Extraction and determination of Iraqi boiled Egg Yolk constituents, and characterization of Lecithin.

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Abstract

This study aimed to extract, determine the Iraqi boiled Egg Yolk constituents and characterize the lecithin. The moisture content of the heated egg yolks was (54%). Egg Yolk protein was extracted using absolute ethanol and the calculated protein percent was (31.31%). The remaining egg yolk was deoiled with acetone using AOCS Official Method Ja 4-46 [13], and the calculated oil percent was (6.72 %). Egg Yolk remaining protein was extracted using chloroform / methanol mixture and the calculated remaining protein percent was (5.7%). Finally Lecithin content was (10.29%). Pure Lecithin was characterized by FT-IR (Shimadzu FT-IR Spectrometer – 30 000:1/ IRAff), U.V-Vis. Analysis (U.V-Vis. Spectrophotometer, Shimadzu 1800), and Powder X-ray diffraction (XRD – 6000 Shimadzu)..

Keywords: boiled Egg Yolk, characterization, Lecithin, constituents, determination.

1. Introduction

An egg yolk is a part of an egg that feeds the developing embryo. The egg yolk is suspended in the egg white (known alternatively as albumen or glair/glair) by one or two spiral bands of tissue called the chalazae. Prior to fertilization, the yolk together with the germinal is a single cell, one of the few single cells that can be seen by the naked eye. As a food, yolks are a major source of vitamins and minerals. They contain all of the egg's fat and cholesterol, and about one-half of the protein [1]. The composition of egg yolk is shown in (Table 1).

Table 1: the composition of egg yolk [8]

Sample preparation	Moisture	Lipids	protein	Ash
Whole yolk	48.7	33.2	16.6	1.5
Yolk after water extraction (pH 5.0)	69.2	20.8	8.9	1.0
Yolk after lipid extraction	10.0	0.8	86.1	3.1

The yolk makes up about 33% of the liquid weight of the egg; it contains approximately 60 calories, three times the caloric content of the egg white. The yolk of one large egg (50 g total, 17 g yolk) contains approximately: 2.7 g protein, 210 mg cholesterol, 0.61 g carbohydrates, and 4.51 g total fat.[2]. All of the fat-soluble vitamins (A, D, E, and K) are found in the egg yolk. Egg yolk is one of the few foods naturally containing vitamin D[3]. The yolk from one large egg contains 2.7 grams of protein. By comparison, the white from the same-sized egg has 3.6 grams of protein. The yolk contains a significant majority of many of the whole egg's nutrients, including calcium, iron, folate, and vitamins B-12.. It also contains all of the egg's fat. You'll get 4.5 grams of total fat, 1.6 grams of saturated fat and 184 milligrams of cholesterol from eating one egg yolk[4,5]. Egg oil also known as egg yolk oil, is derived from the yolk of chicken eggs consisting mainly oftriglycerides with traces of lecithin, cholesterol, xanthophylls such as lutein and zeaxanthin and immunoglobulins. It is free of egg proteins and hence may be used safely by people who are allergic to eggs, for topical applications such as hair and skin care. The product has several historical references in Unani (Greek) medicine for hair care. Chinese traditional medicine uses egg oil for burns, eczema, dermatitis, mouth ulcers, skin ulcers, chapped nipples, tineacapitis, ringworm, nasal vestibulitis, frostbite and hemorrhoids [6]. The fatty acid composition of egg oil is rich in polyunsaturated fatty acids such as omega-3 fatty acids (including docosahexaenoic acid) and omega-6 fatty acids (including arachidonic acid) and closely resembles the fatty acid profile of human milk [7], as well as the lipid profile of human skin. Egg yolk is a source of lecithin as well as egg oil for cosmetic and pharmaceutical applications. Based on weight, egg yolk contains about 9% lecithin.[8].The yellow color is due to lutein and zeaxanthin, which are yellow orange or carotenoids known as xanthophylls.Lecithin is a generic term to designate any group of yellow-brownish fatty substances occurring in animal and plant tissues composed phosphoric acid, choline, fatty acids,of glycerol, glycolipids, triglycerides,and phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol). An example of a phosphatidyl choline, a type of phospholipid in lecithin shown in (Figure 1).

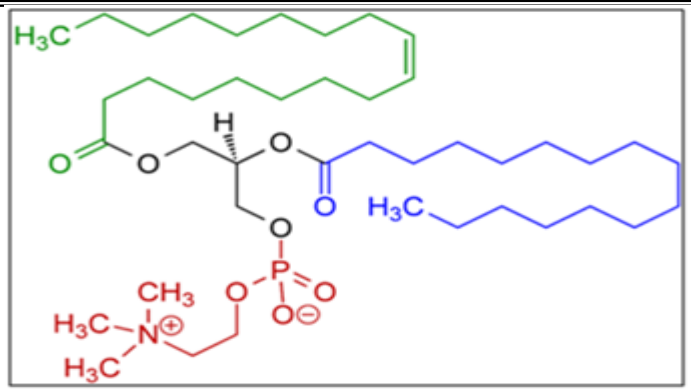

Figure 1. Phosphatidyl choline, a type of phospholipid in lecithin. Red - choline and phosphate group; Black - glycerol; Green - unsaturated fatty acid; Blue - saturated fatty acid.

Lecithin was first isolated in 1846 by the French chemist and pharmacist Theodore Gobley [9]. In 1850, he named the Phosphatidyl choline lecithin [10]. Gobley originally isolated lecithin from yolk Lecithin can easily be extracted chemically (using hexane, ethanol, acetone, petroleum ether, benzene, etc.) or mechanically. It is usually available from sources such soya beans, eggs, milk, marine sources, rapeseed, cottonseed, and sunflower. It has low solubility in water, but is an excellent emulsifier. In aqueous solution, its phospholipids can form liposomes, bilayer sheets, micelles, or lamellar structures, depending on hydration and temperature. This results in a type of surfactant that usually is classified as amphipathic. Lecithin has emulsification and lubricant properties, and is a surfactant. It can be totally metabolized (see Inositol) by humans, so is well tolerated by humans and nontoxic when ingested; some emulsifiers can only be excreted via the kidneys. Composition of lecithin was shown in (Table 2) [11].

Table 2. Lecithin Composition.

Lecithin Composition							
Phosphatidyl choline	Phosphatidyl ethanol amine	Inositol phosphatides	Soybean oil	Sterols	Carbohydrates	Moisture	Other phosphatides
19 – 21 %	8 – 20 %	20 – 21 %	33 – 35 %	2 – 5 %	5 %	1 %	5 – 11 %

Lecithin is used for applications in human food, animal feed, pharmaceuticals, paints, and other industrial applications.

Materials and methods

1.1. Materials and instruments.

Iraqi Egg was purchased from local store, Absolute alcohol (Ethanol 100%), Acetone, Chloroform and Methanol were purchased from BDH Company, also we use Distilled deionized water of 0.01 µs/ cm Electrical conductivity. (Shimadzu FT-IR Spectrometer – 30 000:1/ IRAff), U.V-Vis.

spectrophotometer (Shimadzu 1800), and NMR spectrometer at Al-albiat university in Jordan.

1.2. Egg yolk preparation.

Four eggs were heated in boiling water for 15 min, and the egg yolks were separated from the whites. The complete semi liquid-to solid transformation was taken as an indicator that the yolk protein was heat denatured. The moisture content of the heated egg yolks was determined by using a conventional oven-drying method at 100°C for 4 h.

2.3. Lecithin extraction from undeoiled boiled egg yolks [12].

25 ml of Ethanol (100%) was added to approximately 5 g of boiled egg yolks to a final 5:1 ratio of ethanol to egg yolks (wet weight). The mixture was stirred until the egg yolks were completely dispersed. The sample was then centrifuged at 400 x *g* for 5 min. The protein enriched fraction (supernatant) was transferred to a previously weighed round-bottomed flask and the ethanol was removed by rotary evaporation to determine the protein content. The residual egg yolk was dried at ambient temperature for 2 d and then deoiled with acetone using AOCS Official Method Ja 4-46 [13] to a final 4:1 ratio of acetone to the dried precipitate. The acetone extract was transferred to a previously weighed round-bottomed flask and the acetone was removed by rotary evaporation to determine the oil content. After acetone deoiling, 40 mL of chloroform/methanol (2:1, vol. /vol.) was used to extract residual protein from the remaining egg yolk precipitate.

The (supernatant) was transferred to a previously weighed round-bottomed flask and the chloroform/methanol was removed by rotary evaporation to determine the residual protein content. Water-saturated butanol also was used to extract any remaining polar lipids from the yolk residual. The combined lipids were washed using the method of Folch et al. [14]. This fraction was referred to as the remaining PL fraction. The solid lecithin was determined and characterized by FT-IR (Shimadzu FT-IR Spectrometer – 30 000:1/ IRAff) (Figure 2), U.V-Vis. Analysis (U.V-Vis. Spectrophotometer, Shimadzu 1800) (Figure 3), and Powder X-ray diffraction (XRD – 6000 Shimadzu). (Table 3) show all the determination data.

2. Results and discussion.

2.1. Determination of Iraqi Egg Yolk constituents.

The following (Table 3) show the results of determination of hard-boiled egg yolk constituents.

Table 3 Constituents of boiled Iraqi Egg yolk.

Wt. of 4 boiled Egg yolks	70.7701 g.
Wt. of 4 Egg yolks after drying for 4 hours at 100 ºC	32.5214 g.
Wt. of water in 4 boiled Egg yolks	38.2487 g.
Water %	54 %
Wt. of Protein extracted by Absolute Ethanol	22.1637 g.
Protein %	31.31 %
Wt. of extracted oil by acetone	4.7604 g.
Oil %	6.72 %
Wt. of remaining protein extracted by chloroform; methanol mixture	4.0359 g.
Remaining Protein	5.7 %
Wt. of Lecithin	7.2887 g.
Lecithin %	10.29 %

2.2. ***FT – IR Characterization of Iraqi boiled egg yolk*** *Lecithin.*

Iraqi boiled egg yolk lecithin was characterized by FT –IR spectroscopic analysis (Shimadzu FT-IR Spectrometer – 30 000:1/ IRAff). FT-IR spectrum (Figure 2) showed absorption bands listed in (Table 3) compared with standard absorption bands of lecithin.

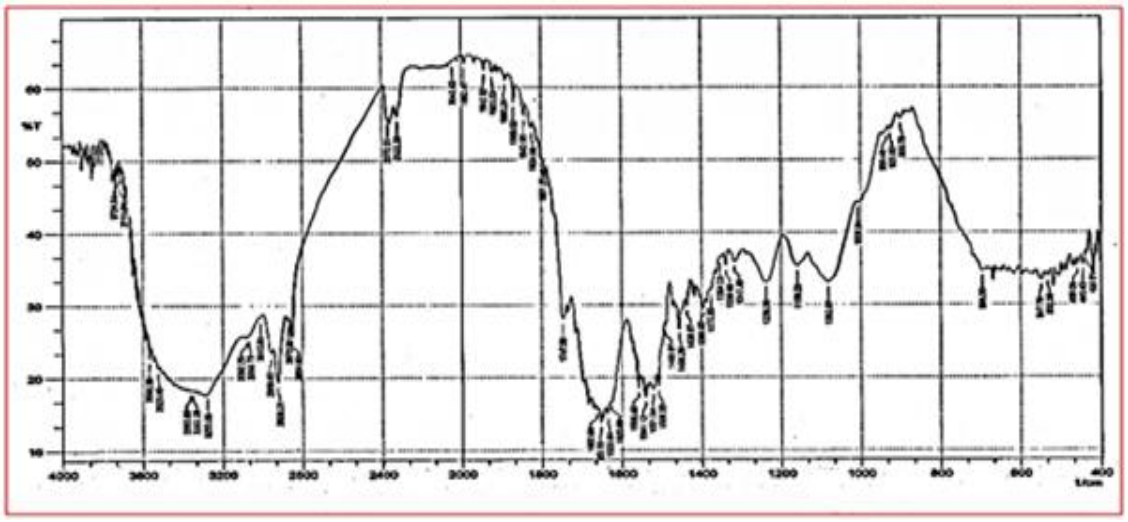

Figure 2. FT-IR Spectrum of boiled Egg Yolk Lecithin.

Table 3 FT –IR bands of Boiled Egg Yolk Lecithin sample.

FT-IR-Band	Standard Egg Yolk lecithin	Boiled Iraqi Egg Yolk Lecithin sample
	Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
C=O (sn-1 / sn-2) – stretching	1742 / 1725	1747
C-O – stretching	1170 + 1070	1159 + 1082
CH_2 – stretching, antisymmetric	2920	2926
CH_2 – stretching, symmetric	2850	2854
CH_2 – deformation (scissoring)	1465	1456
CH_2 – deformation (wagging)	1305	1317
CH_2 – deformation (twisting)	1180-1345	1159-1354
CH_2 – deformation (rocking)	720	696
Terminal CH_3 – stretching, antisymmetric	2956	2958
Terminal CH_3 – stretching, symmetric	2870	2872
=C-H – stretching, antisymmetric	3010	3012
CH_3 – stretching in $N(CH_3)_3$, antisymmetric	3040	3068
$N-(CH_3)_3$ – stretching, antisymmetric	970	1006
C-N – stretching, antisymmetric	945	935
PO_2 – stretching, symmetric	1085-1100	1082
PO_2 – stretching, antisymmetric	1220-1250	1238
-OH – stretching	3200-3600	3277-3566

From the above table we can see that the FT-IR bands of Boiled Iraqi Egg Yolk Lecithin sample Match often with the FT-IR bands of Standard Egg Yolk lecithin.

2.3. U V - Vis. Characterization of Iraqi boiled egg yolk Lecithin.

UV spectra were recorded for lecithin, using U.V-Vis. spectrophotometer (Shimadzu 1800). The sample was dissolved in methanol. The absorbance of solution was scanned in the wavelength range of 225 - 500 nm to obtain the UV spectra as shown in (Figure 3). The maximum absorption peaks of Lecithin was at 235, 271, 355 nm due to the presence of carboxyl groups and amine group in Lecithin structure. It can be observed that the lecithin showed a lower absorbance value due to the absence of π system [15].

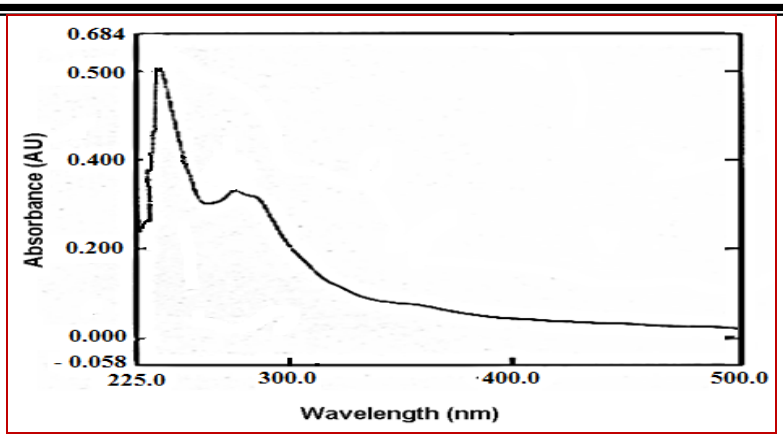

Figure 3. U V - Vis. Spectrum of Iraqi boiled egg yolk lecithin.

2.4. Powder X- ray diffraction Characterization of Iraqi boiled egg yolk Lecithin.

Powder X-ray diffraction pattern was recorded by Powder X-ray Diffractometer (XRD – 6000 Shimadzu). The powder X-ray diffraction patterns of lecithin, is shown in (Figure 4). The powder diffraction pattern of lecithin showed amorphous property lacking crystalline peaks. The diffractogram of lecithin showed characteristic peak at (20) 2θ, consistent with its amorphous character.

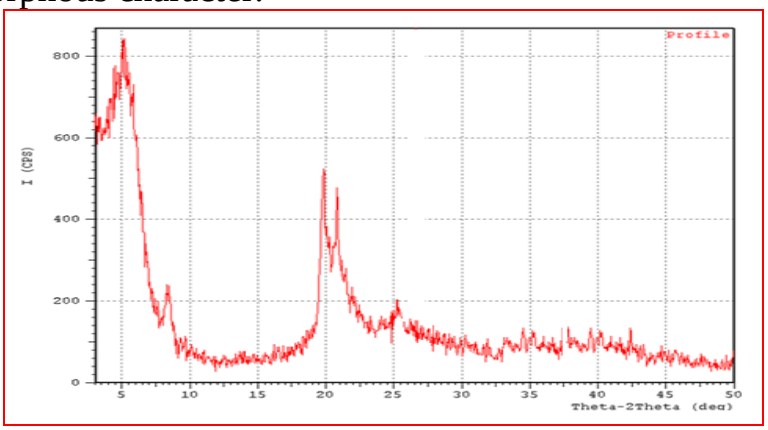

Figure 4: X-ray diffraction patterns of Iraqi boiled egg yolk Lecithin.

3. Conclusions

The method of extraction of boiled egg yolk constituents offers a simple and easy way to determine egg yolk contents. Characterization of boiled egg yolk Lecithin indicates that the FT-IR bands of Boiled Iraqi Egg Yolk Lecithin sample Match often with the FT-IR bands of Standard Egg Yolk lecithin. UV maximum absorption peaks of Lecithin was at 235, 271, 355 nm due to the presence of carboxyl groups and amine group in Lecithin structure. . It can be observed that the lecithin showed a lower absorbance value due to the absence of π system. The diffractogram of lecithin showed characteristic peak at (20) 2θ, consistent with its amorphous character.

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استخلاص وتحديد مكونات صفار البيض العراقي المغلي وتشخيص اللسيثين

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الملخص:

تهدف هذه الدراسة استخلاص وايجاد مكونات صفار البيض العراقي المغلي وتشخيص اللسيثين . المحتوى المائي لصفار البيض هو (٥٤%). استخلص بروتين صفار البيض باستخدام الايثانول وكانت النسبة المئوية للبروتين هي(٣١.٣١%). تم ازالة الدهون من صفار البيض المتبقي باستخدام الاسيتون[١٣] ، وان النسبة المئوية للدهون هي (٦.٧٢%). استخلصت بقايا البروتين من صفار البيض (10.29%) باستخدام مزيج كلوروفورم | ميثانول) وكانت النسبة المئوية هي (٥.٧%). اخيرا كان محتوى اللسثين هو X. تم تشخيص اللسثين النقي بواسطة طيف الاشعة تحت الحمراء ، مطيافية الاشعة الفوق البنفسجية وحيود اشعة

الكلمات المفتاحية - : صفار البيض المغلي ، تشخيص ، اللسثين ، مكونات ، تحديد.