two forms as vitamin D_2 and D_3 that are initially inactive before being metabolized in the liver to calciferol (Farag *et al*., 2018). The first one, named ergocalciferic, is of less bioavailability so it is not widely used than vitamin D_3. On the other hand, vitamin D_3 (cholecalciferol) is the most important one and it could be obtained through synthesis from 7-dehydrocholesterol after exposure to ultraviolet rays or absorption from the diet rich in such vitamin (Gupta and Tripathi, 2015). Human could drive the majority of daily vitamin D through the action of sun light in their skin (Mason *et al*., 2011). Interestingly, vitamin D has a crucial role in calcium-phosphorus metabolism (Holick, 2007) that is required for either bone or teeth development. Additionally, it helps in muscle building due to the presence of its receptors inside the muscle tissue (Schmid and Walther, 2013). Moreover, several previous studies showed a wide range of pharmacological and physiological functions of vitamin D. It could play a vital role as an anti-inflammatory, antioxidant, and antiviral agent together with regulatory properties in the adaptive and innate immune systems (Bishop *et al*., 2020).

Notably, it was reported that the deficiency of vitamin D was quite common among COVID-19 patients (Karahan and Katkat, 2021). A recent clinical study by Castillo *et al*. (Entrenas Castillo *et al*., 2020) investigated that those COVID-19 patients who supplemented with vitamin D showed less severe symptoms of such disease. Furthermore, Demir *et al*. (2021) found that the high vitamin D levels could decrease COVID-19 PCR positivity, D-dimer and C-reactive protein levels and the number of affected lung segments in COVID-19-positive patients which lead to shortening the duration of hospital stays and minimizing the severity of COVID-19.

Vitamin D through exposure to sun light is not always adequate due to the current lifestyle with most activities indoors. Additionally, season may influence the intensity of the sun, so interfere with the amount of vitamin D synthesized inside the body. Hence, the dietary intake of vitamin D becomes increasingly important (Dimartino, 2019; Mason *et al*., 2011). Vitamin D_3 and its metabolites are present only in foods of animal origin such as fish, meat, eggs, and milk (Foote *et al*., 2004; Mandrioli *et al*., 2020; Barnkob *et al*., 2020). The European Food Safety Authority recently set an adequate intake for vitamin D at 10 µg/day for infants aged 7-11 months and 15 µg/day for children aged 1-17 years and adults (EFSA Panel on Dietetic Products and Allergies, 2016).

The vitamin D deficiency is implicated in many health problems such as muscle weakness, rickets, osteomalacia, cardiovas-

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cular disease, cancer, autoimmune disease, diabetes mellitus and hypertension (Ross *et al*., 2011). Furthermore, the deficiency of such vitamin could also affect the normal growth and development of the newly born infants (Principi *et al*., 2013). When the level of vitamin D is above 10.000 IU, it could exert a toxic effect as a result of excessive absorption of calcium from intestine and resorption of calcium from bone. Therefore, it was important to develop an accurate method to estimate the amount of vitamin D in table eggs (Dimartino, 2019). The last decade, high performance liquid chromatography (HPLC) method offers the best technique to measure the level of vitamin D3 accurately in foods (Farag *et al*., 2018).

The stability of vitamin D in different kinds of food was previously reported showing variable losses in the level of vitamin D and its metabolites. In this context, the fortification of food with such vitamin was the possible choice to avoid its loss. For instance, the stability in fortification regiments was examined during spray-drying of milk to produce vitamin D fortified UHT milk and cheese. The loss of vitamin D was insignificant in such products (Wagner *et al*., 2008; Hanson and Metzger, 2010). Moreover, a previous study on fortification of bread with vitamin D_3 investigated a lower retention (Natri *et al*., 2006; Madsen *et al*., 2013). Saghafi *et al*. (2018) examined the effect of time and temperature on stability of added vitamin D_3 in some vegetable oils (corn, sunflower, and canola oils) and identified that the retention rate of the fortified vitamin D_3 was varied from 68.6% to 87.4%. Therefore, the temperature, time, and pH of the food could affect the level of vitamin D retention during cooking (Jakobsen and Knuthsen, 2014). It was emphasized that the current information on the fate of vitamin D metabolites during processing of food is insufficient (Lešková *et al*., 2006).

The current study was designed to determine the level of vitamin D in most consumed table eggs in Egypt. Besides, the retention of vitamin D during cooking of eggs was also studied.

MATERIALS AND METHODS

Collection of samples

A total of 150 fresh hen's egg samples were collected from different outlet retails and supermarket in Assiut Governorate during a period from September to January 2021. Eggs samples were collected from different sources to obtain random and representative samples. They were transferred in plastic bags to the laboratory (The Analytical Chemistry Unit at Assiut University). The samples were protected from air and light during transportation and then kept refrigerated at 4ºC until further analysis. The samples were categorized into two major groups: Farm eggs and balady eggs throughout the study.

Measuring of vitamin D3 in egg samples using HPLC

Reagents and materials

All the used chemicals in the current study were of the highest purity and this experiment were done using HPLC system (Apparatus: Agilent Technologies1200 series, G 1321A FLD, Column: Zorbax Eclipse XDB C18; Analytical 4.6×150 mm 5-Micron), Detector: FLD at 280 nm. (excitation), 360 nm (emission) with flow rate: 1ml/min, temperature: 30 ºC, injection volume: 20 µl, Mobile phase: Methanol: Water (95%: 5%). The total run time was equal to 7 min. The standard solution of Cholecalciferol (vitamin D3) was prepared by dissolving 1mg of standard cholecalciferol in 1ml methanol.

Saponification and extraction

Saponification step was done by weighing 25 g of the pooled egg yolk sample in a measuring flask and was completed with warm deionized water at 40ºC to the mark, then vortexed for 10 min until complete homogenization. 20 ml of 50% KOH solution and 1 g of ascorbic acid were added to the previous mixture in a new 100 ml measuring flask then covered with foil. Ethanol was added to the solution with continuous shaking and then overnight incubated at room temperature in a water bath until the sample was completely saponified. The organic layers were collected and washed twice with cyclohexan for 2 min; then evaporated with liquid nitrogen.

HPLC protocol

For the chromatography step, the residues in the previous step were dissolved in 2 ml methanol and filtered through 0.45 mm filter. Then, 20 ml of the extract was subjected to analysis using HPLC. The level of vitamin D_3 in the examined samples was measured in µg/100g of egg weight.

Stability of vitamin D3 after cooking (boiling and frying) of table eggs

Pooled egg samples were punctured by a sterile needle to aspirate 25 g of egg yolk (control group) for measuring the level of vitamin D_3 before treatments (boiling and frying); and then part of the same pooled samples was put in a beaker on a hot plate to boil the eggs for 10 min. After boiling, 25 g of egg yolk was used to estimate the retention of vitamin D_3 using HPLC method. Other aliquots of pooled eggs were subjected to frying in a pan without adding any greasy substances and then vitamin D_3 was measured. The current experiments were done in three replicates (n = 5 pooled egg samples for each treatment).

Statistical analysis

The observed data were statistically analyzed using SPSS statistics 21 for windows (IBM SPSS, Amonk, NY, USA). Descriptive statistics such as mean and standard deviation were calculated from the obtained data with MS Excel (Microsoft Corporation). The statistical analysis consisted of mean comparison tests, univariate Analysis of variance (ANOVA) followed by Tukey's post-hoc test ($p < 0.05$) to evaluate significant differences between the level of vitamin D_3 in farm and balady eggs before and after cooking procedures.

RESULTS

Levels of vitamin D3 in farm and in balady eggs

The obtained findings in the current study showed the differences between vitamin D_3 content in two major categories of hen's eggs purchased in Assiut city, Egypt including farm and balady eggs. The data presented in Table 1 illustrated that the level of vitamin D_3 in farm eggs, which was ranged from 0.13 to 8.50 µg/100g with an average of 2.03±0.52 µg/100g of the examined sample, while in balady eggs the content of such vitamin was between 0.0.1 and 5.69 µg/100g and the mean value was 1.04±0.49 µg/100g. Vitamin D_3 was higher in farm eggs in comparison to balady eggs however the difference was not significant ($p > 0.05$)

Table 1. Content of Vitamin D_3 in the examined samples (balady eggs and farm eggs).

Groups	Examined samples (No.)	Level of vitamin D3 (µg/100g)
Farm eggs	15	2.03±0.52
Balady eggs	15	1.04±0.49
Total	30	1.53±0.36

Data are presented as Mean±SE

Table 2. Level of vitamin D_3 in farm eggs subjected to different cooking methods (boiling and frying) using HPLC.

Cooking methods	Level of vitamin D3 (µg/100g)	
	Before treatment	After treatment
Boiling	2.53±0.77	1.20±0.13
Frying	2.25±0.91	1.48±0.55

Data are presented as Mean±SE

Table 3. True retention of vitamin D_3 in farm eggs after cooking.

Cooking methods	True retention%
Boiling	40.3 - 60.8
Frying	64.24 - 69.4

Effect of heat treatment on content of vitamin D_3 in table eggs

The results for the retention of vitamin D_3 in the cooked eggs (boiled eggs and fried eggs) were presented in Tables 2 and 3. This part was applied using samples of farm eggs due to the higher level of vitamin D_3 in such kind of eggs than the other one. The boiling process could reduce the content of vitamin D_3 from 2.53±0.77 µg/100g in raw shell egg to 1.20±0.13 µg/100g in boiled eggs. While, frying decreased such vitamin to 1.48±0.55 µg/100g in the fried eggs. Concerning the true retention percentage of vitamin D_3 after cooking; it was ranged from 40.3 to 60.8% and, between 64.24 and 69.4% for boiling and frying procedures, respectively (Table 3). On average, there was a significant difference ($p < 0.05$) in retention rate of vitamin D_3 between boiling (47.43%) and frying (65.78%) methods.

DISCUSSION

Dietary vitamin D may compensate the inadequate exposure to sun lights. There have been few previous investigations on vitamin D_3 content in Egyptian eggs, which used as a base for the current study to present new data for the level of vitamin D_3 in the most consumed table eggs in Egypt.

In the present study, the level of vitamin D_3 was higher in farm eggs than in balady eggs. This difference may be due to several factors such as the breeds of laying hens and vitamin D_3 supplementation in poultry ration. Especially in farms, vitamin and mineral supplementations are one of the basic constituents of poultry feed to enhance the productivity and avoid outbreaks of vitamin and mineral deficiency. Yao *et al*. (2013) found that the content of vitamin D_3 in table eggs increased in a dose-dependent manner by the addition of vitamin D_3 in poultry feed. Furthermore, exposure to sunlight could also affect the level of such vitamins. Vitamin D_3 content usually higher in chickens reared outside cages than that stayed inside cages as reported previously by Roseland *et al*. (2018).

The current result agreed with Dunlop *et al*. (2017) who measured the level of vitamin D_3 in raw free-range egg in Australia and demonstrated that vitamin D_3 content in raw free-range eggs was ranged from 0.3 - 2.4 µg/100g with an average of 1.3 µg/100g. However, a lower level of the vitamin was indicated in caged eggs (average 0.6 µg/100g). On the other hand, a higher level of vitamin D_3 was determined by Mattila *et al*. (2011) who analyzed pooled egg yolk samples from commercial chicken eggs in autumn (4 µg/100g); while in spring, it was 4.9 µg /100g. Moreover, Schmid and Walther (2013) reported that the level of vitamin D_3 in egg yolk ranged between 3.2 and 5.5 µg/100g in Switzerland city. Lower level of vitamin D_3 was measured in Alexandria, Egypt in table eggs by Aborhyem *et al*. (2020) who found that the mean value of such vitamin was 0.161µg/100g. In this study, vitamin D_3 content in the examined egg yolk differed widely between samples; this could reflect the variation in nourishment, species and the production practices due to water clearness, season and location (Mattila *et al*., 2011).

After exposing the egg samples to different cooking methods (boiling and frying), the obtained result illustrated that the highest loss in vitamin D_3 content was observed after frying than boiling. Hence, it is recommended to use boiling method in cooking of eggs to avoid the excessive loss of such vitamins. This finding was in harmony with Mattila *et al*. (1999) who found that when egg exposed to boiling for 10 min, vitamin D_3 content decreased when compared with raw egg. Additionally, Dunlop *et al*. (2017) measured the retention rate of vitamin D_3 during cooking and observed that the reduction percentage was ranged from 39 – 104 % in free range egg and 36 – 113 % in cage eggs. In contrast, Indyk *et al*. (1996), and Schmid and Walther (2013) stated that heat treatment of milk and eggs with ultra-heat treatment, sterilization or pasteurization does not provoke a significant loss of vitamin D_3 level.

CONCLUSION

The results obtained from this study indicate that content of vitamin D_3 is higher in farm eggs than in balady eggs. Cooking of egg by boiling and frying could be implicated in excessive loss in the content of vitamin D_3. Besides, the stability of such vitamin is obvious in cooked eggs than in frying. Hence, it is suggested to use boiled farm eggs as a supplementary source of vitamin D_3.

CONFLICT OF INTEREST

The authors have no conflict of interests to disclose.

REFERENCES

Aborhyem, S., Hamza, D., Agamy, N., 2020. Vitamin D Content in Some Food Items Sold in Egyptian Markets. Canadian Journal of Clinical Nutrition 8, 62-77.

Barnkob, L.L., Argyraki, A., Jakobsen, J., 2020. Naturally enhanced eggs as a source of vitamin D: A review. Trends in Food Science & Technology 102, 62-70.

Bishop, E., Ismailova, A., Dimeloe, S.K., Hewison, M., White, J.H., 2020. Vitamin D and immune regulation: antibacterial, antiviral, anti-inflammatory. Journal of Bone and Mineral Research plus 5, e10405.

Demir, M., Demir, F., Aygun, H., 2021. Vitamin D deficiency is associated with COVID-19 positivity and severity of the disease. Journal of medical virology 93, 2992-2999.

Dimartino, G., 2019. Simultaneous Determination of Cholecalciferol (Vitamin D3) and Ergocalciferol (Vitamin D_2) in Foods by Selected Reaction Monitoring. Journal of AOAC International 92, 511-517.

Dunlop, E., Cunningham, J., Sherriff, J.L., Lucas, R.M., Greenfield, H., Arcot, J., Strobe, N., Black, L.J., 2017. Vitamin D_3 and 25-Hydroxyvitamin D_3 Content of Retail White Fish and Eggs in Australia. Nutrients 9, 647.

EFSA Panel on Dietetic Products N and Allergies., 2016. Dietary reference values for vitamin D. 14, e04547.

Entrenas Castillo, M., Entrenas Costa, L.M., Vaquero Barrios, J.M., Alcalá Díaz, J.F., López Miranda, J., Bouillon, R., Quesada Gomez, J.M., 2020. Effect of calcifediol treatment and best available therapy versus best available therapy on intensive care unit admission and mortality among patients hospitalized for COVID-19: A pilot randomized clinical study". The Journal of steroid biochemistry and molecular biology 203, 105751-105751.

Farag, A.M., Rizk, M.S., Youssif, M.H., El-Bassel, H.A., 2018. Determination of Vitamin D3 Content in High, Low and Zero Fat Food Using High Performance Liquid Chromatography. The Medical Journal

of Cairo University 86, 3911-3918.
Foote, M.R., Horst, R.L., Huff-Lonergan, E.J., Trenkle, A.H., Parrish Jr, F.C., Beitz, D.C., 2004. The use of vitamin D_3 and its metabolites to improve beef tenderness. Journal of Animal Science 82, 242-249.
Gupta, S.,Tripathim A., 2015. An Improved and Sensitive Method for Vitamin D3 Estimation by RP-HPLC. Pharmaceutica Analytica Acta 6, 8.
Hanson, A.L., Metzger, L.E., 2010. Evaluation of increased vitamin D fortification in high-temperature, short-time-processed 2% milk, UHT-processed 2% fat chocolate milk, and low-fat strawberry yogurt. Journal of Dairy Science 93, 801-807.
Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.
Indyk, H.E., Littlejohn, V., Woollard, D.C.J.F.C., 1996. Stability of vitamin D_3 during spray-drying of milk. Food Chemistry 57, 283-286.
Jakobsen, J., Knuthsen, P., 2014. Stability of vitamin D in foodstuffs during cooking. Food Chemistry 148, 170-175.
Karahan, S., Katkat, F., 2021. Impact of Serum 25(OH) Vitamin D Level on Mortality in Patients with COVID-19 in Turkey. The journal of nutrition, health & aging 25, 189-196.
Lešková, E., Kubíková, J., Kováčiková, E., Košická, M., Porubská, J., Holčíková, K., 2006. Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. Journal of Food Composition and Analysis 19, 252-276.
Madsen, K.H., Rasmussen, L.B., Andersen, R., Mølgaard, C., Jakobsen, J., Bjerrum, P.J.,, Andersen, E.W., Mejborn, H., Tetens, I., 2013. Randomized controlled trial of the effects of vitamin D–fortified milk and bread on serum 25-hydroxyvitamin D concentrations in families in Denmark during winter: the VitmaD study. American Journal of Clinical Nutrition 98, 374-382.
Mandrioli, M., Boselli, E., Fiori, F., Rodriguez-Estrada, M.T., 2020. Vitamin D3 in High-Quality Cow Milk: An Italian Case Study. Foods 9, 548.
Mason, R.S., Sequeira, V.B., Gordon-Thomson, C., 2011. Vitamin D: the light side of sunshine. European Journal of Clinical Nutrition 65, 986-993.
Mattila, P., Lehikoinen, K., Kiiskinen, T., Piironen, V., 1999. Cholecalciferol and 25-hydroxycholecalciferol content of chicken egg yolk as affected by the cholecalciferol content of feed. Journal of Agricultural and Food Chemistry 47, 4089-4092.
Mattila, P., Valkonen, E., Valaja, J., 2011. Effect of Different Vitamin D Supplementations in Poultry Feed on Vitamin D Content of Eggs and Chicken Meat. Journal of Agricultural and Food Chemistry 59, 8298-8303.
Natri, A-M., Salo, P., Vikstedt, T., Palssa, A., Huttunen, M., Kärkkäinen, M.U., Salovaara, H., Piironen, V., Jakobsen, J., Lamberg-Allardt, C.J., 2006. Bread Fortified with Cholecalciferol Increases the Serum 25-Hydroxyvitamin D Concentration in Women as Effectively as a Cholecalciferol Supplement. The Journal of Nutrition 136, 123-127.
Principi, N., Bianchini, S., Baggi, E., Esposito, S., 2013. Implications of maternal vitamin D deficiency for the fetus, the neonate and the young infant. European Journal of Nutrition 52, 859-867.
Roseland, J.M., Phillips, K.M., Patterson, K.Y., Pehrsson, P.R., Taylor, C.L., 2018. Chapter 60 - Vitamin D in Foods: An Evolution of Knowledge. In: Feldman D (ed) Vitamin D (Fourth Edition). Academic Press, pp. 41-77.
Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., 2011. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. The Journal of Clinical Endocrinology & Metabolism 96, 53-58.
Saghafi, Z., Nikooyeh, B., Jamali, A., Mehdizadeh, M., Zargaraan, A., 2018. Influence of Time and Temperature on Stability of Added Vitamin D_3 During Cooking Procedure of Fortified Vegetable Oils. Nutrition and Food Sciences Research 5, 43-48.
Schmid, A., Walther, B., 2013. Natural vitamin D content in animal products. Advances in Nutrition (Bethesda, Md.) 4, 453-462.
Wagner, D., Rousseau, D., Sidhom, G., Pouliot, M., Audet, P., Vieth, R., 2008. Vitamin D_3 Fortification, Quantification, and Long-Term Stability in Cheddar and Low-Fat Cheeses. Journal of Agricultural and Food Chemistry 56, 7964-7969.
Yao, L., Wang, T., Persia, M., Horst, R.L., Higgins, M., 2013. Effects of Vitamin D_3-Enriched Diet on Egg Yolk Vitamin D_3 Content and Yolk Quality. Journal of Food Science 78, C178-C183.