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Extraction of Phosphatodylecholine from Egg Yolk

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Abstract: In this paper the phosphatidylcholine is extracted from egg yolk from different egg sources using water, alcohol and hexane. High degree of purity is achieved and this is assisted through many analysis methods.

1. Introduction

The egg yolk contain carbohydartes, lipids, protein, water and cholesterol. The yolk makes up about 33% of the liquid weight of the egg. It contain calories three times the caloric contents. 17 gm yolk contain approximately
2.7g protein, 210mg cholesterol, 0.61g carbohydrates and 4.5g total fates. Figure 1 indicates the major relative contents of the egg yolk and the fates contents in the egg yolk[1,2]. Egg yolk is a source of lecithin as well as egg oilbased on weight, egg yolk contains about 9% lecithin [3]. Retrived 2013-03-20. "egg yolk hase the approximate composition (by weight) of 50% water, 16% protein, 9% lecithin, 23% other fate, 0.3 carbohydrates and 1.7 minerals."

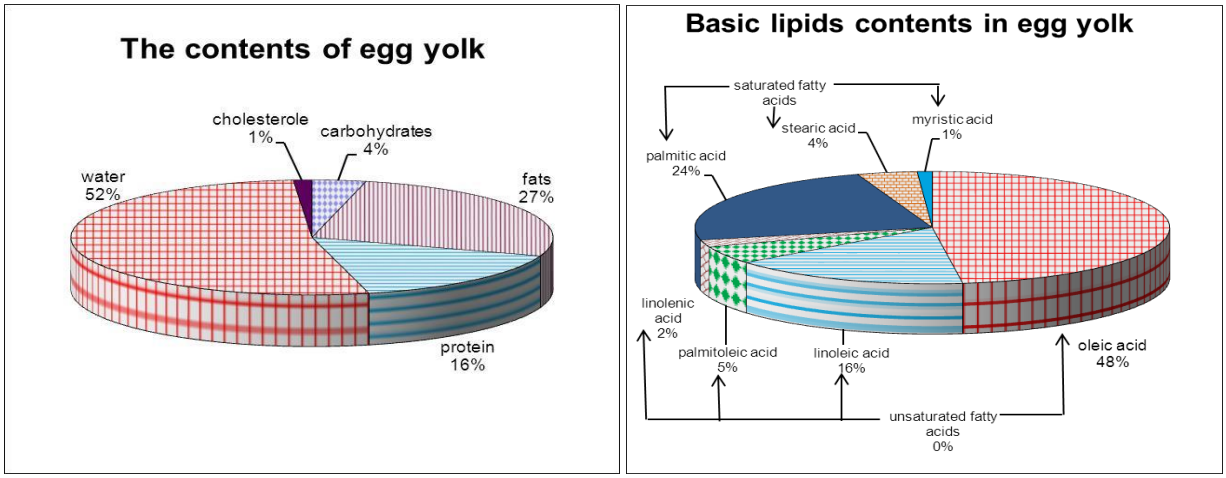

Figure 1: Egg yolk component and s aturated and uns aturated fatty acids compos ition fraction according to USDA National Nutrient Database Phospholipids are the major structural components of the biological cell membranes in the human body and animal"s bodies; phospholipid molecule has one head and two tails. The head is made from three molecular components: choline, phosphate, and glycerol. The head is hydrophilic. Each tail is a long, essential fatty acid chain. These fatty acids are hydrophobic. Phosphatidylcholine the main component used in the preparation of liposomes as drug delivery systems. Figure one shoe the chemical structure and the 3d configuration of the phosphatidylecholine.

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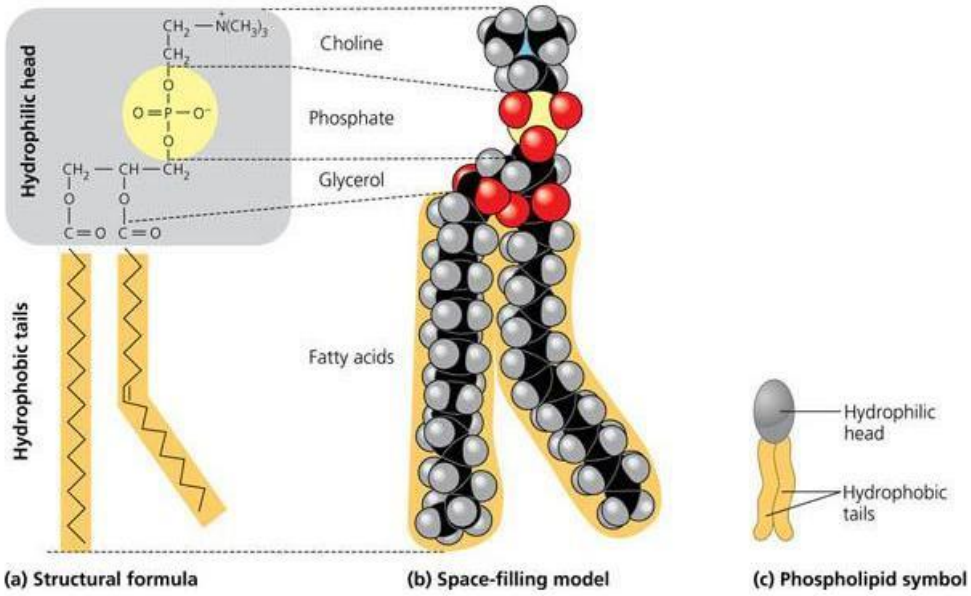


Figure 2: the structural formula, space-filling model and symbol for phos phati dylcholine

2.Material and methods

2.1 extraction of phosphatidylecholine from egg yolk

The egg is weighted using sensitive balance and the egg yolk is separated from egg white by manual method. Then the egg yolk is dissolved in water of PH= 5.0 in order to increase the extracted percentage of the phosphatidylecholine where it isn"t soluble in the water of PH=5.0. The solution is centrifuged at 6000 round per minute. the precipitate is separated from supernatant. The precipitate contain the basic lipids, protein and phosphatydylecholine „components of egg yolk that are not soluble in the water of PH=5.0" the supernatant is dissolved in alcohol (Abs). The protein is insoluble in the alcohol and the basic lipids are also insoluble in the alcohol, but the phosphatodylecholine is soluble in alcohol. Some hexane is added to the alcohol in order to extract any dissolved basic lipids in the alcohol.

The mixture is placed in a separation flask and left for (time) and then the alcohol is separated from hexane.

2.2 purification of the extraction

The separation of impurities was established by column chromatography with radius 2 cm and height 30cm. the stationary phase used is silica gel for column mesh 60-120, and the eluent used is acetone/hexane mixture 3/1. Charcoal and sodium sulphat anhydrous is added to the solution obtained from the colomn chromatography and filtered using filter paper. The solution is inserted in a petridish and an Al foil cover with very large number of holes is used to cover the petridish and left for slowly vaporization in order to obtain crystalline phosphatodylecholine.

The previous method is repeated for different kinds of chickens eggs that are Gallus Gallus Domesticus, Solid White Gallus Gallus Domesticus and duck. A omparison between the contents, crystallization of phosphatidylecholine for each sample is carried out through different analysis spectroscopic methods.

2.3 characterization methods

The purification of our extracted compound (phosphatidylcholine) was carried out by thin layer chromatography (TLC) (0.2 mm thikness) precoated silica gel plates (Merck Kiesegel 60F25u.
The structure of the samples is examined by x-ray diffraction (XRD) at room temperature using a GNR-APD 20000 pro, H423-vertical diffractometer in the range (2θ from 5° to 80°) where the samples were exposed to Cu-Kα radiation (λ = 1.541178 Ao). The particles average size (tave) is calculated by Scherer's equation [135].

$$t_{ave} = \frac{k\lambda}{h_{1/2}\cos\theta_B}$$ Where K = 0.89 is constant, θ_B is the peak location and $h_{1/2}$ is the full width at half maximum of the peak, and λ is the wave length of the X-ray for Cu-Kα radiation.

Fourrier Transition Infrared Red (FTIR) spectra for the extracted phosphatidylecholine were carried out at room temperature by using a PERKIN-ELMER-1430, the infrared spectra was in the wavenumber range 200 to 4000 cm-1. And the results are compared with that obtained by Erhan Suleymanoglu[4].

The purity of the samples are checked by using High pressure liquid chromatography (HPLC) using Agilent 1100 series. The concentration is calculated by comparing the peak area with that for a standared phosphatidylcholine sample. A drop of sample solution is peptided at a sheet of copper using micropepite and left for four hours and then scanned using scanning electronic microscope JXA-840A electron probe microanalyzer. A drop is peptided at a copper grid using micropepite and left for two hours and then photographed using and transition electronic microscope JEOL-JEM-2100.

3.Results and discussion

3.1 Thin layer chromatography (TLC)

The results of thin layer chromatography (TLC) exhibit the presence of only one spot of each sample as shown in Figure 3 indicates which indicates high degree of purity, also it appear that the elution rate is the same for all of them since the distance between the spots and the base line is approximately equal for the three samples.

Figure 3: Thin layer chromatography (TLC) for the extracted phos phati dylcholine from A) Solid White Gallus egg yolk, B) Gallus Gallus Domes ticus egg yolk and C) Duck egg yolk

3.2 X-Ray Diffraction spectra (XRD)

Using X-ray diffraction patterns shown in figure 4 and Scherer's equation, the average size of the particles are calculated. The average, standard deviation, minimum and maximum sizes are shown in table 1. The total average size is 13.0967±3.8907 nm (mean ± standard deviation).

X-ray diffraction pattern

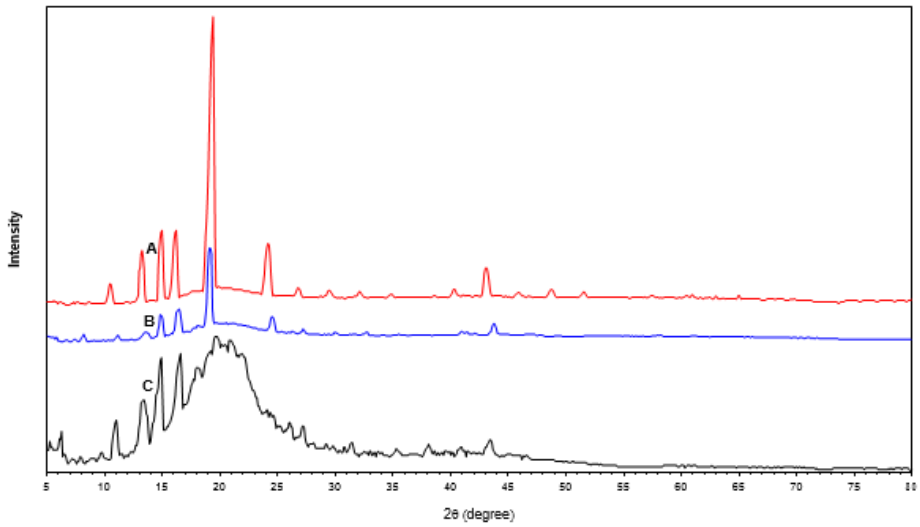


Figure 4: X ray diffraction pattern for the extracted phos phati dylcholine from A) Solid White Gallus egg yolk, B) Gallus Gallus Domes ticus egg yolk and C) Duck egg yolk

	Mean (nm)	**standard deviation**	**minimum**	**maximum**
white gallos	16.4427	2.0660	13.884	19.804
Gallos	14.0201	3.7870	9.6577	18.826
Duck	8.8274	4.0371	1.3395	14.192

Table 1: average sizes of the extracted phos phatidylcholine from Solid White Gallus egg yolk, Gallus Gallus Domes ticus egg yolk and Duck egg yolk

3.3 Fourrier Transition Infra Red spectra (FTIR)

The Fourrier Transition Infra Red spectrum of the extracted samples is shown in figure 5 the bonds present exactly represents the phosphatidylecholine and compared to that obtained by Erhan Suleymanoglu[4]. Table2 show the group bands and the wavenumbers of the peaks shown in figure 5 in the samples compared with that obtained by Erhan Suleymanoglu[4].

FTIR for the extracted phosphatidylcholine

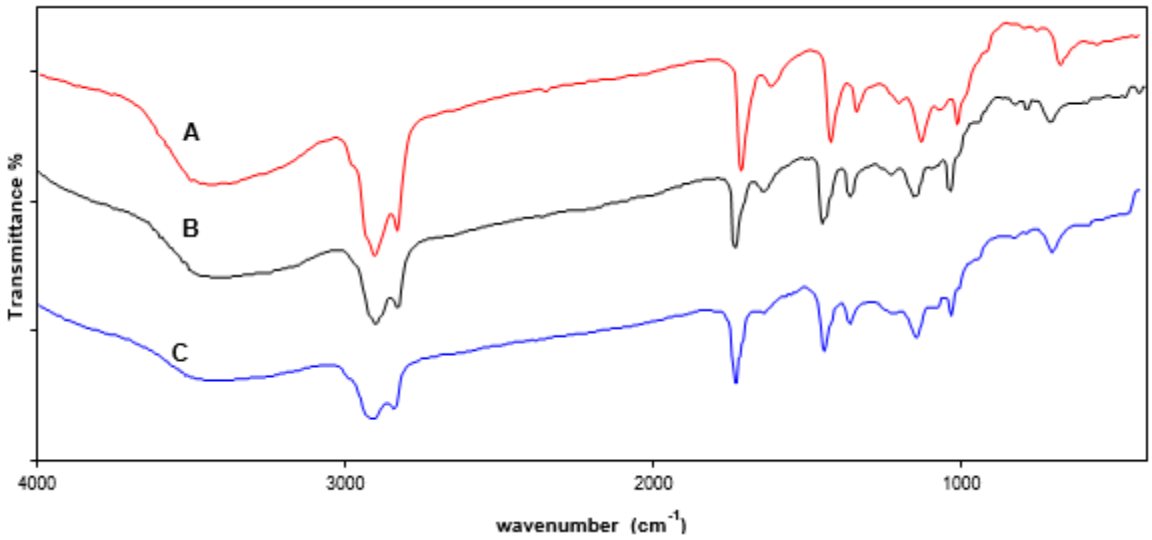


Figure 5: Fourrier trans ition Infra Red for the extracted phos phati dylcholine from A) Solid White Gallus egg yolk, B) Gallus Gallus Domes ticus egg yolk and C) Duck egg yolk

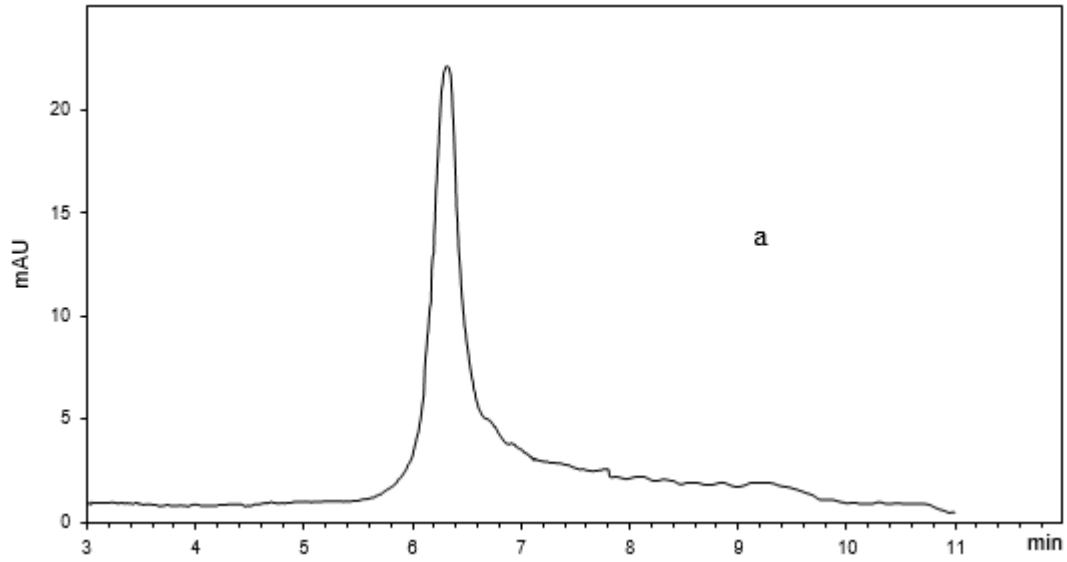
Group bonds	Wavenumber (cm^{-1})	Wavenumber (cm^{-1})	Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
Water band	3439.21	3437.14	3411.15	924243
$v_S CH_3)]$	2903.54	2907.45	2903.91	4344
$v_S CH_3)]$	2830.68	2842.86	2833.32	4.2444
$v_S C=O]$	1715.15	1728.57	1732.40	6.9141
C-H]	700-1500	700-1500	700-1500	700-1500
scissoring $(CH_3)_n]$	1423.00	1444.64	1450.84	621.42
$CH_2]$	1340.18	1358.93	1360.34	69..46
$v_{as}[PO_2-]$	1203.79	1219.64	1224.58	642441
$v[PO_2-]$	1072.02	1032.23	1093.11	6.3642
Asssymetric $[^+N-(CH_2)]s$	1013.00	1032.25	1033.17	3..42
v [C-C-N]	916.25		948.82	34.4.
v [P(-O-C)$_n$]	838.23	838.58	827.14	.4143
rocking v[$(CH_2)_n$]	755.15	705.66	716.65	.464.
[C=C]	1617	1640	1631	

Table2 The group bonds and the wavenumbers of the peaks s hown in figure 4 in the s amples compared with that obtained by Erhan Suleymanoglu[4].

3.4 High Pressure Liquid Chromatography (HPLC)

Figure 6 show the high pressure liquid chromatography (HPLC) spectrum for a) thes standard phosphatidylecholine and b) for the extracted phosphatodylcholine from the three different sources. Table 3 indicate the value of concentrations, injected amount, peak areas and the purity of the samples. Purity ranges from 80 to 93.5 % is obtained.

HPLC for the standard phosphatidylcholine



HPLC for the extracted phosphatidylecholine

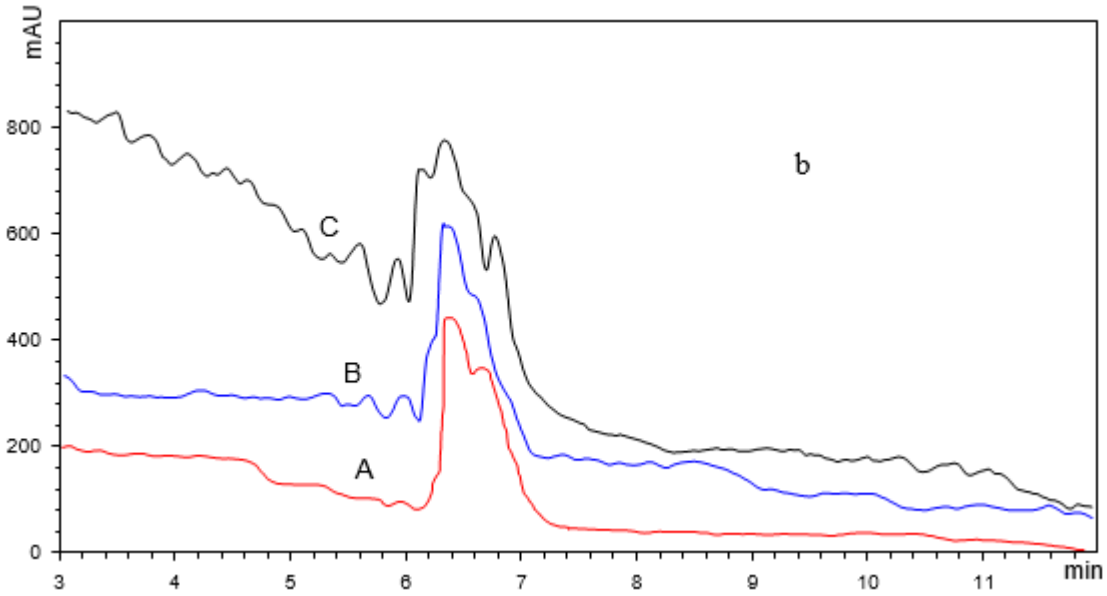


Figure 6: High Pres s ure Liquid Chromatography (HPLC) a) for the s tandard phos phati dylcholine and b)for the extracted phos phati dylcholine from A) Solid White Gallus egg yolk, B) Gallus Gallus Domes ticus egg yolk and C) Duck egg yolk

	peak position	Area (mAU*min)	Concentration (µg/µl)	Injected (µl)	injected mass (µg)	calculated mass (µg)	Purity percentage
standard	6.3132	6.9450	1.3500	10.0000	13.5000	13.5000	100.0000
white gallos	6.3834	193.3015	20.1000	20.0000	402.0000	375.7481	93.4697
gallos	6.3646	183.8186	10.0000	40.0000	400.0000	357.3148	89.3287
duck	6.3413	224.3733	11.5000	40.0000	460.0000	436.1468	94.8145

Table 3 indicate the value of concentrations , injected amount, peak areas and the purity of the s amples .

3.5 Scanning electron microscope (SEM)

Figure 7 show the scanning images for the extracted samples. The grain size and shape are approximately similar. The dendrite structure appear very clear at the surface of the samples

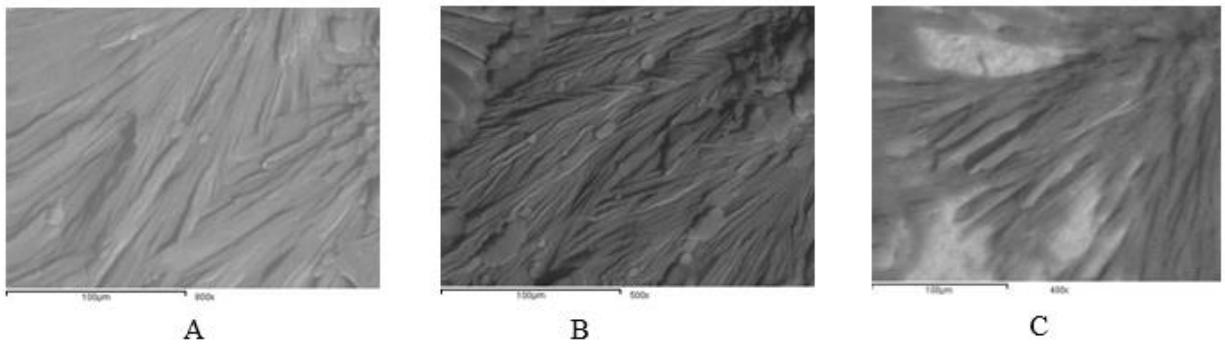


Figure 7: Scanning Electronic Micros cope (SEM) images for the extracted phos phatidylecholine from A) Solid White Gallus egg yolk, B) Gallus Gallus Domes ticus egg yolk and C) Duck egg yolk

3.6 Transition electron microscope (TEM)

Figure 8 show the transition electronic microscope images for the extracted samples. The partciles size is of order of average 20 nm.in case of duck egg yolk the particle size seems to be smaller than the other samples but the difference is insignificant.

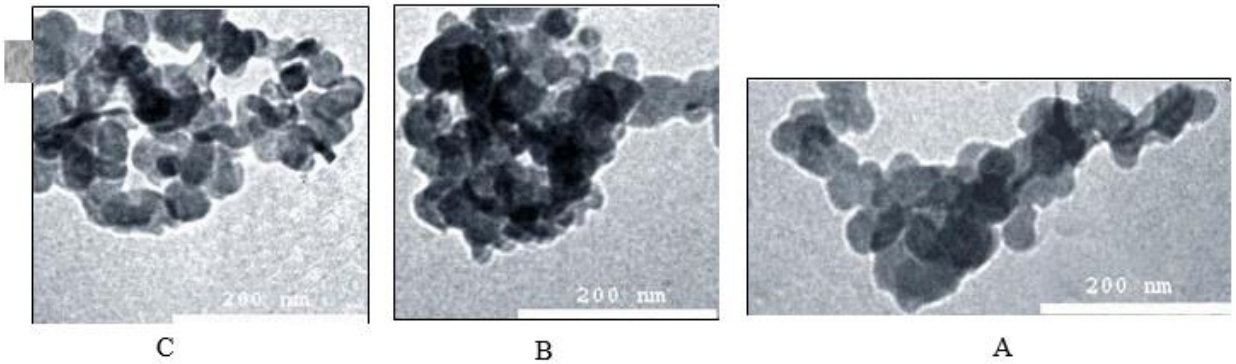


Figure 8: Trans ition Electronic Micros cope (TEM) images for the extracted phos phati dylecholine from A) Solid White Gallus egg yolk, B) Gallus Gallus Domes ticus egg yolk and C) Duck egg yolk

4.Conclusions

In this method of extraction, High purity is achieved with crystalline and very small size of order of 20nm, exclusion of basic lipids using hexane and using silica gel column chromatography to dispose of impurities enable us to achieve this degree of purity approximately 97% purity. There is no significant particle size differences between the phosphatidylcholine extracted from these three different sources.

References

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