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Determination of Lecithin from egg yolk, milk, soyabean seed, sunflower oil Calorimetrically and its FTIR study

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Abstract

Lecithin is a generic term to designate any group of yellow-brownish fatty substances occurring in animal and plant tissues which are amphiphilic – they attract both water and fatty substances, and are used for smoothing food textures, emulsifying, homogenizing liquid mixtures, and repelling sticking materials. Lecithin's are mixtures of glycerophospholipids including phosphatidylcholine, phosphatidyl-ethanolamine, phosphatidylinositol, phosphatidylserine, and phosphatidic acid. Lecithin can easily be extracted chemically using solvents such as hexane, ethanol, acetone, petroleum ether or benzene; or extraction can be done mechanically. Common sources include egg yolk, marine foods, soybeans, milk, rapeseed, cottonseed, and sunflower oil. It has low solubility in water, but is an excellent emulsifier. In aqueous solution, its phospholipids can form either 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 is sold as a food additive and dietary supplement. In cooking, it is sometimes used as an emulsifier and to prevent sticking, for example in non-stick cooking spray. Soy Lecithin Improves Cholesterol Levels, it serves as a source of Choline, helps body deal with physical and mental Stress, relieves Menopause Symptoms. It may Boost Immunity, improve Cognitive Function, prevent Osteoporosis, and help prevent Cancer, Present study deals with determination of Lecithin from Egg Yolk, Soyabean seed, milk and sunflower oil Colorimetrically and FTIR study of Soy-Lecithin. Determination of Lecithin from Egg Yolk, Soyabean seed, milk and sunflower oil Colorimetrically involves preparation of Soy lecithin solution, Egg Yolk solution, Soyabean seed solution, Milk solution and Sunflower Oil solution. Different systems were prepared. Absorbance of standard Soy lecithin system was taken at different wavelength using ethyl alcohol as blank. And λmax was found which is 420 nm. Absorbance of standard Soy lecithin systems were taken at 420 nm and Calibration plot was prepared. Then absorbance of systems of Egg Yolk solution, Soyabean seed solution, Milk solution and Sunflower Oil solution were taken at 420 nm and concentration of

Lecithin present in Egg Yolk, Soyabean seed, milk and sunflower oil were calculated from calibration plot. The method is simple, rapid and precise. FTIR spectra of Soy-Lecithin is obtained at room temperature by using an FTIR Spectrophotometer – Perkin Elmer – Spectrum RX-IFTIR. The spectra is collected in a range from 400 to 4000 cm^{-1}. Interpretation of FTIR Spectra of soy lecithin shows Presence of various functional groups such as C-H bending – Alkane, Aromatic compound; O-H Stretching – Alcohol, Carboxylic acid; C=O stretching – Esters and O=C=O stretching - Carbon dioxide.

Keywords: Lecithin, Soy Lecithin, Egg Yolk, Milk, Soyabean Seed, Sunflower Oil, FTIR study

Introduction

Lecithin

Lecithin is a generic term to designate any group of yellow-brownish fatty substances occurring in animal and plant tissues which are amphiphilic – they attract both water and fatty substances (and so are both hydrophilic and lipophilic), and are used for smoothing food textures, emulsifying, homogenizing liquid mixtures, and repelling sticking materials. [1][2] Lecithins are mixtures of glycerophospholipids including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and phosphatidic acid. [3] Lecithin was first isolated in 1845 by the French chemist and pharmacist Théodore Gobley. [4] In 1850, he named the phosphatidylcholine lécithine.[5] Gobley originally isolated lecithin from egg yolk and established the complete chemical formula of phosphatidylcholine in 1874; [6] in between, he demonstrated the presence of lecithin in a variety of biological materials, including venous blood, human lungs, bile, roe, and brains of humans, sheep and chicken. Lecithin can easily be extracted chemically using solvents such as hexane, ethanol, acetone, petroleum ether or benzene; or extraction can be done mechanically. Common sources include egg yolk, marine foods, soybeans, [7] milk, rapeseed, cottonseed, and sunflower oil. It has low solubility in water, but is an excellent emulsifier. In aqueous solution, its phospholipids can form either 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 is sold as a food additive and dietary supplement. In cooking, it is sometimes used as an emulsifier and to prevent sticking, for example in non-stick cooking spray.

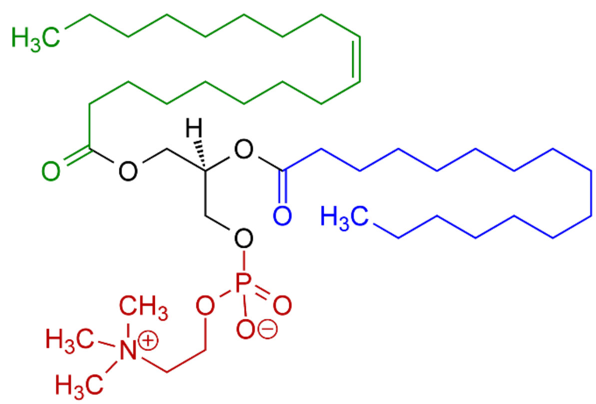


Figure 1. An example of a phosphatidylcholine, a type of phospholipid in lecithin. Shown in red – choline residue and phosphate group; black – glycerol residue; green – monounsaturated fatty acid residue; blue – saturated fatty acid residue. [8]

Lecithins have emulsification and lubricant properties, and are a surfactant. They can be completely metabolized (see inositol) by humans, so are well tolerated by humans and nontoxic when ingested. The major components of commercial soybean-derived lecithin are 33–35% soybean oil, 20–21% phosphatidylinositols, 19–21% phosphatidylcholine, 8–20% phosphatidylethanolamine, 5–11% other phosphatides, 5% free carbohydrates, 2–5% sterols and 1% moisture. Lecithin is used for applications in human food, animal feed, pharmaceuticals, paints, and other industrial applications. [9]

In the pharmaceutical industry, it acts as a wetting agent, stabilizing agent and a choline enrichment carrier, helps in emulsification and encapsulation, and is a good dispersing agent. It can be used in manufacture of intravenous fat infusions and for therapeutic use. In animal feed, it enriches fat and protein and improves pelletization. In the paint industry, it forms protective coatings for surfaces with painting and printing ink, helps as a rust inhibitor, is a colour intensifying agent, catalyst, conditioning aid modifier, and dispersing aid; it is a good stabilizing and suspending agent, emulsifier, and wetting agent, helps in maintaining uniform mixture of several pigments, helps in grinding of metal oxide pigments, is a spreading and mixing aid, prevents hard settling of pigments, eliminates foam in water-based paints, and helps in fast dispersion of latex-based paints. Lecithin also may be used as a release agent for plastics, an anti-sludge additive in motor lubricants, an anti-gumming agent in gasoline, and an emulsifier, spreading agent, and antioxidant in textile, rubber, and other industries.

Soya Lecithin

Lecithin is a fat that can be found in many foods like soybeans and egg yolks. It is also known as Egg Lecithin, Lecitina, Ovolecithin, Soy Lecithin, Soy Phospholipid, Soybean Lecithin, Vegilecithin, Vitellin, Vitelline, and other names.

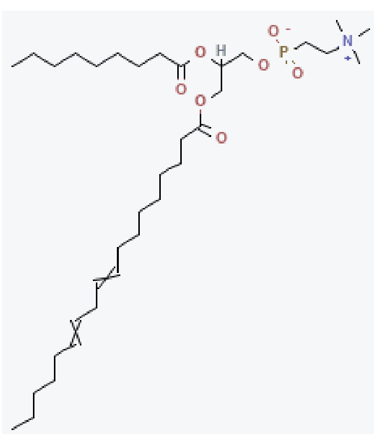


Figure 2. Lecithin from Soybean $C_{35}H_{66}NO_7P$ [10] (2-nonanoyloxy-3-octadeca-9,12-dienoyloxypropoxy)-[2-(trimethylazaniumyl) ethyl] phosphinate

Lecithin has been used in alternative medicine as a possibly effective aid in treating liver disease. Lecithin has also been used to treat gallbladder disease, dementia related to Alzheimer's disease, age related loss of memory, and head injuries. However, research has shown that lecithin may not be effective in treating these conditions.

Potential Benefits of Soy Lecithin

1. Improves Cholesterol Levels

Dietary soy lecithin supplementation is most strongly connected with decreasing hyperlipidemia and influencing lipid metabolism. It's known for its important role in processing fat and cholesterol, which is why people sometimes take soy lecithin supplements to lower cholesterol naturally. Research suggests that properties of lecithin have the ability to reduce the excess of LDL cholesterol and promote the synthesis of HDL in the liver. Study suggests that soy lecithin may be used as a dietary supplement for the treatment of hypercholesterolemia.

2. Serves as a Source of Choline

Soy lecithin contains phosphatidylcholine, which is one of the primary forms of choline, a macronutrient that plays an important role in liver function, muscle movement, metabolism, nerve function and proper brain development. Phosphatidylcholine supplementation has been found to support healthy cholesterol levels, liver function and brain function. Many of the potential benefits of soy lecithin powder or supplements come from the choline content.

3. May Boost Immunity

Soy lecithin supplementation has been shown to significantly boost immune function among diabetic rats. More research is needed to conclude the role of soy lecithin in the human immune system.

4. Helps Body Deal with Physical and Mental Stress

One of the many keys to soy lecithin's health benefits is a compound known as phosphatidylserine — a common phospholipid that helps make up part of the cell membranes in plants and animals. Known to affect stress hormones adrenocorticotropic hormone (ACTH) and cortisol, phosphatidylserine derived from cow brains has been shown to dampen response to physical stress. Specific properties in soy lecithin may have a selective stress-dampening effect and may even be used in the natural treatment of stress-related disorders.

5. May Improve Cognitive Function

Soy lecithin-derived phosphatidylserine and phosphatidic acid may have a positive influence on memory, cognition and mood among the elderly and those suffering from cognitive conditions.

6. May Prevent Osteoporosis

Although the research is mixed, there are studies indicating that soybean and soy-based ingredients, including soy lecithin, act as antiresorptive and bone-enhancing agents in preventing osteoporosis. This is due to the isoflavones found in soy, specifically the glycosides. Soy-based products could "potentially lower the bone loss rate and decrease the risk of fracture." This may be due to soy's estrogenic effects, as estrogen deficiency induced by menopause has been shown to accelerate bone loss in older women. It also may be due to properties in soy (notably the glycosides) that have antioxidant, antiproliferative, estrogenic and immune-modulating effects.

7. Relieves Menopause Symptoms

In addition to its potential benefit for osteoporosis, research suggests that soy lecithin supplements may help improve menopause symptoms by improving vigor and blood pressure levels in menopausal women. The improvements in fatigue symptoms, diastolic blood pressure and cardio-ankle vascular index (to measure arterial stiffness) were greater in the high-dose group compared with the placebo group.

8. May Help Prevent Cancer

There may be a reduced risk of breast cancer associated with lecithin supplement use. This link between soy lecithin and decreased breast cancer risk may be due to the presence of phosphatidylcholine in soy lecithin, which is converted to choline when ingested. [11]

An experiment was performed to assess the inclusion of soybean lecithin (**SL**) in the replacement of soybean oil (**SO**), for grower and finisher broiler chicken diets (up to 15 d of life), and its effects on performance, fatty acid (**FA**) absorption, gut health, and saturation degree of the abdominal fat pad (**AFP**). [12] The search of alternatives for soybean oil, as a dietary energy source, has generated a lot of interest in broiler feeding due to economic and supply reasons. Soybean lecithin, as a co-product derived from the soybean oil degumming process, and its blending with other by-products derived from the vegetable oil refining process such as acid oils, may represent an alternative energy source for broiler chicken diets formulation. Study has demonstrated that soybean lecithin high in free fatty acids can be included in grower–finisher diets, as a partial replacer of soybean oil or in combination with an acid oil, without impairing performance or fatty acid digestibility and causing minor changes in the fatty acid composition of the abdominal fat pad. [13]

Present study deals with determination of Lecithin from Egg Yolk, Soyabean seed, milk and sunflower oil Colorimetrically and FTIR study of Soy-Lecithin.

Methodology

1. **Determination of Lecithin from Egg Yolk, Soyabean seed, milk and sunflower oil Colorimetrically**

Determination of Lecithin from Egg Yolk, Soyabean seed, milk and sunflower oil Colorimetrically involves preparation of Soy lecithin solution, Egg Yolk solution, Soyabean seed solution, Milk solution and Sunflower Oil solution. Different systems were prepared. Absorbance of standard Soy lecithin system was taken at different wavelength using ethyl alcohol as blank. And λmax was found which is 420 nm. Absorbance of standard Soy lecithin systems were taken at 420 nm and Calibration plot was prepared. Then absorbance of systems of Egg Yolk solution, Soyabean seed solution, Milk solution and Sunflower Oil solution were taken at 420 nm and concentration of Lecithin present in Egg Yolk, Soyabean seed, milk and sunflower oil were calculated from calibration plot. The method is simple, rapid and precise.

Preparation of Soy lecithin solution:

0.409 gm of Soy lecithin Capsule sample was taken. To it 50ml of ethyl alcohol was added. This is Soy lecithin solution A. Different systems were prepared as follows:

System No.	1	2	3	4	5
Soy lecithin Solution	1	2	3	4	5
Dichloromethane	1	1	1	1	1
Ethyl Alcohol	8	7	6	5	4

Preparation of Egg Yolk solution:

0.5 g of egg yolk was taken. To it 20 ml of ethyl alcohol was added. This is Egg yolk solution B. Different systems were prepared as follows:

System No.	6	7	8	9	10
Egg Yolk Solution	1	2	3	4	5
Dichloromethane	1	1	1	1	1
Ethyl Alcohol	8	7	6	5	4

Preparation of Soyabean seed solution:

2 Soyabean seeds were taken. Weight of 2 seeds of Soyabean was taken. Then it was crushed and its powder was formed. To it 20 ml of ethyl alcohol was added. This is Soyabean seed solution C. Different systems were prepared as follows:

System No.	11	12	13	14	15
Soyabean seed Solution	1	2	3	4	5
Dichloromethane	1	1	1	1	1
Ethyl Alcohol	8	7	6	5	4

Preparation of Milk solution:

2 ml Milk was taken. To it 20ml of ethyl alcohol was added. This is Milk solution D.

System No.	16	17	18	19	20
Milk Solution	1	2	3	4	5
Dichloromethane	1	1	1	1	1
Ethyl Alcohol	8	7	6	5	4

Preparation of Sunflower Oil solution:

2 ml Sunflower Oil was taken. To it 20ml of ethyl alcohol was added. This is Sunflower Oil solution E.

System No.	21	22	23	24	25
Sunflower Oil Solution	1	2	3	4	5
Dichloromethane	1	1	1	1	1
Ethyl Alcohol	8	7	6	5	4

Absorbance of System No. 1 was taken at different wavelength using ethyl alcohol as blank. And λmax was found which is 420 nm.. Then Absorbance of System No. 1 to 5 were observed at 420 nm. Absorbance of System No. 6 to 25 was also taken at 420 nm. Calibration graph was plotted between Concentration of Lecithin and Absorbance from System No 1 to 5. Then from calibration plot concentration of lecithin in Egg Yolk, Soyabean Seed, Milk and Sunflower Oil was calculated.

2. FTIR study of Soy- Lecithin

FTIR can be routinely used to identify the functional groups and identification/quality control of raw material/finished products. Spectrum RX-I offers fast throughput and rapid access to reliable and dependable IR results. High signal to noise ratio makes FTIR more useful for difficult samples. It has resolution of 1 cm1 and scan range of 4000 cm-1 to 250 cm-1. In the normal mode around 10 mg sample is required in the form of fine powder. The sample can be analyzed in the form of liquid, solid and thin films also. FTIR spectra of Soy-Lecithin is obtained at room temperature by using an FTIR Spectrophotometer – Perkin Elmer – Spectrum RX-IFTIR. The spectra is collected in a range from 400 to 4000 cm^{-1}.

Observation

1. Determination of Lecithin from Egg Yolk, Soyabean seed, milk and sunflower oil Colorimetrically

Absorbance of System No. 1 taken at different wavelength using ethyl alcohol as blank.

Wave Length	Absorbance
400	0.05
420	0.06
470	0.04
500	0.03
530	0.03
620	0.02
660	0.03
700	0.02

Absorbance of System No. 1 to 25 at . λmax 420 nm

System number	Absorbance at 420 nm	System number	Absorbance at 420 nm
1	0.05	16	0.31
2	0.07	17	0.60
3	0.08	18	0.84
4	0.11	19	0.80
5	0.14	20	0.98
6	0.96	21	0.01
7	0.10	22	0.04
8	0.08	23	0.10
9	0.13	24	1.15
10	0.12	25	1.32
11	0.04		
12	0.06		
13	0.08		
14	0.10		
15	0.11		

1. FTIR study of Soy- Lecithin

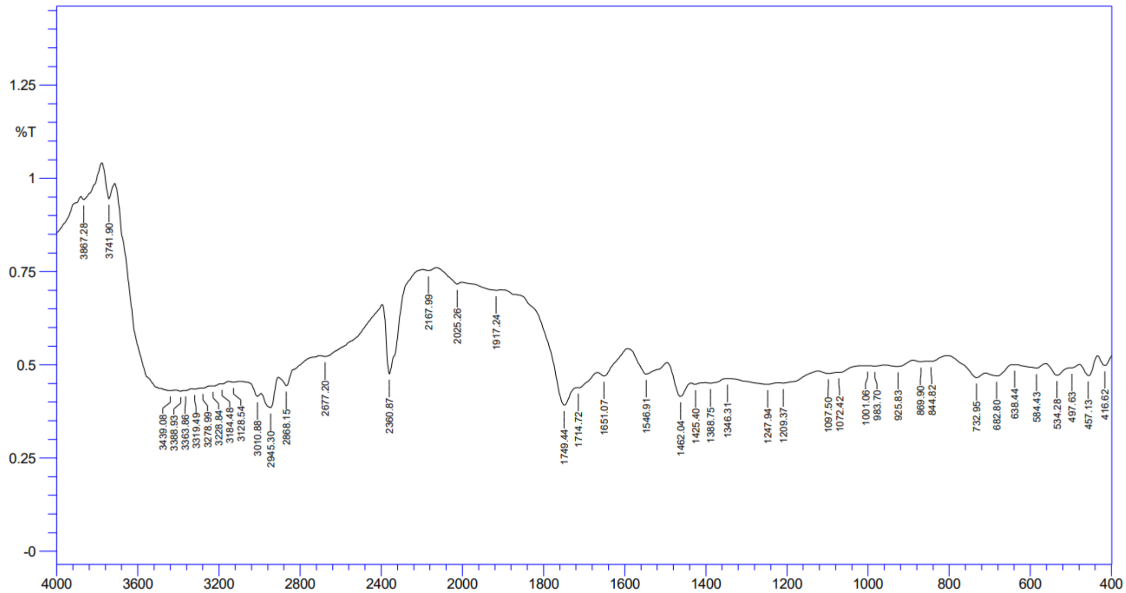
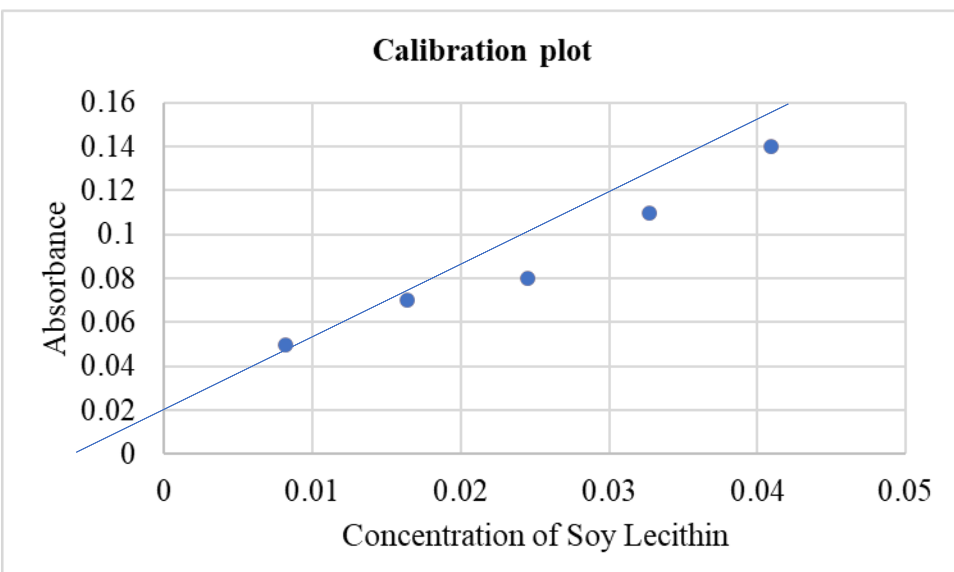


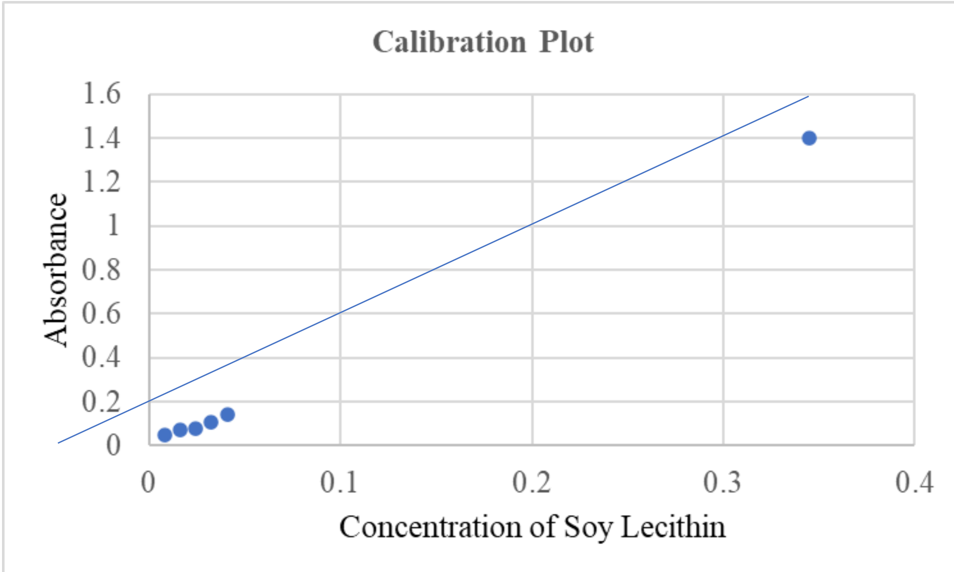
Figure 3 FTIR spectra of Soy lecithin

Results and Discussion

1. Determination of Lecithin from Egg Yolk, Soyabean seed, milk and sunflower oil Colorimetrically

System number	Soy Lecithin Solution A taken, ml	Concentration of Lecithin per 50 ml	Absorbance
1	1	1×0.409 / 50 = 0.00818	0.05
2	2	2×0.409/ 50 = 0.01636	0.07
3	3	3×0.409/ 50 = 0.02454	0.08
4	4	4×0.409 / 50 = 0.03272	0.11
5	5	5×0.409 / 50 = 0.0409	0.14





System number	Absorbance	Concentration from Calibration Plot	Volume of Solution taken	Concentration of Lecithin found / 20 ml	Average Concentration of Lecithin found / 20 ml
Egg Yolk solution					
6	0.96	0.028	1	$\frac{0.028 \times 20}{1} = 0.56$	$\frac{0.56 + 0.29 + 0.16 + 0.19 + 0.14}{5} = \frac{1.34}{5} = 0.268$
7	0.10	0.029	2	$\frac{0.029 \times 20}{2} = 0.29$	
8	0.08	0.024	3	$\frac{0.024 \times 20}{3} = 0.16$	
9	0.13	0.038	4	$\frac{0.038 \times 20}{4} = 0.19$	
10	0.12	0.035	5	$\frac{0.035 \times 20}{5} = 0.14$	
Soyabean seed solution					
11	0.04	0.012	1	$\frac{0.012 \times 20}{1} = 0.24$	$\frac{0.24 + 0.17 + 0.16 + 0.145 + 0.13}{5} = \frac{0.845}{5} = 0.169$
12	0.06	0.017	2	$\frac{0.017 \times 20}{2} = 0.17$	
13	0.08	0.024	3	$\frac{0.024 \times 20}{3} = 0.16$	
14	0.10	0.029	4	$\frac{0.029 \times 20}{4} = 0.145$	

15	0.11	0.0325	5	$\frac{0.0325 \times 20}{5} = 0.13$	
Milk solution					
16	0.31	0.091	1	$\frac{0.091 \times 20}{1} = 1.82$	$\frac{1.82 + 1.48 + 1.38 + 0.98 + 0.96}{5}$ $= \frac{6.62}{5} = 1.324$
17	0.60	0.148	2	$\frac{0.148 \times 20}{2} = 1.48$	
18	0.84	0.207	3	$\frac{0.207 \times 20}{3} = 1.38$	
19	0.80	0.196	4	$\frac{0.196 \times 20}{4} = 0.98$	
20	0.98	0.24	5	$\frac{0.24 \times 20}{5} = 0.96$	
Sunflower Oil solution					
21	0.01	0.003	1	$\frac{0.003 \times 20}{1} = 0.06$	$\frac{0.06 + 0.12 + 0.193 + 1.4 + 1.292}{5}$ $= \frac{3.065}{5} = 0.613$
22	0.04	0.012	2	$\frac{0.012 \times 20}{2} = 0.12$	
23	0.10	0.029	3	$\frac{0.029 \times 20}{3} = 0.193$	
24	1.15	0.28	4	$\frac{0.28 \times 20}{4} = 1.4$	
25	1.32	0.323	5	$\frac{0.323 \times 20}{5} = 1.292$	

2. FTIR study of Soy- Lecithin

Spectral region wave number cm^{-1}	Pattern and intensity of Band	Bond causing Absorption	Compound Class
3867.28	Broad and low intensity	-	-
3741.90	Sharp and low intensity	-	-
3439.08	Broad and Strong intensity	O-H Stretching	Alcohol
3388.93	Broad and Strong intensity	O-H Stretching	Alcohol
3363.86	Broad and Strong intensity	O-H Stretching	Alcohol
3319.49	Broad and Strong intensity	O-H Stretching	Alcohol
3278.99	Broad and Strong intensity	O-H Stretching	Carboxylic acid
3228.84	Broad and Strong intensity	O-H stretching	Carboxylic acid
3184.48	Broad and Strong intensity	O-H stretching	Carboxylic acid
3128.54	Broad and Strong intensity	O-H stretching	Carboxylic acid
3010.88	Broad and strong intensity	O-H stretching	Carboxylic acid
2945.30	Broad and strong intensity	O-H stretching	Carboxylic acid
2868.15	Broad and Strong intensity	O-H stretching	Carboxylic acid
2677.20	Broad and Strong intensity	O-H stretching	Carboxylic acid

2360.87	Sharp and Strong intensity	O=C=O stretching	Carbon dioxide
2167.99	Broad and low intensity	-	-
2025.26	Broad and low intensity	-	-
1917.24	Broad and low intensity	C-H bending	Aromatic compound
1749.44	Sharp and Strong intensity	C=O stretching	Esters
1714.72	Broad and low intensity	-	-
1651.07	Broad and low intensity	-	-
1546.91	Broad and medium intensity	-	-
1462.04	Broad and medium intensity	C-H bending	Alkane
1425.40	Broad and low intensity	-	-
1388.75	Broad and low intensity	-	-
1346.31	Broad and low intensity	-	-
1247.94	Broad and low intensity	-	-
1209.37	Broad and low intensity	-	-
1097.50	Broad and low intensity	-	-
1072.42	Broad and low intensity	-	-
1001.09	Broad and low intensity	-	-
983.70	Broad and low intensity	-	-
925.83	Broad and low intensity	-	-
869.90	Broad and low intensity	-	-
844.82	Broad and low intensity	-	-
732.95	Broad and low intensity	-	-
682.80	Broad and low intensity	-	-
638.44	Broad and low intensity	-	-
584.43	Broad and low intensity	-	-
534.28	Broad and low intensity	-	-
497.63	Broad and low intensity	-	-
457.13	Broad and low intensity	-	-
416.62	Broad and low intensity	-	-

Conclusion

1. Determination of Lecithin from Egg Yolk, Soyabean seed, milk and sunflower oil Colorimetrically

Determination of Lecithin from Egg Yolk, Soyabean seed, milk and sunflower oil shows 0.5 g of egg yolk contains 0.268 g Lecithin, 2 Soyabean seeds contains 0.169 g Lecithin, 2 ml Milk contains 1.324 g Lecithin and 2 ml Sunflower Oil contains 0.613 g Lecithin.

2. FTIR study of Soy- Lecithin

Interpretation of FTIR Spectra of soy lecithin shows Presence of various functional groups such as C-H bending – Alkane, Aromatic compound; O-H Stretching – Alcohol, Carboxylic acid; C=O stretching – Esters and O=C=O stretching - Carbon dioxide.

Conflicts of interest: The author stated that no conflicts of interest.

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