The Impact of the Use of Freeze-Dried Eggs on some Liver Bioindicators and Histological in Cirrhosis Male Albino Rats upon feed it in their diets

تأثير استخدام البيض المجفف على بعض المؤشرات الحيوية والنسيجية للكبد في حالات تليف الكبد لدى ذكور الجرذان البيضاء عند تغذيته في وجباتها الغذائية

by

[1] Eman Ahmed

Supervised

Ahmed abdelfattah Aly* Ahmed Mohamed Hamoda**

ABSTRACT

This investigation aims to study the protective effects of freeze-dried white, yolk and whole eggs on induced cirrhosis for male albino rats. White and yolk eggs separated from whole eggs and dried with freeze-drier to obtain three treatments (Freeze-dried white, yolk and whole eggs powders). Proximate chemical composition examined for freeze-dried white, yolk and whole eggs powders. Twenty five male albino rats divided to five groups: G_1 control group of rats feed on basal diet, G_2 group of rats with hepatotoxicity with CCl_4 fed on basal diet, G_3 group of rats with abnormal liver function fed on Freeze-dried white egg. G_4 group of rats with abnormal liver function fed on Freeze-dried yolk egg. G_5 group of rats with abnormal liver function fed on Freeze-dried whole egg. The results showed that GPT and GOT enzymes of male albino rats

[1] Researcher at benha university.

* Department of Home Economics, Faculty of Specific Education, Benha University, Benha, Egypt, Food Technology.

** AgricultureResearch center- Food Technology Research Institute.

reduced for G_3 group of rats followed by G_5 group of rats followed by G_4 group of rats compared with G_2 group of rats with hepatotoxicity with CCl4. The deceleration observed in the physiological indicators caused by specific protein variations in. Histological examination of liver of rats under investigation takes the same manner. It can be concluded that use the freeze-dried white egg for diet enhance and improve the biological and histological parameters for rats fed it.

Keywords: Freeze-dried eggs, Biological and Histopathology parameters, and Cirrhosis rats.

Practical Application: Freeze-dried eggs show promise in improving liver function for rats with induced cirrhosis, with reduced enzyme levels and positive physiological and histological effects.

Introduction

The consumption of chicken eggs is a fundamental part of the global human diet. In recent times, there has been a notable increase in the utilization of different components of eggs in various food products like cakes, desserts, confectionaries, pies, and powdered soups. The whole egg consists of three main parts: the shell, the yolk, and the white (**Chang et al., 2018**).The composition of the entire egg includes proteins, water, carbohydrates, fats, ash, and cholesterol. The egg yolk is a valuable ingredient in food due to its nutritional qualities, such as essential fatty acids, vitamins, minerals, and phospholipids, as well as its sensory properties (**Anton, and Rorres 2013**). It mainly comprises water, protein, lipids, and carbohydrates. Egg white, which constitutes the majority of the egg's volume, is composed of water, proteins, carbohydrates, ash, and lipids. Ovalbumin, ovotransferrin, ovomucoid,

ovomucin, lysozyme, globulin, and avidin are some of the proteins found in egg white, each possessing unique structures and functional properties. These egg white proteins are widely used in the food industry for their essential amino acid content, high bioavailability, and exceptional functionality (**Campbell, et al., 2003**). Eggs offer the benefits of being nutritious and affordable, containing essential amino acids required by humans. They are widely consumed as a daily food source and have been shown to possess bioactive compounds that contribute to treating and preventing chronic diseases. Studies indicate that whole eggs, as opposed to just egg whites, have a more positive impact on individuals with diabetes, leading to decreased blood sugar levels, improved blood lipids, and reduced blood pressure (**Diez-Espino, et al., 2017**). However, excessive consumption of egg yolks can negatively affect blood cholesterol levels that have the potential to modulate the body's immune status (**Benede & Molina, 2020**). However, there is limited research on the specific immunomodulatory effects of egg whites versus egg yolks. Understanding the synergistic effects of egg whites and yolks in their entirety is crucial for developing appropriate dietary guidelines for immunocompromised individuals. This study aims to assess the immunomodulatory effects of three different components (egg white, egg yolk, and whole egg) on immunocompromised mice induced by cyclophosphamide (CY). The findings will provide valuable insights into enhancing immunity through dietary choices. The use of egg yolk has gained significant popularity in the food industry due to its rich nutritional content and functional properties (**Primacella et al., 2020**). Egg yolk is a complex combination of water, lipids, and proteins,

constituting approximately 50%, 30%, and 16% respectively (**Wang et al., 2020**), particularly for individuals with hyperlipidemia (**Spence et al., 2012**). Interestingly, the consumption of both egg whites and yolks in the form of whole eggs does not raise cholesterol levels in obese patients. Furthermore, egg consumption has been associated with a decreased risk of diabetes and atherosclerosis **(Krittanawong et al., 2021**). Whole eggs have also been found to aid in weight loss for obese patients, suggesting a synergistic effect between egg whites and yolks in promoting physical health (**Wright et al., 2018**). While both egg yolks and whites are easily digested proteins, they differ significantly in their nutrient composition. Egg whites are rich in ovalbumin, while yolks contain yolk protein, neutral lipids, phospholipids, fat-soluble vitamins, and micronutrients. Researchers have explored the health-promoting properties of various egg components as bioactive substances **(Moreno-Fernández et al., 2020**). Eggs have been shown to contain several biologically active substances. The separation of two key components of egg yolk, namely plasma and granules, can be easily achieved through a simple centrifugation process. The plasma, accounting for about 78% of the dry matter in egg yolk, encompasses 50% of yolk proteins and 90% of yolk lipids, including carotenoids. It primarily consists of 85% LDL and 15% yolk livetin. On the other hand, granules constitute 22% of the dry matter and consist of approximately 16% phosvitin, 70% high-density lipoprotein (HDL), and 12% low-density lipoprotein (LDL) (**Li et al., 2021**). Due to concerns surrounding the elevated cholesterol levels in eggs and their potential adverse effects on lipid profiles, eggs have not been recommended for individuals

with a higher risk of heart disease. However, recent research has indicated that egg consumption does not actually lead to an increase in total cholesterol or LDL cholesterol levels. In fact, it may enhance the cholesterol efflux capacity of HDL particles. This finding is of particular importance for postmenopausal women, who have a greater risk of heart disease compared to premenopausal women. In a previous study involving overweight, mildly hypercholesterolemic postmenopausal women, it was discovered that consuming two eggs per day improved the cholesterol efflux capacity of HDL particles without causing any changes in lipid profiles or other cardiometabolic risk factors. Hypothesis was that the intake of two eggs per day would raise plasma TMAO concentration by affecting the composition of the gut microbiome in postmenopausal women. We recruited twenty overweight to obese, mildly hypercholesterolemic yet otherwise healthy women for this randomized cross-over study (**Zhu, et al., 2020**). The objective of this study is to explore the potential benefits of freeze-dried eggs by harnessing their bioactive compounds to improve the biological parameters in rats consuming them. Additionally, the research aims to assess the impact of incorporating these freeze-dried eggs on various biological indicators and to conduct histological examinations on male albino rats fed with eggs.

2. Materials and method

2.1. Materials

Eggs were sourced from the nearby market, while the chemicals utilized in the research were acquired from Al-Amiriya, Cairo, Egypt, through the Arab Company for Pharmaceuticals and Chemical Industries

2.1.1. Preparing Freeze-dried eggs powder

Initially, eggs were acquired from the market and their weight was measured. Subsequently, the eggs were carefully separated into whites, yolks, and whole eggs, each type placed in distinct trays containers. These containers were then frozen at -40 ± 3 ˚C for preservation purposes. Next, the whites, yolks, and whole eggs were subjected to a drying or lyophilizing process using a specialized device (Zirbus Technology, S/N: COM98754, model: VaCo 5-D, Germany). The primary objective of employing this device was to transform the whites, yolks, and whole eggs into a powdered form, which could be preserved for approximately three days. Once the drying process was complete, the powdered eggs were weighed to compare their weight before and after drying. The resulting powder was then placed in plastic bags to serve as feed for the mice during the biological experiments as the methods of **Aly et al. (2022)**.

2.2. Analytical methods

2.2.1. Chemical analysis of freeze-dried eggs

The analysis of freeze-dried egg samples included the determination of moisture, total lipids, ash and crude protein using the **AOAC method (2019)**. To calculate total carbohydrates, the approach described by **Aly et al. (2021a)** was employed, which involves taking the difference of 100 and the sum of moisture, ash, protein, and fat percentages.

2.2.2. Biological examination

2.2.2.1. Animals

Twenty-five male albino rats weighing approximately 250 ± 10 g were utilized for the biological assessment conducted at the Animal House Agricultural Research Center in Giza, Egypt.

The rats were organized into five groups: G_1, consisting of male albino rats fed only on the basal diet as a negative control; G_2, composed of male albino rats with hepatotoxicity induced by CCl_4 and fed on the basal diet; G_3, consisting of male albino rats with abnormal liver function fed with white egg; G_4, comprising male albino rats with abnormal liver function fed with yolk egg; and G_5, including male albino rats with abnormal liver function fed with whole egg. Liver cirrhosis was induced in the rats by injecting Carbon tetrachloride (CCl_4) and paraffin oil at 5% V/V (2 ml/kg by weight) with the first dose administered at the beginning of the week and the second dose in the middle of the week, following the method described by **Aly et al. (2021c)**.

2.2.2.2. Separation of serum blood samples and organs

At the end of the experimental feeding period, blood samples were extracted from the rats following the procedure outlined by **Aly et al. (2022)**. The rat livers were subsequently isolated, cleaned, and placed in a 10% formalin solution for preservation until histopathological examination.

2.2.2.3. Biological determination

The levels of various parameters in rats fed with freeze-dried eggs were examined using the method described by **Aly et al., (2021b)**. The parameters included liver enzymes (GOT and GPT).

2.2.3. Statistical analysis

The method employed for statistical analyses was carried out in accordance with **Ismail et al., (2020)**, utilizing one-way analysis of variance (ANOVA).

3. Results and dissection

3.1. Proximate composition freeze-dried eggs samples

Table 1 illustrates the comparative analysis of the chemical composition of freeze-dried egg samples. The moisture content in egg whites was observed to be higher than that in yolk eggs, with a ratio of 7.19±0.25% for egg whites and 2.07±0.57% for yolk eggs. The total lipids in egg whites were lower than those in yolk eggs, with percentages of 2.66±0.14% and 33.00±1.63%, respectively. The ash content also varied, being 5.75±0.34% for egg whites and 3.95±0.36% for yolk eggs, likely attributed to the composition of whole eggs. Notably, egg white protein showed a higher concentration, at 82.58±0.05%, compared to yolk eggs, which had a protein ratio of 55.18±0.85%. While whole eggs, their chemical composition was found to be 3.70±0.20 % moisture, 25.83±1.43% fat, 4.37±0.44% ash, and 64.66±0.87% protein.

These findings align with the research conducted by **Campbell et al., (2003)** they both noted that the composition of egg white primarily includes water (88%), protein (10.5%), carbohydrate (0.5%), ash (0.8%), and lipids (0.2%). Similarly, indicated that the egg yolk is a complex amalgamation of water, lipids, and proteins, comprising roughly 50%, 30%, and 16% respectively. Notably, **Wang et al. (2020)** emphasized that egg yolk, recognized as the most nutrient-dense portion of the egg, contains approximately 50% water, 30% lipids, 16% protein, along with minor quantities of carbohydrates and minerals. Hen eggs, as a cost-effective and nutrient-rich dietary option, have been and continue to be widely consumed globally (**Lesnierowski & Stangierski, 2018**). This same foundation is supported by **Li et al., (2021)** reported that the egg yolk, with its composition of 51.1% water, 16% proteins, 30.6% lipids, 1.7% minerals, and 0.6% carbohydrates, emerges as a valuable

source of protein, providing well-balanced amino acids essential for human protein metabolism.

Table 1 Chemical composition of freeze-dried eggs samples

Constituents (%)	Samples		
	Freeze-dried white egg	Freeze-dried yolk egg	Freeze-dried whole egg
Moisture	7.19±0.25	2.07±0.57	3.70±0.20
Total lipids*	2.66±0.14	33.00±1.63	25.83±1.43
Crud protein*	82.58 ± 0.05	55.18±0.85	64.66±0.87
Ash*	5.75±0.34	3.95±0.36	4.37±0.44

* Data are indicated as: average for three replicates ± standard deviation (SD), % on dry weight basis.

3.2. Effect of feeding with freeze-dried eggs treatments for 2 months on GPT, GOT of male albino rats

Data presented in the table 2 revealed the effect of feeding rats with freeze-dried eggs on the serum level of alanine amino transferase (GPT/ALT) enzyme. It could be noticed from the present table that rats without treatment, positive group G2 the mean value of GPT enzyme was 50.66±1.24 U/L while the normal rats G1 group was 18.33±2.05 U/L and showed a significant differences at ($p \leq 0.05$). G3, G4, and G5 showed an improvement in GPT enzyme level, meanwhile G4 which rats were fed on Freeze-dried yolk egg recorded the highest score, while G3 which rats were fed on Freeze-dried white egg and recorded the lowest level in serum GPT compered other treatments groups. Data presented in same table 2 revealed the without treatment positive groupG2 indicated enzyme activity as 46±3.26 U/L while negative group G1 was 28±3.74 U/L. For

rats fed on treatments G3, G4, and G5 it could be noticed that the mean value of serum GOT of these showed significant difference and special rats in G3 recorded the lowest level in serum GOT compered to all other treatments groups.

Table 2 Effect of feeding with eggs treatments for 2 months on (GPT/ALT) and (GOT/AST) enzymes.

Groups of male albino rats	Parameters	
	GPT/ALT(U/L)	GOT/AST (U/L)
G1	18.33±2.05	28±3.74
G2	50.66±1.24	46±3.26
G3	29±1.63	30±5.09
G4	36.66±3.85	38±8.60
G5	30.3±14.35	35.56±28.76

G: Group; %Var.: Variation; Different within column show significant differences between values ($p < 0.05$). Data are indicated as: average for three replicates ± standard deviation (SD).

G_1: Group of male albino rats fed on basal diet only as a negative control.

G_2: Group of male albino rats with hepatotoxicity with CCl4 fed on basal diet.

G_3: Group of male albino rats with abnormal liver function fed on with Freeze-dried white egg
G_4: Group of male albino rats with abnormal liver function fed on Freeze-dried yolk egg
G_5: Group of male albino rats with abnormal liver function fed on Freeze-dried whole egg

3.3. Examination of rat hepatic cells subjected to freeze-dried egg consumption (H & E X 400)

Figures 1-4 illustrate photomicrographs of rat liver samples from the studied groups (Groups 1-5). In the negative control group (G1), consisting of male albino rats fed solely on a basal diet, the liver presents a typical portal area with a regular portal vein, bile duct, and portal artery. The adjacent hepatocytes exhibit normal characteristics, including typical nuclei.

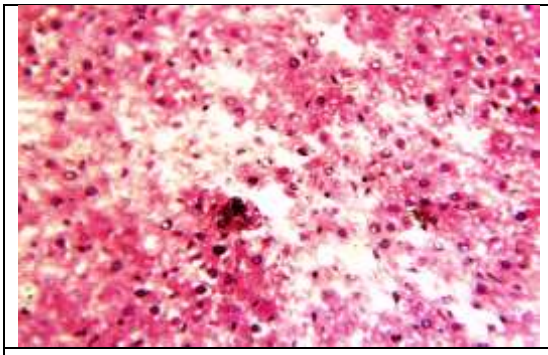
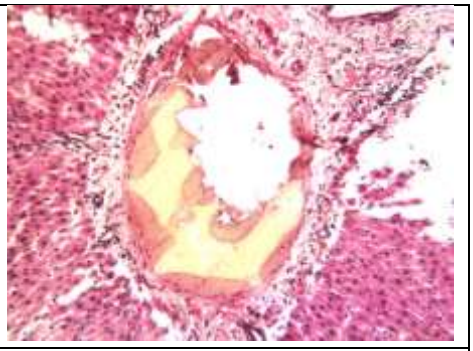
Moving on to the group of male albino rats with hepatotoxicity induced by CCl4 and fed on a basal diet (G2), the liver reveals a portal vein containing a thrombus blockage (indicated by a blue arrow) accompanied by iron deposits resulting from hemochromatosis. These iron deposits are observed within the vein's wall and at its center, as marked by black arrows.

In the group of male albino rats with abnormal liver function fed on freeze-dried white egg (G3), the liver displays emboli suspected to be fat emboli within the portal vein (blue arrow), along with hepatocytes containing iron deposits due to hemochromatosis. A black arrow points to an area with necrotic surrounding hepatocytes.

For the group of male albino rats with abnormal liver function fed on freeze-dried yolk egg (G4), the liver exhibits a shrunken portal area surrounded by fibrosis, and hepatocytes have undergone necrosis.

Finally, in the group of male albino rats with abnormal liver function fed on freeze-dried whole egg (G5), the central vein is observed with occluded thrombi, accompanied by hemochromatosis and a slight precipitation of iron within the vein's wall and center.

In summary, the histopathological examination of rat hepatic cells fed freeze-dried eggs suggests positive impacts on rats due to their nutritional ingredients. These findings align with data from **Diez-Espino et al. (2017)**, which reported that whole eggs, as opposed to just egg whites, have a more positive impact on individuals with diabetes, leading to decreased blood sugar levels, improved blood lipids, and reduced blood.

	
The histopathological analysis of the livers from male albino rats in the control group, which were exclusively fed a basal diet, revealed a negative result. The examination showed a portal area with a normal portal vein, bile	Histological analysis of the livers from a cohort of male albino rats subjected to hepatotoxicity induced by CCl4 and fed a basic diet reveals findings of a thrombus blocking the portal

duct, and portal artery. The hepatocytes in the surrounding area appeared normal, exhibiting regular nuclei (H & E X 400).	vein (indicated by the blue arrow). Additionally, there is evidence of hemochromatosis, with iron deposits observed in both the vein wall and the central region (indicated by black arrows) under an H & E stain at 400x magnification.

Figure 1Histopathological analysis of the livers in two groups: male albino rats on a standard diet and rats with hepatotoxicity induced by CCl4, also fed on a standard diet.

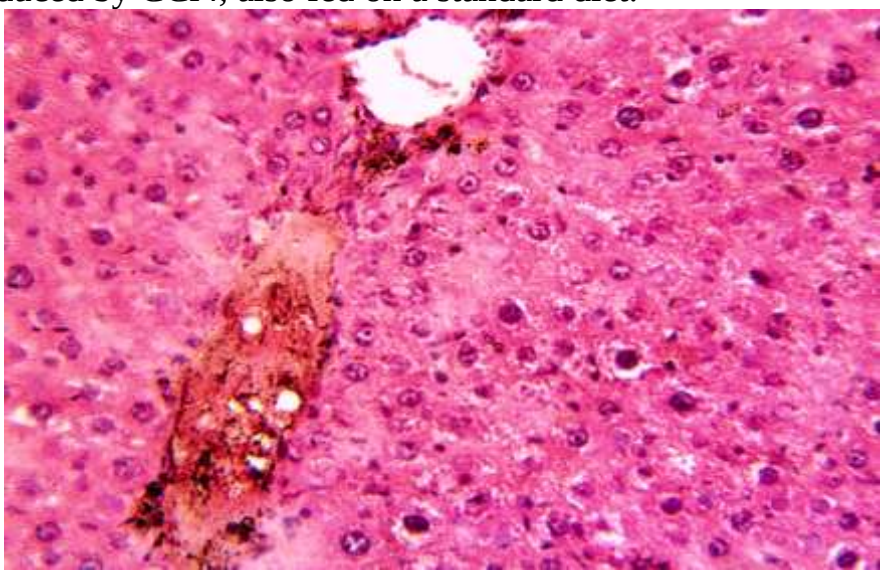


Figure 2 displays the histopathological analysis of the livers in a group of male albino rats exhibiting impaired liver function.

These rats were fed freeze-dried white egg. The examination reveals the presence of emboli, potentially fat emboli, in the portal vein (indicated by the blue arrow). Additionally, there is evidence of hemochromatosis in hepatocytes, characterized by iron deposition (depicted in parentheses). The black arrow points to necrotic hepatocytes surrounded by necrotic tissue (H & E X 400).

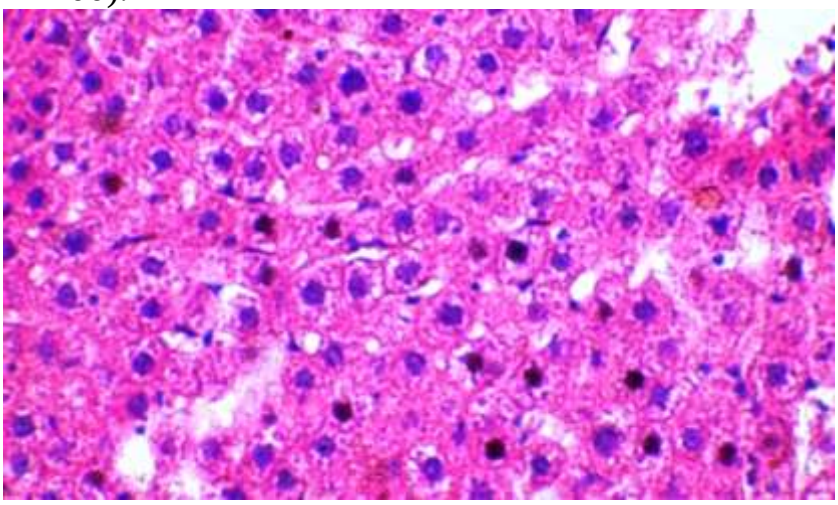


Figure 3 Histopathological analysis of the livers from a group of male albino rats with impaired liver functions, which were fed freeze-dried yolk egg. The image reveals a diminished portal area surrounded by fibrosis and necrotic hepatocytes (H & E X 400).

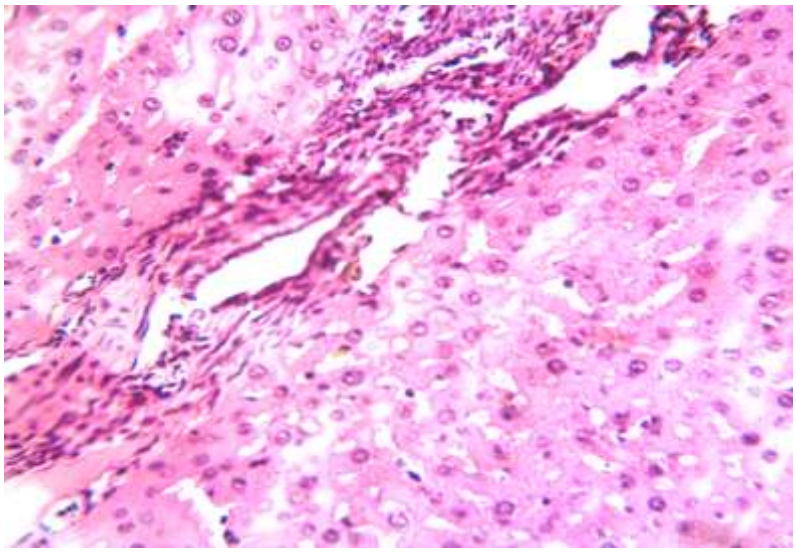


Figure 4 Histopathological analysis of the livers from male albino rats with impaired liver function, who were fed freeze-dried whole egg, revealed findings in Figure 4. The examination displayed occluded thrombi in the central vein, along with evidence of hemochromatosis. Additionally, there was a mild presence of iron precipitates observed in both the vein wall and its central region (H & E 400X)

4. Conclusions

In this investigation, eggs were processed and their weights were measured for. Next, the eggs were divided into whites, yolks, and whole eggs. Subsequently, the eggs underwent freeze-drying for 72 hours, transforming them into powder form and storing them in plastic bags and analyzed for chemical composition. Twenty-five male albino rats were then prepared and divided into five groups. These rats received injections of substance CCL_4 to examine the impact of egg whites, egg yolks, and whole eggs on their liver properties. The first group received a normal diet, the second group served as

the control and received injections without egg consumption, and the third group was fed with egg whites, the fourth with egg yolks, and the fifth group with whole eggs. The experiment continued for duration of two months. The selected analysis focused on various indicators, including GOT and GPT. The key finding revealed that the rat group fed on egg whites exhibited lower levels of GOT and GPT of blood serum compared to the other groups, specifically the fourth group fed on yolks and the fifth group fed on whole eggs. This outcome highlighted the significance of egg whites in promoting liver health and nutrition, along with the observation that the livers of rats fed egg whites were lighter compared to the livers of the fourth and fifth groups. Additionally, histological examinations indicated an improvement in liver cells among the rats treated with carbon tetrachloride and fed on egg whites, in contrast to the other samples.

Acknowledgements

The authors extend their appreciation to the Benha University, Egypt.

References

Aly, A. A., Ali, I. M., Khalil, M., Hameed, A. M., Alrefaei, A. F., Alessa, H., . . . Ammar, M. S. (2021a). Chemical, microbial and biological studies on fresh mango juice in presence of nanoparticles of zirconium molybdate embedded chitosan and alginate. *Arabian Journal of Chemistry, 14*(4), 103066. doi:https://doi.org/10.1016/j.arabjc.2021.103066

Aly, A. A., El-Deeb, F. E., Abdelazeem, A. A., Hameed, A. M., Abdulaziz Alfi, A., Alessa, H., & Alrefaei, A. F. (2021b) . Addition of Whole Barley Flour as a Partial Substitute of Wheat Flour to Enhance the Nutritional Value of Biscuits.

Arabian Journal of Chemistry, 14(5), 103112. doi:https://doi.org/10.1016/j.arabjc.2021.103112

Aly, A. A., Zaky, E. A., Khatab, N. R ،.Hameed, A. M., & Kadasah, S. (2022). The Biological and Chemical Ameliorative Effects of Bread Substituted with Dried Moringa Leaves. *Arabian Journal of Chemistry, 15*(7), 103875. doi:https://doi.org/10.1016/j.arabjc.2022.103875

Aly, A. A., Zaky, E. A., Mahmoud, H. A., Alrefaei, A. F., Hameed, A. M., Alessa, H., . . . Kadasah, S. (2021c). The Impact of Addition Oats (Avena sativa) and Cinnamon on Cookies and their Biological Effects on Rats Treated with Cirrhosis by CCL4. *Saudi Journal of Biological Sciences, 28*(12), 7142-7151. doi:https://doi.org/10.1016/j.sjbs.2021.08.010

Anton, H., & Rorres, C. (2013). *Elementary linear algebra: applications version*: John Wiley & Sons.

AOAC. (2019). Official Methods of Analysis (OMA). Association of Official Analytical Chemists. *Official Methods of Analysis. AOAC, Washington, DC, USA* .

Benedé, S., & Molina, E. (2020). Chicken egg proteins and derived peptides with antioxidant properties. *Foods, 9*(6), 735 .

Campbell, L., Raikos, V., & Euston, S. R. (2003). Modification of functional properties of egg-white proteins. *Food/Nahrung, 47*(6), 369-376 .

Chang, C., Lahti, T., Tanaka, T., & Nickerson, M. T. (2018). Egg proteins: Fractionation, bioactive peptides and allergenicity. *Journal of the Science of Food and Agriculture, 98*(15), 5547-5558 .

Díez-Espino, J., Basterra-Gortari, F. J., Salas-Salvadó, J., Buil-Cosiales, P., Corella, D., Schröder, H., . . . Arós, F. (2017). Egg

consumption and cardiovascular disease according to diabetic status: The PREDIMED study. *Clinical Nutrition, 36*(4), 1015-1021 .

Ismail, H. A., Hameed, A. M., Refaey, M. M., Sayqal, A., & Aly, A. A. (2020). Rheological, physio-chemical and organoleptic characteristics of ice cream enriched with Doum syrup and pomegranate peel. *Arabian Journal of Chemistry, 13*(10), 7346-7356. doi:https://doi.org/10.1016/j.arabjc.2020.08.012 .

Krittanawong, C., Rogers, A. J., Johnson, K. W., Wang, Z., Turakhia, M. P., Halperin, J. L., & Narayan, S. M. (2021). Integration of novel monitoring devices with machine learning technology for scalable cardiovascular management. *Nature Reviews Cardiology, 18*(2), 75-91 .

Li, J., Zhai, J., Gu, L., Su, Y., Gong, L., Yang, Y., & Chang, C. (2021). Hen egg yolk in food industry - A review of emerging functional modifications and applications. Trends in Food Science & Technology, *115*, 12-21. doi:https://doi.org/10.1016/j.tifs.٢٠٢١.٠٦.٠٣١

Lesnierowski, G., & Stangierski, J. (2018). What's new in chicken egg research and technology for human health promotion?-A review. *Trends in food science & technology, 71*, 46-51 .

Moreno-Fernández, S., Garcés-Rimón, M., & Miguel, M. (2020). Egg-derived peptides and hydrolysates: a new bioactive treasure for cardiometabolic diseases. *Trends in Food Science & Technology, 104*, 208-218 .

Primacella, M., Acevedo, N. C., & Wang, T. (2020). Effect of freezing and food additives on the rheological properties of egg

yolk. *Food Hydrocolloids*, *98*, 105241. doi:https://doi.org/10.1016/j.foodhyd.2019.105241

Spence, J. D., Jenkins, D. J., & Davignon, J. (2012). Egg yolk consumption and carotid plaque. *Atherosclerosis*, *224*(2), 469-473 .

Wang, G., Wan, Z., & Yang, X. (2020). Induction heating by magnetic microbeads for pasteurization of liquid whole eggs. *Journal of Food Engineering*, *284*, 110079. doi:https://doi.org/10.1016/j.jfoodeng.2020.110079

Wright, C. S., Zhou, J., Sayer, R. D., Kim, J. E., & Campbell, W. W. (2018). Effects of a high-protein diet including whole eggs on muscle composition and indices of cardiometabolic health and systemic inflammation in older adults with overweight or obesity: a randomized controlled trial. *Nutrients*, *10*(7), 946 .

Zhu, C., Sawrey-Kubicek, L., Bardagjy, A. S., Houts ،H., Tang, X., Sacchi, R., . . . Zivkovic, A. M. (2020). Whole egg consumption increases plasma choline and betaine without affecting TMAO levels or gut microbiome in overweight postmenopausal women. *Nutrition Research*, *78*, 36-41. doi:https://doi.org/10.1/٠١٦j.nutres.2020.04.002