

**ASSESSMENT OF PHOSPHOLIPID CONTENT OF LOCALLY PRODUCED
CHICKEN EGGS FOR PHOSPHOLIPID COMPOSITION IN TORORO DISTRICT**

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DECLARATION

I, SSEBUTINDE KARIM, registration number BU/UP/2023/1852. Declare that this research dissertation is my own work, that all content presented is original, except for the references, and that the same work has not been submitted to any other university or institution of higher learning or for publication.

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APPROVAL

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ABBREVIATIONS

PC Phosphatidylcholine

PE Phosphatidylethanolamine

PI Phosphatidylinositol

PL Phospholipids

PLA₂ Phospholipase A₂

PS Phosphatidylserine

PUFA Polyunsaturated Fatty Acids

SM Sphingomyelin

TAG Triacylglycerols

ACE Angiotensin-Converting Enzyme

Chol Cholesterol

CO₂ Carbon Dioxide

CRP C-reactive Protein

CVD Cardiovascular Disease

DNA Deoxyribonucleic Acid

FA Fatty Acids

GI Gastrointestinal

TG Triglycerides

TNF α Tumor Necrosis Factor Alpha

HDL High-Density Lipoprotein

LPC Lysophosphatidylcholine

LPE Lysophosphatidylethanolamine

MetS Metabolic Syndrome

L-1, L-2, L-3 Local egg lecithin replicates 1, 2, and 3

E-1, E-2, E-3 Exotic egg lecithin replicates 1, 2, and 3

HPLC High-Performance Liquid Chromatography

LC-MS Liquid Chromatography–Mass Spectrometry

ELSD Evaporative Light Scattering Detection

ESI Electro-Spray Ionization

Rf Retention Factor

MS Mass Spectroscopy

FTIR Fourier Transform Infrared Spectroscopy

GC Gas Chromatography

NMR Nuclear Magnetic Resonance

TLC Thin-Layer Chromatography

UV Ultraviolet

ABSTRACT

This study investigated the extraction, characterization, and oxidative stability of phospholipids obtained from local and exotic chicken eggs collected from Tororo District. Lecithin was extracted from (30 g) of egg yolk using the Folch extraction method. Phospholipid classes were analyzed by thin-layer chromatography (TLC) with ninhydrin and iodine vapor visualization, and oxidative stability was assessed spectrophotometrically at (570 nm) over 7 days. Functional groups present in the extracted phospholipids were identified using Fourier Transform Infrared (FT/IR) spectroscopy. The results showed that local eggs produced (10.094 g) of crude lecithin from 30 g of yolk, whereas exotic eggs produced (7.760 g), indicating a higher lecithin yield in local eggs. TLC analysis confirmed the presence of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in both egg types. However, local egg lecithin consistently exhibited higher R_f values, suggesting the presence of more hydrophobic and possibly more saturated fatty acid chains. Oxidation studies revealed that local egg lecithin had lower absorbance values over 7 days, increasing from 0.712 on day 0 to 1.819 on day 7, compared to exotic egg lecithin, which increased from 0.973 to 2.335. These findings indicated that local egg phospholipids were more resistant to oxidation. FTIR analysis further confirmed the presence of characteristic phospholipid functional groups, including O-H, C-H, C=O, P=O, and P-O-C stretches in both samples. The sharper carbonyl absorption band observed in local egg lecithin suggested a greater degree of fatty acid saturation. Overall, the study demonstrated that local chicken eggs contain more stable phospholipids and may therefore provide advantages in nutritional and industrial applications.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Bird eggs are a common food and one of the most versatile ingredients used in cooking. They are well accepted as a safe and nutritious food for all ages and are important in many branches of the modern food industry; the most commonly used eggs are those from chicken(Adeyeye, 2012)(Cheng et al., 2025a). Hen's eggs are also considered a functional food, since they are a source of high-quality proteins, minerals, and lipids such as phospholipids and polyunsaturated fatty acids (PUFA)(Omri et al., 2019). Lipids in eggs are synthesized in the hen liver and are present exclusively in the yolk.(Cherian, 2022). The egg yolk is a single massive cell, weighing about 17-20 g in an average egg, and is composed of 51-52 % water, 16-17 % proteins, and 31-33 % lipids. An average egg has over 5.5-6 g of total lipids, and is present in the lipoprotein in the yolk (Cherian, 2015, 2022). Lipids, particularly phospholipids (PLs), are of substantial medical and industrial interest. Theoretically, PLs can be obtained from all kinds of biomass. However, the main commercial sources of PLs include vegetable oilseeds such as soybeans, rapeseeds, sunflower seeds, and animal sources such as egg yolk, milk, or marine organisms such as fish, roe, or krill.

In a commercial sense, a complex mixture of lipids such as phospholipids, cholesterol, and triglycerides is called lecithin(Kiełbowicz et al., 2015). Egg yolk lecithin is an important source of phospholipids, which makes the eggs ideal for industrial and biomedical applications. The main components of egg yolk lecithin are phosphatidylcholine (PC) and phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS)(Ali, Zou, Abed, Korma, Jin, & Wang, 2019), as well as lysophosphatidylcholine (LPC), sphingomyelin (SM), and neutral lipids

(triacylglycerols, TAG and cholesterol, Chol) in minor quantities (Cherian, 2022). Lecithin is widely used in the food industry as an emulsifier, lubricant, viscosity reducer, wetting, and release agent. Egg yolk lecithin has also found its use in cosmetics and pharmaceutical applications, especially in emulsion and liposome formation. Lecithin is also used as a dietary supplement, positively influencing the functioning of the nervous system and memory processes (Peterson & Cummings, 2006).

1.2 Problem Statement

Phospholipids in egg yolk are good fats essential for human brain health, heart function, and embryonic development (Nimalaratne et al., 2011). In Tororo District, poultry farmers rely on locally available feeds that may not provide adequate nutrients for hens to produce eggs rich in these compounds. However, there is little or no published data comparing the phospholipid levels in eggs from indigenous chicken breeds versus exotic commercial layers raised under local farming conditions in Tororo District. Therefore, farmers, agricultural extension workers, and policy-makers lack scientific evidence to determine which breed under reliable conditions produces eggs with higher phospholipid content. This information gap prevents informed decision-making regarding breed selection, feed improvement, and egg marketing strategies aimed at improving nutritional quality.

1.3 Objectives of the Study

The overall objective was to assess the phospholipid content of locally produced chicken eggs (indigenous vs exotic) in Tororo district.

The overall objective will be achieved by the following specific objectives;

- i) To identify the different phospholipid classes, present in the egg yolk extract of both chicken breeds using TLC
- ii) To determine which type of chicken egg produces phospholipids that resist oxidation longer when exposed to air.
- iii) To find out the functional groups in egg yolk lecithin present in chicken eggs.

1.4 Justification of the Study

The information obtained from this study will help poultry farmers to obtain information on the importance of phospholipids in eggs and also get knowledge on how different chicken breeds and rearing systems affect egg lipid quality. This will help provide data to Ugandan nutritionists and policymakers to develop better guidelines for regional poultry management.

1.5 Literature Review

1.5.1 Egg Lipids

The egg yolk is the primary source of lipids in an egg, and it contains high concentrations of lipids compared to the egg white as well as nutrients such as phospholipids, proteins, vitamins, and minerals(Cheng et al., 2025a)(Zhang et al., 2025). Egg lipids are an important component of eggs and play a crucial role in the development of the embryo, providing energy and essential nutrients(Nimalaratne et al., 2011). The lipid composition of eggs includes various classes of lipids such as triglycerides, phospholipids, cholesterol, and free fatty acids(Berger & Structure, 2014). The chicken eggs contain approximately 28 % of total lipids by weight as phospholipids, with the remaining 66 % as triglycerides (TG) and 5% as cholesterol. The average egg contains approximately 1.3 g of PL, which is almost exclusively found in the yolk.

1.5.2 Phospholipid content and composition of the Chicken Eggs

Phospholipids refer to phosphorus-containing lipids that were first separated from egg yolk by Gobley in 1844 and named lecithin in Greek (Zhao et al., n.d.). They are often referred to as polar lipids (van Hoogevest & Wendel, 2014). These lipids are essential components in cell membranes and consist of a hydrophilic head and a hydrophobic tail (Chen et al., 2019; Jacquot et al., 2014), allowing them to form a stable bilayer structure in an aqueous environment (Lordan, n.d.; Wood et al., 2019). They participate in cell metabolism and have unique structures and properties, ensuring the normal functioning of the cell (Berger & Structure, 2014; Zhao et al., n.d.). Phospholipids are one of the basic components of the cell membrane, which acts as a protective barrier for the cell and a channel for the exchange of environmental substances inside and outside the cell. The membrane is a site where many enzyme systems carry out biochemical reactions, which are the basis of living substances.

Phospholipids are key components of all biological membranes and are abundantly found in the diet, primarily as glycerophospholipid and sphingolipid classes. Dietary Glycerophospholipids are made up of two fatty acids (FA), a glycerol backbone, a phosphate group, and a polar organic molecule (choline, serine, inositol, or ethanolamine). Dietary glycerophospholipids are primarily absorbed in the gastrointestinal (GI) tract as lysophospholipids and free FA after pancreatic phospholipase A₂ (PLA₂) hydrolyses the fatty acyl bond at the sn-2 position. Glycerophospholipids are absorbed into the GI tract with high efficiency. Dietary sphingolipids are primarily in the form of sphingomyelin (SM), which consists of a ceramide with a phosphorylcholine head group. (Blesso, 2015).

1.5.3 Prospects of egg yolk lecithin

Lecithin is not only a high nutritional value substance, but also a highly bioactive functional component. For over 30 years, extensive and in-depth research has been conducted on transmitters of sustained-release drugs, maintenance of function, development of functional foods, and means to improve industrial production. In addition, as a natural emulsifier and wetting agent, lecithin is often used as an emulsifier for intravenous fat injection and the main embedding material for liposomes.

It has unique membrane permeability, and it is of high value to the packaging industry. At present, with the continuous improvement in the lecithin extraction and preparation processes (Zhao et al., n.d.). Currently, high-purity lecithin is in high demand in the international market, and usually refers to phospholipid products containing approximately 95 % PC. Because high-quality lecithin products are pure, have no peculiar smell, strong emulsification, and are easy to dissolve in water, they can be added and used in large quantities in the food industry and can also be made into health products and pharmaceuticals.

1.5.3.1 Egg Yolk Lecithin composition

Egg yolk lecithin is not a single chemical but a mixture of phospholipids, triglycerides, and sterols. The variability in the composition of the egg yolk depends on several factors like diet and breed. It is composed of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and lysophosphatidylcholine. (Blesso, 2015) Phosphatidylcholine (PC) is the main component of lecithin; the content of lecithin in egg yolk is three times higher than the content of lecithin in soybean. (Cheng et al., 2025b; Lordan, n.d.; Zhao et al., n.d.). It has also been shown that the content of lecithin in egg yolk and duck egg yolk is large, accounting for approximately 10 % of total lecithin.

Component	Content %
Phosphatidylcholine	73.0
Phosphatidylethanolamine	15.0
Phosphatidylserine	0.9
Phosphatidylinositol	0.6

Table 1: Phospholipid composition of egg yolk

1.5.3.2 Egg yolk lecithin liposome

Lipid hydration successfully produces liposomes containing different lipid compositions, size, polydispersity index, and zeta potential.(Mutalik et al., 2014). Egg yolk lecithin liposomes are drug carriers with distinct targeting. They are important in the preparation of drug delivery systems. Fatty acid composition and species of egg yolk lecithin have a great influence on liposome properties. Saturated fatty acids in the lecithin structure enhance the firmness and non-permeability of the liposome membrane. Unsaturated fatty acids in lecithin structures make liposomes have a lower phase transition, good fluidity, and low viscosity. Four types of egg yolk lecithin liposomes, which are conventional liposomes, PEG- modified liposomes, multifunctional liposomes, and ligand-targeted liposomes, currently exist. Each liposome has its own advantages and application fields. Both the main components of liposomes (e.g., lecithin, glycerin, and cholesterol) and the component proportions influence the structure of liposomes.(Zhao et al., n.d.).

1.5.3.3 Application of yolk lecithin

The present commercial egg yolk lecithin is mainly used in cosmetics, medications, and nutrition, and can be used in the preparation of liposomes and fat emulsions. The performance of liposomes from egg yolk, soybean, and porcine lecithin has been compared, and the results showed that liposomes made of egg yolk lecithin had the best performance. Fat milk injection is a type of nutritional injection used in the clinical setting. Fat milk is an energy supplement administered outside the gastrointestinal tract. Fat milk can supplement essential fatty acids and energy for patients, and egg yolk lecithin is used as an important component emulsifier. Because lecithin is an important component of biofilms, it can also be used as a drug carrier to form complexes with other drugs, thereby directly delivering them to diseased sites and improving bioavailability (Zhao et al., n.d.). Egg yolk lecithin is widely used in the feed industry and has significant effects. Adding phospholipids to broiler feed improves growth, increases storage in the liver, and promotes bone growth.

1.5.4 Natural Sources of Phospholipids

Natural phospholipids are defined as phospholipids isolated from natural sources; they are widely distributed in animal and plant tissues, and the main sources include vegetables such as soybeans, rapeseed, cotton seeds, corn and sunflower seeds, wheat germ, and flax seeds, while in animal tissues include egg yolk, milk or krill, and bovine brain(Cui & Decker, 2016). These raw materials are produced worldwide at a very large scale. The worldwide harvest of soybeans in 2013 was 280 million tons, and annual worldwide production is about 42 million tons. During 2022, 53.4 million tons of table eggs were produced; this represents approximately 17 million tons of egg yolk(van Hoogevest & Wendel, 2014). Although part of these huge amounts is used for the production of natural phospholipid excipients, it is clear that there is an adequate supply for this purpose. The

phospholipid and fatty acid profile are dependent on the raw material sources. In terms of production, egg yolk and soybean are the most important sources of phospholipids(Ali, Zou, Abed, Korma, Jin, Wang, et al., 2019); however, they have different phospholipid content, where egg yolk lecithin contains higher amounts of PC, and it has long-chain polyunsaturated fatty acids, unlike in soya bean lecithin.

Natural phospholipids may be further converted to saturated phospholipids by means of hydrogenation or further treated with enzymes to remove partial fatty acids(van Hoogevest & Wendel, 2014).

1.5.5 Health-beneficial functions of egg yolk lipids

Beneficial effects of dietary phospholipids (PLs) have been mentioned since the early 1900's in relation to different illnesses or diseases and symptoms, such as coronary heart disease, inflammation, cancer, and cholesterol absorption. The previous studies indicated that dietary PLs have positive influences on various diseases, seemingly without side effects. Additionally, PLs can decrease the side effects of several medications(Ali, Zou, Abed, Korma, Jin, & Wang, 2019).

Lipids are considered one of the six major nutrients required for embryonic development and survival and the biological activities of infants and adults. Neutral lipids and cholesterol in the yolk are directly transferred into embryo tissues without degradation and resynthesis, but some phospholipids can be synthesized de novo in the developing chick embryo. However, most of the phospholipids in the embryo and its organs originate from the yolk phospholipids, and the transfer rate becomes faster at later stages of embryo development. Phospholipids are major components in cell membranes, facilitate cell fusion, cause hemolysis, and affect the permeability of phosphatidylcholine liposomes. They also affect the pathways related to inflammation and

metabolism, HDL, and cholesterol. They can also be used widely as a drug delivery agent due to their good biocompatibility and amphiphilicity(Abeyaratne et al., 2022).

PC is an important component of the cell membrane and plays a vital role in the functions of nerve cells, cholinergic neurotransmission, and lipid transport in the liver. It is also highly effective in controlling Alzheimer's disease in humans.

In humans, the dietary deficiency of choline causes fatty liver, birth defects, and neurological dysfunctions. Choline increases lymphocyte apoptosis and DNA and muscle damage in humans, and its supplementation during pregnancy enhances hippocampal neurogenesis in the fetus. The choline intake of 450-550 mg/day is recommended for pregnant and lactating women. Pregnant women with less choline in their diet have four times higher risks of giving birth to a child with a neural tube defect. Choline in blood, plasma, and serum helps to detect acute coronary syndromes in human, helps in predicting cardiac disorders, and therefore can be considered a good agent in future medicine.

1.5.6 Current and potential uses of egg yolk lipids and their derivatives

Almost all phospholipids used in food and non-food applications at the industry level are from the soybean, and only limited amounts of phospholipids in egg yolk are used. However, the number of phospholipids in eggs is more than tenfold (dry-weight basis) higher than that in soybean. In this way, many methods used to separate egg yolk lipids are currently available; thus, finding new areas for egg yolk lipids will be important to increase egg consumption(Abeyrathne et al., 2022).

1.5.6.1 As a food supplement

Chicken egg yolk is an excellent food emulsifier and is important in many foods such as mayonnaise, salad dressings, ice cream, and bakery products. Dietary intake of egg PL may lead

to a reduction in inflammation. Consuming three eggs per day for 12 weeks resulted in a reduction in plasma C-reactive protein (CRP) and an increase in adiponectin in overweight men, changes which were not observed with yolk-free egg substitute. Egg consumption has also led to improvements in circulating plasma inflammatory markers in adults with Metabolic Syndrome (MetS). In combination with moderate carbohydrate restriction, the addition of three eggs per day led to decreases in plasma TNF α and serum amyloid A in men and women with MetS, whereas no changes were observed in subjects consuming a yolk-free egg substitute. Egg yolk contains a significant amount of choline as PC. Dietary choline intake is inversely associated with serum inflammation markers in healthy adults. Some studies have suggested that dietary PC may reduce inflammation in the GI tract. Therapeutic usage of oral PC (>1 g daily) in ulcerative colitis is well-documented and improves inflammation. This GI anti-inflammatory effect of PC may be of significance to MetS, as there is evidence linking GI inflammation to the development of obesity and insulin resistance in animal models.

1.5.6.2 As pharmaceutical and nutraceutical agents

Phospholipids are used in pharmaceutical technology as wetting agents, emulsifiers, and builders or components of mesophases like liposomes, micelles, mixed micelles, among others. These functional properties are used in many formulation types, like suspensions, various types of emulsions, solid dispersions, drug-phospholipid, etc. In addition, the hydrolysis products of phospholipids (Lys phospholipids) can be used as nutraceuticals and pharmaceutical agents to improve fertility, maintain pregnancy, promote cell proliferation and cell growth, and cure/prevent various diseases, including cancer. Liposomes prepared with phospholipids are used as a drug carrier for drug delivery systems because they can protect drugs from digestion in the stomach,

improve bioavailability in the gut, reduce drug toxicity, and deliver drugs to the targeted sites(van Hoogevest & Wendel, 2014).

These yolk components are also useful for gene delivery to cells in culture and gene therapy in preclinical trials because cationic liposomes can easily form stable complexes with DNA. The use of liposomes facilitates the oral administration of drugs that can bypass the stomach and slowly release the compounds. Carotenoids can be used as a functional food component to prevent many non-communicable diseases, including Alzheimer's, cardiovascular disease (CVD), and breast cancer in women(Abeyrathne et al., 2022). The high-density lipoprotein (HDL) in egg yolk has antioxidant, anti-inflammatory, and arteriosclerosis activities, which are closely associated with the composition of PC and phosphatidylinositol. Since yolk HDL reduces oxidative stress, it can be an alternative compound against CVD(Zhao et al., n.d.).

1.5.7 Basic properties of egg yolk lecithin

1.5.7.1 Physical and chemical properties of egg yolk lecithin

High- purity egg yolk lecithin products are white, waxy solids. When lecithin is in a liquid form, it is light yellow and slippery with a peculiar smell. Egg yolk lecithin contains an abundance of unsaturated fatty acids, and most of the lecithin products appear slightly darker than they are supposed to; this is due to the unsaturated fats being oxidized. In the production of lecithin, the dosage forms are in different forms due to the concentration.

Egg yolk lecithin is a natural phospholipid mixture extracted and refined from egg yolk, and is an amphiphilic molecule. The volume of the egg yolk is 30-32 % of the whole egg. The whole egg contains fats (30 %), proteins (15 %), moisture (50 %), and other chemicals.(Zhao et al., n.d.).

1.5.8 Physiological function of egg yolk lecithin

1.5.8.1 Strengthening nerve conduction

Brain nerve cells contain a large amount of lecithin. The content of lecithin accounts for approximately one- fifth of brain nerve cell mass. Lecithin in brain cells is transformed to release choline, which combines with acetyl- CoA to produce acetylcholine. Acetylcholine is an important chemical transmitter for information transmission between various nervous systems, so lecithin can increase the degree of brain cell activation and improve memory and intelligence. Using the brain for a long time in life and work will consume a large amount of lecithin in the body, leading to a decline in brain function. Relevant studies have shown that with age, brain function gradually declines(Zhao et al., n.d.). Consuming lecithin products effectively prevents further aging of the brain and strengthens brain function. During the growth stage, children should eat foods rich in lecithin nutrients, such as eggs. Nuts and animal liver have a positive effect on brain development, learning, and memory as well. Alzheimer's disease is a neurodegenerative disease characterized by a loss of memory and cognitive impairment. Eating egg yolk lecithin improves memory and cognitive function and delays the onset of neurodegenerative disease.

1.5.8.2 Regulation of blood lipids

In recent years, hyperlipidemia became a common metabolic disorder, causing cardiovascular and cerebrovascular diseases that negatively impact healthful living. The molecular structure of egg yolk lecithin has dual characteristics (hydrophilicity and lipophilicity). Therefore, an emulsification reaction occurs in the body, combining lipid substances with water and emulsifying the accumulated cholesterol and neutral fats in the blood vessels. Egg yolk lecithin serves as some kind of “garbage” (i.e., cholesterol and neutral fats) remover in blood vessels, making blood circulation in the blood vessels of the entire body smoother, promoting blood flow, and penetration

into the body, thereby reducing the viscosity of human blood. Thus, egg yolk lecithin can effectively reduce hyperlipidemia and coronary heart disease. Epidemiologic studies have shown that hypertension is a major cause of heart-blood diseases, such as sudden death and coronary heart disease.(Zhao et al., n.d.). Egg yolk lecithin lowers blood pressure by inhibiting angiotensin-converting enzyme (ACE) reported that 15mL of lecithin fed to patients with metabolic syndrome three times a day significantly improved symptom.

1.5.8.3 Repair biofilms and delay body aging

Biofilms are structured communities of microorganisms, such as bacteria, fungi, and algae, that adhere to surfaces and are embedded in a self-produced extracellular matrix. This matrix is primarily composed of polysaccharides, proteins, and nucleic acids, providing structural stability and protection to the microorganisms within.

Lecithin is very important for the composition of animal somatic cells. Without lecithin, cell membranes will be damaged to varying degrees. PC promotes the synthesis and regeneration of lipoproteins, repairs damaged cell membranes, increases desaturation of cell membrane fatty acids, softens and rejuvenates the cell membrane, protects mitochondria and microsomal membranes of somatic cells, and maintains cell structure. In the process of individual aging, the function of the antioxidant defense system in the body gradually weakens, and free radicals accumulate, which will cause excess free radicals to react with unsaturated fatty acids to form a peroxide and eventually deform the organelles through a series of interactions in which neurons are damaged, and lecithin can scavenge free radicals, which improves the metabolic capacity. Lecithin can also promote skin regeneration, make the skin shiny, and prevent hair loss, which makes gray hair darker and slows the aging process(Zhao et al., n.d.).

1.5.9 Methods used during the extraction of egg yolk lecithin

Egg yolk lipids are composed of phospholipids, neutral lipids, cholesterol, and other minor lipids, and many methods have been developed to separate them separately or together from the rest of the egg components. The two most common methods of separating lipid components from the egg yolk are solvent and supercritical extraction. Organic solvents can be classified into two main groups, polar and non-polar. Among the solvents used, ethanol, methanol, dichloromethane, and ethyl acetate are considered polar, while hexane, heptane, butane, and chloroform are non-polar solvents.(Abeyrathne et al., 2022).

1.5.9.1 Organic solvent extraction method

Solvent extraction uses single solvents or solvent combinations and can scale up easily to industrial levels, and the separated compounds using solvents can be used in food, pharmaceutical, nutraceutical, and cosmeceutical industries. The conventional lipid extraction method that uses solvents is called the Bligh and Dyer method, which uses chloroform and methanol to extract lipids from an organic source(Abeyrathne et al., 2022). In addition, it is a method used to separate and extract lecithin by using the difference in the selectivity of the solvent to the components of phospholipids. Solvent extraction is also the earliest method used for phospholipid extraction. There are two methods (single and mixed organic solvent extraction methods). During single organic solvent extraction, ethanol is used to extract lecithin from fresh egg yolk, and the extraction rate can be 93.38% under suitable process conditions. The mixed organic solvent extraction method uses the characteristics of lecithin that is soluble in ethanol, but insoluble in acetone to extract lecithin from eggs(Zhao et al., n.d.). The organic solvent extraction method is characterized by a simple operation and low requirements for conditions, but the organic solvent extraction method takes too long, the purity is not high, and there may be residual organic solvents.

1.5.9.2 Super-critical and sub-critical extraction

Supercritical extraction is a new type of separation technology that separates substances by changing the temperature and pressure according to the difference in the properties of substances in the supercritical state. Supercritical carbon dioxide extraction technology can extract lecithin with higher purity (95–98%) from egg yolk powder. Because phospholipids are insoluble in supercritical CO₂, it is necessary to add an entrainer to grade phospholipids(Zhao et al., n.d.).

Subcritical extraction refers to a fluid extraction technology that uses a thermodynamic state at the edge of a supercritical state and is above the critical pressure and below the critical temperature. This technology is used to extract valuable compounds from plant materials, such as essential oils, flavors, and pharmaceuticals(Abeyrathne et al., 2022). Compared with the supercritical fluid method, the subcritical fluid method has milder conditions and less stringent requirements for high-pressure equipment(Zhao et al., n.d.). On the other hand, the subcritical fluid method can better retain the natural active ingredients in the extracted product with a lower cost.

Other extraction methods include the column lamination method, enzymatic hydrolysis extraction method, and membrane separation methods.

1.6.0 Analysis of phospholipids

Investigations of lipid composition of the lipid mixtures such as lecithin require accurate methods for the separation and quantification of lipid classes. Because of different chemical compositions, it is often difficult to characterize one class of lipids in the presence of another, especially using rapid methods. However, analytical tools for analyzing phospholipids based on chromatographic techniques include high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), mass spectrometry (MS), nuclear magnetic resonance (NMR)(Ali, Zou, Abed, Korma, Jin, & Wang, 2019)gas chromatography (GC), and Fourier transform infrared spectroscopy

(FTIR)(Latif et al., 2014). TLC is used for initial separation, while HPLC and GC are employed in quantitative analysis(Kiełbowicz et al., 2015). Thin-layer chromatography (TLC) was the earliest chromatographic method used to assess phospholipids, and is frequently used today(Liu et al., 2018). Gas chromatography (GC) has applications for identifying individual fatty acids present in phospholipids, but is generally not used to assess phospholipids. HPLC is the most popular technique used currently for separating lipid classes. However, advances in liquid chromatography–mass spectrometry (LC-MS)(Wang et al., 2023), especially those tied to electrospray ionization (ESI), have resulted in a decrease in studies using HPLC to assess phospholipids. Except for TLC, there is a variety of detectors that can be coupled to the above chromatographic methods, including refractive index (RI), ultraviolet (UV), fluorescence, and evaporative light scattering detection (ELSD)(Peterson & Cummings, 2006).

CHAPTER TWO

EXPERIMENTAL

2.1 Apparatus

Eggs obtained from two different places in Tororo district were weighed using an AX523 model, S/N: B735600861, NJ 07054 USA, 12V--- 0.8A, OHAUS Corporation, OHAUS Europe GmbH, (www.Ohaus.com), analytical balance. A constant temperature during extraction was maintained using a C.012.01/WNB10/NT, 230V-50/60Hz, DIN12878-3-W.:1, Schutz art DIN EN 60529-1P20, Type: WNB10, water bath made in Germany. The mixture was vigorously stirred using a C.OO2.01/SA8/NT, Serial N^o series R800011055, Power Puissance 20W, vortex designed in the

UK, assembled in PRC (www.bibbly.scientific.com). Chloroform under reduced pressure was removed by a C.034.01/WNB10/NT, NEO.Tech.S.A. rotary evaporator made in China. Beakers were used to hold samples during the experiment. The Fourier transform infrared spectrometer (FTIR) was used to identify the different functional groups present in the isolated phospholipids. A sterile knife was used to crack the egg; a syringe was used to remove the top methanol layer during extraction.

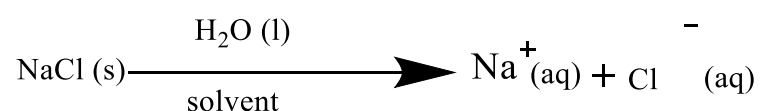
2.2 Materials

All chemicals to be used were analytically graded. They included chloroform, methanol, ethanol, distilled water, hexane, sodium chloride solution, potassium iodide solution, starch indicator, and ninhydrine reagent.

2.2.1 Sodium chloride solution

Sodium chloride solution was prepared by dissolving analytical-grade solid sodium chloride (9.0 g) in distilled water (1000 mL).

Equations for the reaction



2.2.2 Folch reagent

A mixture of chloroform and methanol was measured in the ratio of a 2:1 volume ratio (v/v), that is, (200 mL) chloroform and (100 mL) methanol

2.2.3 Potassium iodide solution

Potassium iodide solution was prepared by dissolving analytical-grade solid potassium iodide (1.00 g) in distilled water (20 mL).

2.2.4 Starch indicator

Soluble starch (0.5 g) was mixed with a few milliliters of cold water to make a smooth paste. The solution formed was then transferred into a beaker containing (100 mL) of distilled water. The mixture was stirred and boiled for (3 minutes) until it became clear.

2.2.5 Ninhydrin solution

Ninhydrin solution was prepared by dissolving analytical-grade ninhydrin (0.2 g) in ethanol (100 mL) and was stored in a cool, dark condition.

2.2.6 Standard solvent system for phospholipid separation

In a fume hood, a mixture of chloroform, methanol, and water was measured in the ratio of (65:25:4 v/v/v)

2.2.7 Solvent system for peroxide test

In a fume hood, a mixture of acetic acid and chloroform was measured in the ratio of (3:2 v/v)

2.2.8 Blank solution used in the determination of peroxide

To a mixture of the solvent (acetic acid: chloroform) (3 mL), 5 drops of glacial acetic acid were added, followed by 5 drops of potassium iodide solution; to the resultant solution (3 mL) of distilled water was added, followed by 5 drops of starch indicator.

2.3 Sample preparation

2.3.1 Procedure

Fresh chicken eggs (4-10) were collected from different parts of Tororo District, specifically in Nagongera Town Council. They underwent a gentle washing process using distilled water to avoid contamination; they were then weighed accurately one at a time using an analytical balance. The egg content was separated from the egg and albumen by cracking the egg using a sterile knife to

avoid contamination. Approximately (30.0 g) of the egg content was weighed using an analytical balance and then placed into a clean small glass beaker and transferred into a mortar and pestle to create a smooth paste and later placed into a boiling tube. Folch reagent was added to the paste formed. The resultant mixture was stirred up vigorously for (2-3 minutes) using a vortex mixer to ensure that the egg yolk penetrated the yolk matrix. Furthermore, the mixture was left to stand for about (10-15 minutes) at room temperature, as this allowed it to separate into two distinct layers, the lower chloroform layer containing lipids and the upper methanol/water layer containing non-lipid impurities like salts. A syringe was used to carefully collect the upper layer, leaving the lower chloroform layer, which was transferred into a clean glass container. During the washing process, the chloroform layer was mixed with an equal volume of sodium chloride solution (0.9 %), which was shaken gently and allowed to separate; the lower layer was collected again and placed in another glass beaker. A rotary evaporator was used to remove the chloroform under reduced pressure, keeping the temperature low (below 40°C) to avoid degradation of phospholipids.

2.4 Determination of phospholipid class using TLC

2.4.1 Procedure

The solvent was poured into the developing TLC tank to a depth of (0.5-1.0 cm), and the lid was carefully closed to allow the chamber to saturate for (30 minutes). The plate marked with spots using a capillary tube (local egg yolk lecithin) along the line (1-2 cm from the bottom) was carefully placed into the developing chamber, ensuring that the solvent did not touch the spots, and the tank was closed tightly. The solvent was allowed to rise to the top of the plate, which was later removed, and the solvent front was marked using a pencil. The plate was allowed to dry in a fume hood for (15 minutes) to remove the solvent. The plate was sprayed with ninhydrin solution and placed on a hot plate at (100-120 °C) for (5 minutes) until the purple-pink spots appeared. The

spots were marked with a pencil and used to calculate the R_f values of each spot. The same procedure was used on exotic egg yolk lecithin.

2.5 Determination of oxidation stability of local eggs vs exotic eggs using a spectrophotometer

2.5.1 Procedure

The spectrophotometer was set at a wavelength of (570 nm) zeroed using the blank solution in a cuvette (1 cm) to determine the absorbance of the samples that had been stored for seven days. In cuvettes named (L-1, L-2, L-3 and E-1, E-2, E-3), (0.1 g) of the lecithin sample for both chicken eggs (Local –L and Exotic-E) was added to all the 6 cuvettes, respectively. To the cuvettes, the solvent (3 mL) was added to dissolve the lecithin, followed by 5 drops of glacial acetic acid. To the resultant solution, 5 drops of potassium iodide solution were added, followed by (3 mL) of distilled water, and finally 5 drops of starch indicator were added to the solution formed and allowed to mix for 3 minutes. The cuvettes were then carefully put in the spectrophotometer to measure the absorbance of the different replicates of the egg yolk lecithin (L and E)

2.6 Identification of functional groups

Both local and exotic egg yolk lecithin samples were analyzed by Fourier Transform Infrared spectroscopy (FT/IR) to identify the key functional groups present in the isolated phospholipids by detecting specific absorption bands associated with the different chemical bonds present in different phospholipid fractions. The spectra obtained from the chicken egg provided valuable information about the specific functional groups present in isolated phospholipids.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Yield of egg yolk lecithin extracted from chicken egg contents

Sample	Weight of egg yolks(g)	Weight of extract (g)
Local	30.00	10.094
Exotic	30.00	7.760

Table 2: Shows the weight of egg yolk lecithin extracted from the egg contents

The extraction of lecithin from both local and exotic chicken egg yolks was carried out using the Folch method, which is a standard and widely accepted technique for total lipid extraction from biological samples; therefore, the extracted weights represent crude lecithin (a mixture of phospholipids, neutral lipids, and sterols). From the initial yolk weight of 30.0 g for each sample, the local chicken eggs yielded 10.094 g of crude lecithin extract, while the exotic yielded 7.760 g under identical experimental conditions. The local chickens used in this study were indigenous breeds raised in Tororo District under free-range conditions. The birds fed naturally, fed on insects, green vegetables, seeds, and household food scraps, thus increasing the total lipids contained in the egg yolk lecithin. In contrast, the exotic commercial layers were likely fed standardized commercial poultry feeds formulated to maximize the number of eggs produced rather than the nutritional density of each egg.

3.1.1 Thin layer chromatography (TLC) profile of egg lecithin lipids

Sample	Solvent front (cm)	Lower spot (cm)	Lower spot Rf values	Upper spot (cm)	Upper spot Rf value
Local	4.70	0.800	0.170	2.90	0.617
Exotic	4.90	0.790	0.161	2.80	0.571

Table 3: Shows Rf values of lipid components from local and exotic chicken egg lecithin visualized by spraying ninhydrin solution

Sample	Solvent front (cm)	Lower spot (cm)	Lower spot Rf values	Upper spot (cm)	Upper spot Rf values
Local	3.80	1.10	0.289	3.50	0.921
Exotic	3.80	1.00	0.263	3.30	0.868

Table 4: Shows Rf values of lipid components from local and exotic chicken egg lecithin visualized with iodine vapor

$$\text{Retention factor (Rf)} = \frac{\text{Distance moved by the spot}}{\text{Distance moved by the solvent front}}$$



Figure 1: Shows a TLC plate sprayed with ninhydrin solution for local



Figure 2: Shows a TLC plate sprayed with ninhydrin solution for exotic



Figure 3: Shows a TLC plate stained in iodine vapor for both local and exotic egg samples

Thin layer chromatography was used to separate the lipid components, and 2 different visualization methods were employed, that is, ninhydrin solution and iodine vapor.

3.1.1.1 Ninhydrin solution

Ninhydrin solution reacts specifically with primary amine groups. Therefore, it only detects phospholipids that contain a free amine. In egg yolk lecithin, the two main phospholipids that react with ninhydrin are phosphatidylethanolamine, PE, and phosphatidylserine, PS. When the TLC plates were sprayed with ninhydrin, both local and exotic samples showed two distinct purple-pink spots. The lower spot, which stayed closer to the origin where the sample was applied, had R_f values of 0.170 for local eggs and 0.161 for exotic eggs. This lower spot is tentatively identified as phosphatidylethanolamine. PE is more polar than other phospholipids because its ethanolamine head group can form strong hydrogen bonds with the silica gel stationary phase, so it does not

travel far up the plate. The upper spot had Rf values of 0.617 for local eggs and 0.571 for exotic eggs. This upper spot is tentatively identified as phosphatidylserine. PS contains both an amine group and a carboxyl group, giving it intermediate polarity and thus migrating further than PE but not as far as neutral lipids. The local egg sample showed higher Rf values for both spots compared to the exotic sample. This means that the amino-containing phospholipids from local eggs traveled further up the plate.

3.1.1.2 Iodine vapor

Iodine vapor stains all lipids non-specifically. It does not react chemically but rather dissolves reversibly into the hydrophobic regions of lipid molecules, producing a yellow-brown color. Therefore, iodine vapor provides a complete profile of all lipid classes present in the crude lecithin, including phospholipids that do not react with ninhydrin such as phosphatidylcholine, as well as neutral lipids such as triglycerides and cholesterol esters. When the plate was exposed to iodine vapor, both local and exotic samples showed two distinct spots. The lower spot on the iodine-stained plates had Rf values of 0.289 for local eggs and 0.263 for exotic eggs. This spot is tentatively identified as phosphatidylcholine, which is the most abundant phospholipid in egg yolk. PC does not react with ninhydrin, which is why it did not appear on the ninhydrin plates at this Rf position. The upper spot on the iodine-stained plates had Rf values of 0.921 for local eggs and 0.868 for exotic eggs. Such high Rf values indicate compounds that are highly non-polar and travel very close to the solvent front. These are almost certainly neutral lipids, primarily triglycerides and cholesterol esters, which are also present in crude lecithin extracts.

The local egg sample showed higher Rf values for both spots compared to the exotic sample. The local phosphatidylcholine spot traveled further with an Rf of 0.289 compared to 0.263 for the

exotic. The local neutral lipids traveled further with an Rf of 0.921 compared to 0.868 for exotic. This consistent pattern across both visualization methods and across all spots is highly significant. It strongly suggests that the local egg lecithin contains fatty acids that are generally more hydrophobic, meaning longer chain lengths or higher degrees of saturation, across all lipid classes.

3.2 Oxidation absorbance profile of local (L) and exotic (E) egg lecithin

Table 5: shows absorbance of local and exotic chicken egg lecithin during oxidation over 7 days

SAMPLE	DAY 0	DAY 3	DAY 7
L1	0.758	1.079	1.982
L2	0.805	1.003	2.247
L3	0.572	0.949	1.227
Average	0.712	1.010	1.819
E1	0.928	1.266	2.283
E2	0.998	1.086	2.458
E3	0.944	1.294	2.264
Average	0.973	1.215	2.335

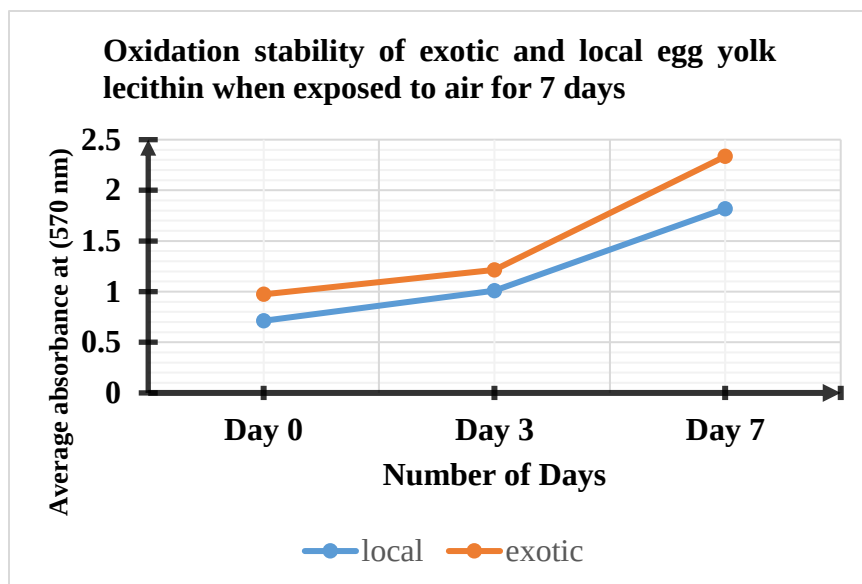


Figure 4: shows oxidation stability of exotic and local egg yolk lecithin we exposed to air for 7 days

To investigate which chicken egg is more resistant to oxidation, crude lecithin extracted from both local and exotic chicken eggs was exposed to air at room temperature, and the extent of oxidation was monitored using a spectrophotometer at a wavelength of 570 nm over a period of 7 days. The principle of this method is based on the formation of primary oxidation products such as hydroperoxides during phospholipid oxidation.

On day 0, immediately after extraction, the local egg lecithin samples had an average absorbance of 0.712, whereas the exotic egg lecithin samples showed a higher average absorbance of 0.973. This initial difference is important because it suggests that the exotic egg lecithin already contained a greater amount of pre-existing oxidation products before the deliberate exposure to air began. This could be due to feeding hens with diets rich in polyunsaturated fatty acids, which are more prone to oxidation during storage and handling; also, local egg lecithin naturally contains higher

concentrations of endogenous antioxidants that provide greater protection against oxidation before experimentation.

By day 3, both local and exotic egg lecithin samples showed increased absorbance values, indicating continued oxidation. The average absorbance for local egg lecithin increased to 1.010, while that of exotic egg lecithin rose to 1.215.

By day 7, the distinction between the two egg types became more evident. The average absorbance of local egg lecithin reached 1.819, and the exotic egg lecithin reached an average absorbance of 2.335. This finding indicates that, after 7 days of exposure to air, exotic egg lecithin produced significantly greater quantities of oxidation products than local egg lecithin.

3.3 Characterization of egg phospholipids

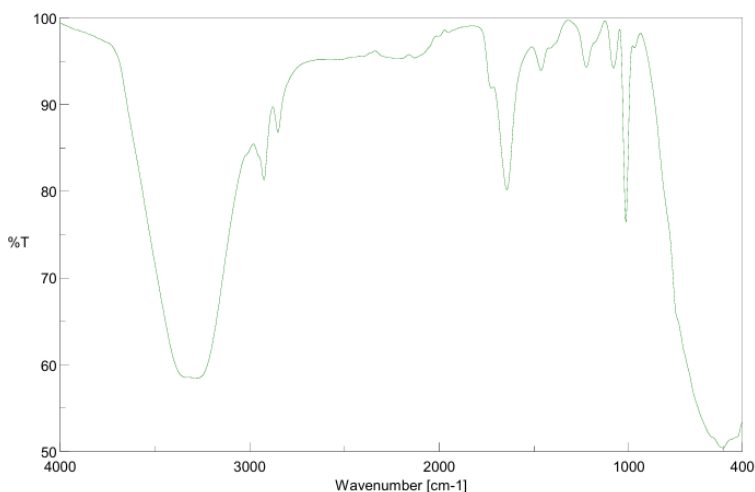


Figure 5 Shows the FT/IR absorption spectra for exotic chicken egg yolk lecithin

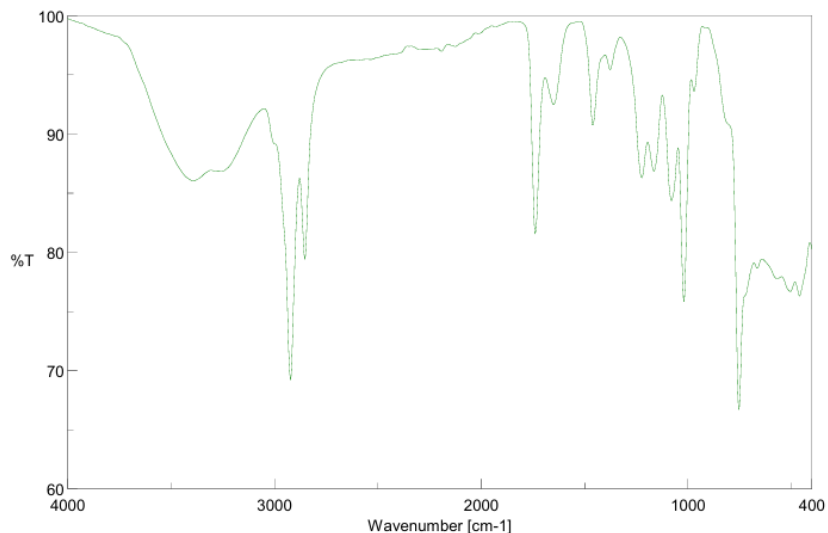


Figure 6: Shows the FT/IR absorption spectra for local chicken egg yolk lecithin

Fourier Transform Infrared (FTIR) spectroscopy was utilized to determine the functional groups of the extracted egg yolk lecithin from both local and exotic chicken breeds. The spectra for both samples (Figures 4 and 5) revealed characteristic absorption bands corresponding to the primary functional groups of phospholipids.

The findings presented in Figures 4 and 5 indicated the presence of common functional groups in the lecithin extract, which include an O-H stretch at $\sim 3400\text{ cm}^{-1}$ (broad band). This broad band is characteristic of hydrogen-bonded hydroxyl groups. The broadness indicates hydrogen bonding, typical of water and alcohols. C-H asymmetric stretch (CH_2 , CH_3) at $\sim 2923\text{ cm}^{-1}$ (strong, sharp). This strong, sharp band comes from the methylene ($-\text{CH}_2-$) and methyl ($-\text{CH}_3$) groups of the long-chain fatty acids esterified to the glycerol backbone. The high intensity reflects the abundance of fatty acyl chains in lecithin. C=O stretch (ester carbonyl) at $\sim 1737\text{ cm}^{-1}$ (sharp, strong). This sharp, intense band is characteristic of the ester carbonyl group where fatty acids are covalently bonded to the glycerol backbone via ester linkages. This is a key feature of both phospholipids and

triglycerides (neutral lipids) present in crude lecithin. Its Slightly sharper and has a higher wavenumber in the local egg yolk lecithin (Fig. 5). The sharpness indicates a well-defined carbonyl environment and more saturated fatty acids. P=O asymmetric stretch (phosphate) at $\sim 1240\text{ cm}^{-1}$ (strong, broad). This is a diagnostic band for phospholipids, and the phosphate group (P=O) in the phospholipid head region gives this strong, broad absorption, which is unique to phosphorus-containing lipids and confirms the existence of phospholipids in both extracts.

P-O-C stretch (phosphodiester) at $\sim 1050\text{-}1080\text{ cm}^{-1}$. This band corresponds to the phosphodiester linkage of the hydrophilic head group such as choline or serine, confirming the amphiphilic nature of the extracted lecithin.

CHAPTER FOUR

CONCLUSIONS AND RECOMMENDATION

4.1 Conclusions

This study demonstrated that local chicken eggs from Tororo produced a higher yield of crude lecithin and phospholipids with greater oxidative stability than exotic chicken eggs. TLC analysis showed that local egg phospholipids had higher R_f values, suggesting a more hydrophobic and likely more saturated fatty acid composition. FTIR analysis confirmed the presence of characteristic phospholipid functional groups in both egg types. The oxidation study further revealed that local egg lecithin was more resistant to oxidation during 7 days of exposure to air, as shown by consistently lower absorbance values compared to exotic egg lecithin. These findings suggest that local chicken eggs contain more stable phospholipids, making them potentially more beneficial for nutritional, pharmaceutical, and food industry applications.

4.2 Recommendations

The TLC analysis was conducted without the use of authentic phospholipid standards such as pure phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), or phosphatidylinositol (PI) as positive controls. Consequently, the identification of lipid spots was based only on comparison of R_f values with those reported in the literature. This limitation is important because, in the absence of authentic standards, the identification of the spots as PE, PS, and PC remains tentative. Other phospholipids or lipid degradation products may potentially migrate to similar R_f positions on the TLC plate, leading to possible misidentification. To improve the accuracy of future studies, I recommend using authentic phospholipid standards and running

them alongside the egg samples on the same TLC plate. Running the TLC plate with standards alongside the samples would allow more reliable confirmation of phospholipid identities. In cases where standards are not available, advanced analytical techniques such as Liquid Chromatography-Mass Spectrometry (LC-MS) should be employed for definitive phospholipid identification.

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