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Quantification of the components of the Iraqi Chicken wet egg yolk, and characterization of Lecithin.

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Abstract
The target of this study is to determine the Iraqi wet egg yolk constituents, and to characterize the Lecithin of this wet egg yolk. The protein of Egg yolk was extracted using and was to be (55.27 %) while water content represent (25 %). Oil content was (13.23 %) isolated from the remaining egg yolk using acetone according to AOCS Official Method Ja 4-46 [1]. Finally Lecithin content was (22.4 %). Pure Lecithin was characterized by FT-IR, U.V-Vis. Analysis, and Powder X-ray diffraction.
Keywords*:* Wet Egg Yolk, characterization, Lecithin, Quantification, Iraqi Chicken.

1. Introduction

In the avian egg, the 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 is a single cell, the ovum or egg cell, one of the few single cells that can be seen by the naked eye.[2] This fact was discovered by Hoyer in 1858, [3]. 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.[4]. 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. The composition (by weight) of the most prevalent fatty acids in egg yolk is typically as shown in (Table 1) [5].

Table 1. The composition of fatty acids in egg yolk.

Unsaturated fatty acids			
Oleic acid 47%	Linoleic acid 16%	Palmitoleic acid 5%	Linolenic acid 2%
Saturated fatty acids			
Palmitic acid 23%	Stearic acid 4%	Myristic acid 1%	

The yellow color is due to lutein and zeaxanthin, which are yellow or orange carotenoids known as xanthophylls. The color of an egg yolk is directly influenced by the makeup of the chicken feed, [6]. The major constituents of egg yolk are protiens and lipids. These fractions play important role in cosmotic formulations and food processing industry and they also act as bioactive compounds in pharmaceutical products [7]. Generally eggs are considered to be a rich source of lecithin [8], and in this current research an attempt has been made to isolate and characterize lecithin from Iraqi wet egg yolk. Lecithin is a group of yellowbrownish fatty substances occurring in animal and plant tissues, and in egg yolk [9]. It has been used as a matrix to improve the lipophilic property and targeted delivery of bioactive compounds [10&11]. It is expected that diosmetin combined with lecithin might result in the improvement of its lipophilic property. In this study, the complex of diosmetin with lecithin was prepared for the first time, and the physicochemical properties of the complex investigated. Lecithin is the common name for a series of related compounds called phosphatidylcholines. Lecithin is a phospholipid mixture of phosphatides consisting mainly of phosphatidylcholines, Phosphatidyl ethanolamine, Phosphatidyl serine, Phosphatidyl inositol combined with various other substances, including fatty acids and carbohydrates. Lecithin also contain phosphorous and nitrogenous (e.g., choline) compounds [12] as shown in (Figure 1).

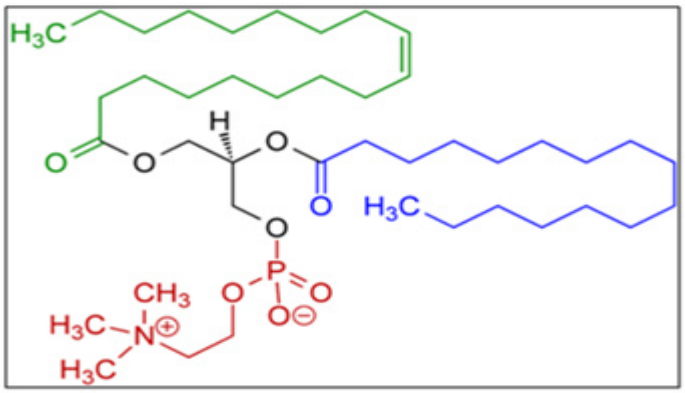

Figure 1: Phosphatidyl choline, a type of phospholipid in lecithin. Red - choline and phosphate group; Black - glycerol; Green - unsaturated fatty acid; Blue - saturated fatty acid.

Lecithin 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) [13].

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 %

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 Powder X-ray Diffractometer (XRD – 6000 Shimadzu).

1.2. Egg yolk preparation.

Four eggs were carefully broken to separate the yolks from the whites, and the combined yolks were kept in a cold room (5°C) before use. The water content of the wet egg yolks was determined by using a conventional oven-drying method at 100°C for 4 h. Grinded, and stored in a desiccator until use.

2.3. Lecithin extraction from undeoiled boiled egg yolks [14].

25 ml of Ethanol (100%) was added to approximately 5 g of prepared powdered 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 [1] 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. [15]. This fraction was referred to as the remaining PL fraction. The solid lecithin was determined and characterized by FT-IR (Figure 2), U.V-Vis. Analysis (Figure 2), and Powder X-ray diffraction. (Table 3) show all the determination data.

2. Results and discussion.

2.1. Determination of Iraqi wet egg yolk constituents.

The following (Table 3) show determination results of Iraqi wet egg yolk constituents.

Table 3 Constituents of Iraqi wet egg yolk.

Wt. of 4 fresh Egg yolks	72.0385 g.
Wt. of 4 Egg yolks after drying for 4 hours at 100 ºC	54.0067 g.
Wt. of water in 4 fresh Egg yolks	18.0318 g.
Water %	25 %
Wt. of Protein extracted by Absolute Ethanol	29.8485 g.
Protein %	55.27 %
Wt. of extracted oil by acetone	7.1497 g.
Oil %	13.23 %
Wt. of remaining protein extracted by chloroform; methanol mixture	4.9071 g.
Remaining Protein	9.1 %
Wt. of Lecithin	12.1014g.
Lecithin %	22.4 %

2.2. FT – IR Characterization of Iraqi wet egg yolk Lecithin.

Iraqi wet egg yolk lecithin was characterized by FT –IR spectroscopic analysis (Shimadzu FT-IR Spectrometer – 30 000:1/ IRAff). IR spectra of lecithin, was collected between 4000 and 400 cm^{-1} by the KBr method. FT-IR spectrum (Figure 2) showed absorption bands listed in (Table 4) compared with standard absorption bands of lecithin.

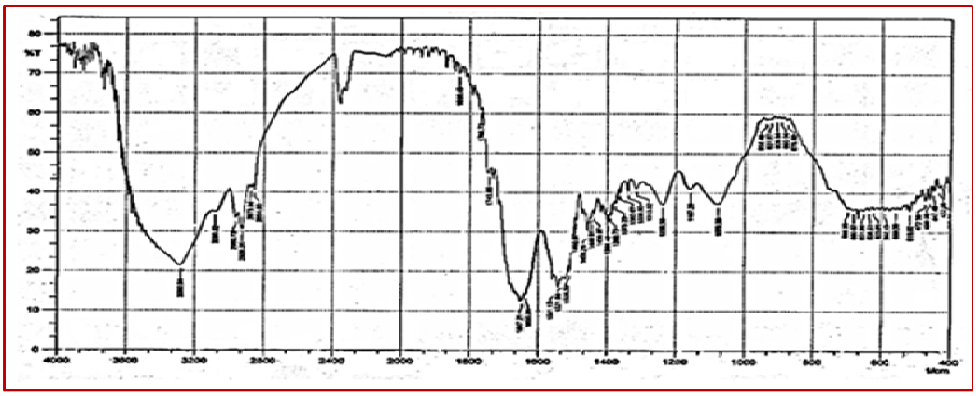

Figure 2: FT-IR Spectrum of Iraqi wet Egg Yolk Lecithin.

Table 4 FT –IR bands of Iraqi wet Egg Yolk Lecithin sample.

FT-IR-Band	Standard Egg Yolk lecithin	Iraqi wet Egg Yolk Lecithin sample
	Wavenumber (cm^{-1})	Wavenumber (cm-1)
C=O (sn-1 / sn-2) – stretching	1742 / 1725	1743
C-O – stretching	1170 + 1070	1157+1076
CH_2 – stretching, antisymmetric	2920	2926
CH_2 – stretching, symmetric	2850	2854
CH_2 – deformation (scissoring)	1465	1456
CH_2 – deformation (wagging)	1305	1313
CH_2 – deformation (twisting)	1180-1345	1157- 1338
CH_2 – deformation (rocking)	720	696
Terminal CH_3 – stretching, antisymmetric	2956	2960
Terminal CH_3 – stretching, symmetric	2870	2873
=C-H – stretching, antisymmetric	3010	2960
CH_3 – stretching in $N(CH_3)_3$, antisymmetric	3040	3080
$N-(CH_3)_3$ – stretching, antisymmetric	970	970
C-N – stretching, antisymmetric	945	935
PO_2 – stretching, symmetric	1085-1100	1076
PO_2 – stretching, antisymmetric	1220-1250	1238
-OH – stretching	3200-3600	3262

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

2.3. U V - Vis. Characterization of Iraqi wet 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 each solution was scanned in the wavelength range of 220 - 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 [16].

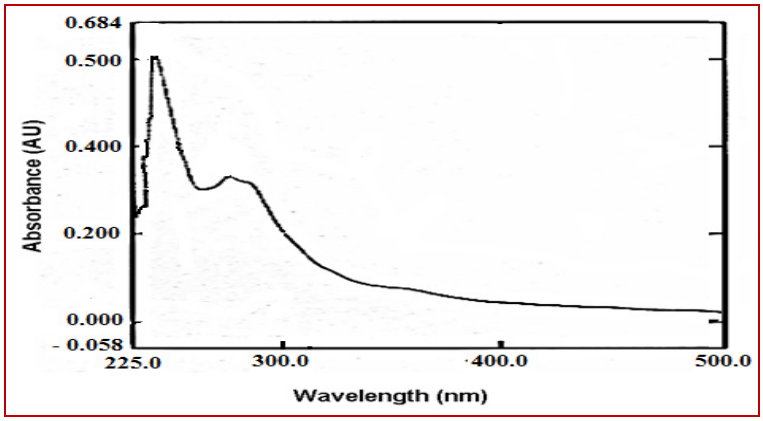

Figure 3: UV spectra of Iraqi wet egg yolk Lecithin.

2.4. Powder X- ray diffraction Characterization of Iraqi wet 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 gives information about the amorphous property lacking crystalline peaks. The diffractogram of lecithin showed characteristic peak at (20) 2θ, consistent with its amorphous character.

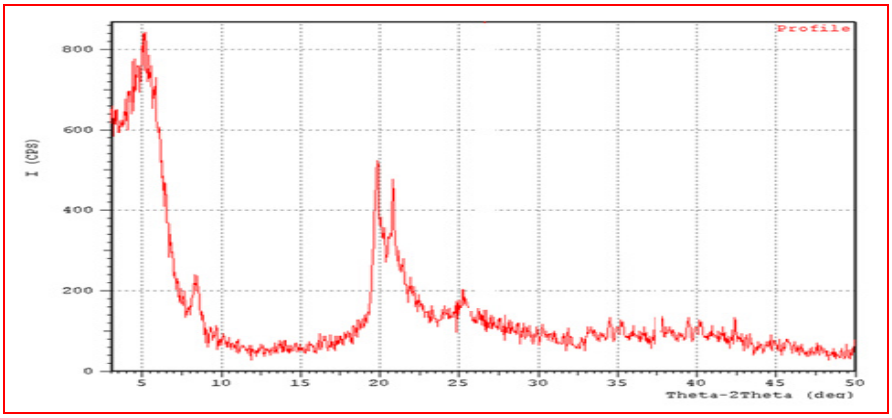

Figure 4: X-ray diffraction patterns of lecithin

3. Conclusions

The method of extraction of wet egg yolk constituents offers a simple and easy way to determine egg yolk contents. Characterization of wet egg yolk Lecithin indicates that the FT-IR bands of wet 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. The diffractogram of lecithin showed characteristic peak at (20) 2θ, consistent with its amorphous character.

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