



## **A Comparative Study on Isolation and Characterization of Lecithin from *Gallus gallus* and *Gallus Domesticus* Using Analytical Methods**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### **ABSTRACT**

The present study mainly focuses on the extraction and purification of lecithin from both *Gallus gallus* and *Gallus gallus domesticus*. The comparative study shows a great significance in the amount and quality of lecithin extracted from both the sources. The egg lecithin was standardized by Thin Layer Chromatography (TLC) analysis and Fourier transform infrared spectroscopy (FTIR) spectroscopy. TLC analysis was done and calculated for both standard and the samples. It was found to be the same for the test sample and standard soya lecithin. FTIR analysis shows the presence of Methyl group, alkane, carbonyl group, alkenes, hydroxyl alkyl ketone. FTIR helps to compare the intensity of functional groups in both lecithin isolated from *Gallus gallus* and *Gallus domesticus*.

**Keywords:** Lecithin; *Gallus gallus*; *Gallusgallus Domesticus*; TLC; FTIR.

## 1. INTRODUCTION

Lecithin is a fat that is basic in the cells of the body. It tends to be found in numerous nourishments, including soybeans and egg yolks. Lecithin is taken as a medication and is likewise utilized in the assembling of meds. Lecithin is utilized for treating memory problems, for example, dementia and Alzheimer's disease [1]. When lecithin is consumed, it is broken down into choline, which the body uses to transport fat, control metabolism, maintain cell structural integrity, and promote nerve transmissions (by synthesizing a neurotransmitter called acetylcholine). Choline isn't easily created by the body, so we have to get it from our food [2,3]. It is likewise utilized for treating gallbladder disease, liver diseases, high cholesterol, and anxiety. Some individuals apply lecithin to the skin as a moisturizer [4]. You will regularly consider lecithin to be a food added substance. It is utilized to shield certain fixings from isolating out. You may likewise consider lecithin to be fixing in some eye drugs. It is utilized to help keep the medication in contact with the eye's cornea. Studies of lecithin intellectual debilitation have utilized a wide scope of measurements, from 1 to 35g every day. A functional lipid found in egg yolk, likewise known as phosphor lipid, since its structure contains phosphorous. Examination study zeroed in on the relative investigation of lecithin in *Gallus gallus* and *Gallusgallusdomesticus*. Generally, eggs are viewed as a rich wellspring of lecithin and in this momentum research an endeavor has been made to separate and describe lecithin from egg //

## 2. MATERIALS AND METHODS

### 2.1 Lecithin Crude Extract Preparation

Three fresh egg yolks were separated, carefully washed with water, and passed through a sieve to remove the membranes. 50ml of cold acetone (4 °C) was added to the yolk suspension and the mixture was homogenized with a mechanical homogenizer equipped with a glass helix for 5 min. The suspension was filtered and the precipitate was collected. The acetone filtrate, which contains neutral fats and pigments, was discarded and the residual solvent was eliminated under vacuum. Centrifugation was done at 4000 rpm for 5min and the precipitate was collected so as to separate acetone from the egg Yolk. Remove the precipitate from the

centrifugal tube and wash it with acetone three to four times to remove soluble triglycerides and other pigments and filter the content using filter paper. Repeat the process until the yellow coloured egg yolk turns to white colour. Phospho lipid extraction is done from the washed sample by treating it with extracting solvent. Extracting solvent is the mixture of chloroform: ethanol in the ratio of 2:1<sup>5</sup>. 60ml of this solvent mixture is added into the precipitate and incubate it at room temperature for 3 hrs. Filter the content and the filtrate is transferred into a clean petridish and is incubated at room temperature or under nitrogen stream for 24hrs. The solvent gets evaporated. The crude lecithin is obtained by adding 30ml of petroleum ether. Lecithin gets dissolved in petroleum ether completely. 50ml of acetone is added and incubate for 3hr at room temperature. The Lecithin gets precipitated and will get completely settle down at the bottom and mixture of petroleum ether and acetone is /

## 2.2 Characterization Techniques

### 2.2.1 Thin Layer Chromatography (TLC)

Glassware used to perform the experiment was cleaned thoroughly using alcohol and is followed by washing with refined water and air-dried. Appropriate amount of silica powder was mixed with refined for to get slurry of silica. The readied slurry was spread consistently and equitably on the glass slide and dried to get the TLC plate. The solvent system required for the detection of Lecithin is prepared in the ratio of 65:25:4 using Chloroform: Methanol: Water [7]. To carry out the procedure a TLC chamber was made and is saturated with the solvent system. The phospholipids were dispersed in chloroform and is applied in the TLC plate as a small spot. The disengaged phospholipids were scattered in the fitting dissolvable (chloroform) and stacked like a little right on target the readied TLC plate, which is about 1.5mm from the base. For correlation, unadulterated soy lecithin was taken as the standard [8]. The TLC plate is then kept in the saturated framework for the solvent run. The solvent has to travel through the plate till it had voyaged three-fourths of the plate. At that point, the plate was taken out, air-dried, and kept in an immersed iodine chamber for 15 minutes for sample detection.

The different spots developed in each solvent system were identified by means of detecting agent and the  $R_f$  value are correspondingly calculated [9,10].

Calculation of Retardation factor (Rf value)

Retardation factor was calculated using the formula:

Retardation factor = (Distance travelled by compound in the substance / Distancetravelled by the solvent) [11].

### 2.3 FT-IR Spectroscopic Analysis

Biochemical [12], structural and functional groups can be analyzed using spectroscopy [13-16], HPLC [17-19], FTIR respectively [20]. In this study functional groups analysis for an sample was done on FTIR [21]. Fourier Transform Infrared Spectrophotometer (FTIR) analysis can be done to know the compound bonds/utilitarian gatherings present in the lecithin. The frequency of light retained is the notable component of the compound bonds found in the explained range. By deciphering the infrared ingestion range, the synthetic bonds in a compound can be resolved. The extricated lecithin was utilized for FTIR butt-centric as is. 10mg of the lecithin was epitomized in 100mg of KBr pellet, to plan clear example circles. The powdered example of each concentrate was stacked an FTIR spectroscope, with a Scan range from 400 to 4000  $\text{cm}^{-1}$  with a goal of 4  $\text{cm}^{-1}$  [22,23].

## 3. RESULT AND DISCUSSION

With the Thin layer of chr/omatography, the brownish-yellow spots were seen after the plate was kept in the iodine chamber. Rf factor was calculated for both standard and the samples. It was found to be the same for the test sample and standard soya lecithin. From this, we can say that the test sample is having lecithin. Rf value was found to be 0.78 for *Gallus gallus* and 0.77 for *Gallus gallusdomesticus* and the standard is having an RF value of 0.78.

### 3.1 FT-IR Spectrum Analysis

Characterization of Stabilized gel concerning the pertained functional group distribution was carried out by FTIR spectroscopy. The strong and broad intensity of the band at 2870  $\text{cm}^{-1}$  in the sample is assigned to a methyl group. The absorption band at 1730  $\text{cm}^{-1}$  confirms the presence of the carbonyl group. The presence of Alkyl amide, alkanes, alkyl ketone, and alkenes are also identified through this FTIR analysis. When comparing the Band intensity of lecithin in *Gallus gallus* and *Gallus gallusdomesticus*, the intensity was strong and broad in *Gallus gallus*.

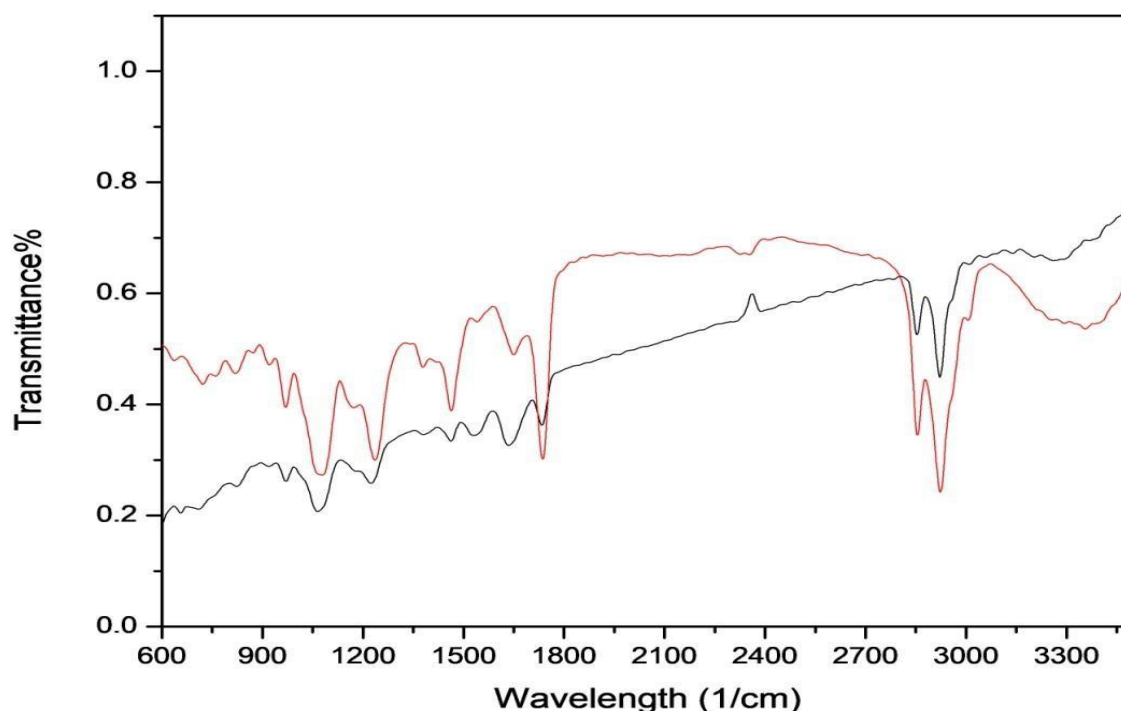


Fig. 1. FTIR spectrum curve

**Table 1. Functional group intensity**

| <b>S.no</b> | <b>Functionalgroup</b> | <b><i>Gallus gallus</i></b> | <b><i>Gallus gallusdomesticus</i></b> |
|-------------|------------------------|-----------------------------|---------------------------------------|
| 1           | Methylgroup            | Strong intensity            | Strong intensity                      |
| 2           | Carbonylgroup          | Strong intensity            | Strong intensity                      |
| 3           | Alkylamide             | Strong intensity            | Very less intensity                   |
| 4           | Alkanes                | Strong intensity            | Less intensity                        |
| 5           | Alkylketone            | Strong intensity            | Less intensity                        |
| 6           | Alkenes                | Strong intensity            | Very poor intensity                   |

#### 4. CONCLUSION

The present work has been performed to establish the TLC and FTIR parameters, which could serve as important and has commercial importance in both research institutes and pharma companies for the manufacturing of innovative medicines. With the above-obtained data, we could conclude that the lecithin was isolated successfully from eggs of *Gallus gallus* and *Gallus gallusdomesticus*. The eggs are having a high concentration of lecithin. Comparative FTIR analysis of both *Gallus gallus* and *Gallus gallusdomesticus* shows the higher concentration of lecithin in *Gallus gallus*. This data can be applied to the pharmaceutical companies for the preparation of potential lecithin drugs.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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