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COMPARATIVE STUDY OF PHOSPHOLIPID CONTENT IN EGGS OF DUCKS AND TURKEYS

BY

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NOVEMBER, 2023

DECLARATION

I NAMBUYA LILIAN registration number BU/UP/2020/0629 declare that this work has been done by myself. I really confirm that the work is my own. My contribution and those of other authors to this work have been indicated below. Appropriate credit has been granted to the origin of some work in the thesis where reference has been made to the creation of others.

Signature: ..Nb[signature]..................................Date: ..29th / 11 / 2023.........

NAMBUYA LILIAN

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APPROVAL

This research work has been submitted for examination and has been approved by my supervisor

Signature: ......[signature]............................ Date: ...07.12.2023...

DR ANDIMA MOSES

SUPERVISOR

DEDICATION

This research is dedicated to the almighty God for the divine knowledge, love, mercy, financial support, health, protection and divine direction and how I pray He may remain with me forever.

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ABSTRACT

Background information: The aim of this research was to conduct a comparative study on the amount of egg yolk lecithin extracted from eggs of ducks and turkeys. Lecithin is an important phospholipid found in egg yolks, which has various functional and nutritional properties. Therefore, understanding the amount of lecithin present in different types of eggs can have significant implications for the food and pharmaceutical industries.

Methods and materials: The research involved the collection of fresh duck and turkey eggs from local farms. The eggs were separated, and the yolk was extracted using standard techniques. The lecithin was then isolated from the yolk using a solvent extraction method, and analyzed using Fourier Transform Infrared spectroscopy.

Results: the percentage yield from eggs of ducks and turkeys were 10.7 % and 8.6 % respectively. Analysis of phospholipid samples using Fourier Transform Infrared Spectrometer showed vibrational frequencies at wave numbers 3300 cm^{-1}, 2922 cm^{-1} and 1733 cm^{-1} from which the presence of phospholipids in the samples was inferred.

Conclusion: Duck egg yolks contain phospholipids with a higher concentration than turkey eggs.

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CHAPTER ONE: INTRODUCTION

1.1 Background

Egg yolk lecithin is a natural emulsifier that is widely used in various industries such as food, cosmetics, and pharmaceuticals due to its unique properties and benefits. Lecithin extracted from egg yolk has been reported to have a wide range of health benefits, including improving liver function, reducing cholesterol levels, and preventing cognitive decline(Elkin & Kim, 2010).Ducks and turkeys are common poultry birds that are widely consumed for their meat and eggs. However, there is limited research on the comparative amount of lecithin extracted from their eggs. Several studies have compared the lecithin content of eggs from different bird species, but there is limited research on duck and turkey eggs. A study by (Y. H. Kuo, Wang, C. P, Chen, S. Y & Shieh, 2017) reported that duck egg yolk had a higher lecithin content than chicken egg yolk. However, there is limited research on the lecithin content of turkey egg yolk. Therefore, there is a need to investigate the lecithin content of duck and turkey eggs and compare them. The extraction of lecithin from egg yolk is typically done using the Bligh and Dyer method (Bligh & Dyer, 1959), which is a widely accepted method for lipid extraction. The amount of lecithin extracted can be quantified using various methods, such as spectrophotometry (Liu & Li, 2015).The results of this study provided valuable information on the lecithin content of duck and turkey eggs, which can be used in various industries. The information can also be used to promote the consumption of duck and turkey eggs, which are often overlooked compared to chicken eggs.

1.2 Problem statement

Egg yolk phospholipids are valuable ingredients in the food and pharmaceutical industries due to their emulsifying, stabilizing, and nutritional properties. However, the amount of egg yolk phospholipids extracted from different bird species, such as ducks and turkeys, is not well-documented in literature, and there is growing need of natural emulsifiers according to (Ferreira and Barreto,2018), due to their wide applications in various fields including the industry of cosmetics.so these study aims to explore eggs for both ducks and turkeys to analyses the phospholipid content in them such that eggs with a higher level of phospholipids can be recommended to meet the diverse needs of various fields.

1.3 General objective

The general objective of this research was to determine the amount of egg **yo**lk lecithin extracted from the eggs of ducks and turkeys.

1.4 Specific objectives.

The objectives of the research are as follows:

1.To determine the amount of egg yolk lecithin present in ducks and turkeys' eggs.

2.To compare the amount of egg yolk lecithin present in ducks and turkeys' eggs.

3.To find out the functional groups present in egg yolk lecithin present in ducks and turkeys' eggs

1.5 Significance

Contribution to the knowledge on egg yolk lecithin extraction: The study contributed to the knowledge on egg yolk lecithin extraction, particularly from ducks and turkeys' eggs. According to (Y. H. Kuo & Lee, 2012), there is a need for more research on egg yolk lecithin extraction, including the optimization of extraction conditions and the evaluation of different egg sources.

Potential for the development of new sources of egg yolk lecithin: The study led to the development of new sources of egg yolk lecithin. According to (Ferreira & Barreto, 2018), there is a growing demand for natural emulsifiers, including egg yolk lecithin, in the food industry.

Potential for the optimization of egg yolk lecithin extraction methods: The study could lead to the optimization of egg yolk lecithin extraction methods. According to (J. H. Kim, Kim, K. S & Kim, 2017), the optimization of extraction methods can improve the yield and quality of egg yolk lecithin.

CHARPTER TWO: LITERATURE REVIEW

Lecithin is a natural emulsifier found in egg yolks, which has multiple applications in the food, pharmaceutical, and cosmetic industries. It is a complex mixture of phospholipids, glycolipids, and triglycerides, making it a valuable ingredient for various applications. The extraction of lecithin from egg yolks is a crucial step in its commercial production (Djeridane & Gaydou, 2016).

The amount of lecithin in egg yolks varies between different bird species, with ducks and turkeys being two of the most commonly used species in the food industry. According to a study by (Sujka & Chojnacka, 2019), the content of lecithin in duck egg yolks ranged from 3.6% to 5.3

However, the method of lecithin extraction can also impact the yield of lecithin from egg yolks. According to a study by (Djeridane & Gaydou, 2016), different extraction methods such as acetone, ethanol, and hexane, can produce varying yields of lecithin. Therefore, it is essential to determine the optimal extraction method for each bird species to obtain the highest yield of lecithin.

2.1 EGG LIPIDS

Egg lipids are an important component of eggs, providing essential nutrients and functional properties. The lipid composition of eggs includes various classes of lipids such as triglycerides, phospholipids, cholesterol, and free fatty acids (pikul et al., 1984) The yolk is the primary source of lipids in an egg, and it contains a higher concentration of lipids compared to egg white. Egg lipids play a crucial role in the development of the embryo, providing energy and essential nutrients (Nimalaratne et al., 2011), and also the lipid content in an egg depends on several factors like diet and breed

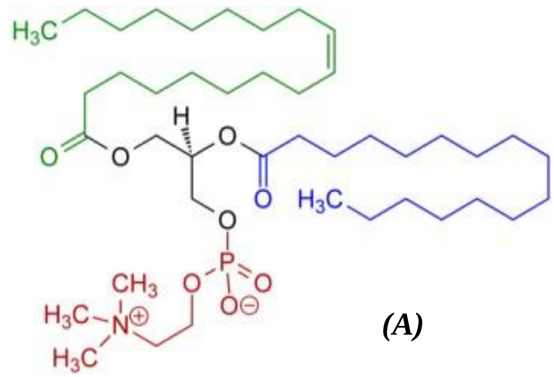
2.2 PHOSPHOLIPIDS

Phospholipids are a class of biomolecules that play a crucial role in the structure and function of the cell membranes. They are amphipathic molecules, meaning they have both hydrophilic (water loving) and hydrophobic (water repelling) regions, allowing them to form a stable bilayer structure in aqueous environments. (Hershey, J.W.B, and Matsudaira, p, 2000)

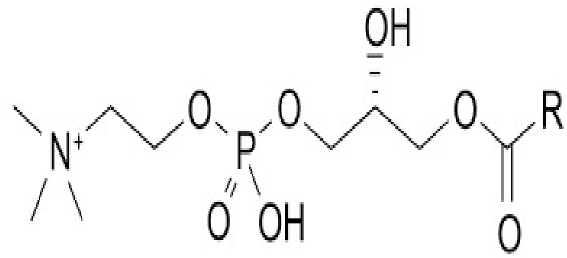
The egg yolks of both ducks and turkeys are good sources of phospholipids .these phospholipids represent approximately 10% of the wet weight of the egg yolk equivalent to about 22% of the total egg yolk solids(Yujie & Shuying, 2002). Phospholipids are a type of lipids that contain phosphorus; they were first isolated by Gobley in1844 and named Lecithin which is derived from the Greek language(Sinanoglou VJ, Strati IF, & Miniadis-Meimaroglou, 2011).

2.3 Composition of egg yolk lecithin

Egg yolk lecithin is a complex mixture of several components which include phospholipids, triglycerides, cholesterol and proteins which include lipoproteins and enzymes (K.N. Houlihan and J.M. Newsome,1976). However the variability in the composition of the egg yolk lecithin depends on several factors like diet and breed (S.M. Hargis et al., 1991)Egg yolk lecithin is composed of several components and these include phosphatidylcholine (PC)*(figure 1)*, phosphatidylethanolamine (PE), and lysophosphatidic choline*(figure 1)*(Marta Pokora et al.). Phosphatidylcholine (PC) is the primary component of lecithin, making up approximately 73.0% of its content(Feng Zhao et al., 2023). The amount of lecithin present in egg yolk is three times higher than that found in soya beans(Feng Zhao et al., 2023). Egg yolks and some oil crop seeds (soybeans and rape seeds) contain the most abundant amount of lecithin(Feng Zhao et al., 2023). As compared to plant derived phospholipids, egg yolk phospholipids have a well-balanced and unique composition of phospholipids. Additionally, egg yolk phospholipids contain specific fatty acids that are not found in plant derived phospholipids(Katarzyna Sko´ rkowska-Telichowska et al., 2016). Egg yolk lecithin is particularly a component of the granular part of egg yolk, making up approximately 70% of all phospholipids in egg yolk(Sahar Navidghasemizad, Feral Temelli, & Wu, 2015).



(A)



(B)

Figure 1:chemical structures of phosphatidylcholine(A) and lysophosphatidylcholine(B)

2.4 Importance of phospholipids

Phospholipids are a crucial class of lipids that play a vital role in various biological processes. They are the major components of the cell membranes and are involved in cell signaling and lipid metabolism. Phospholipids consist of a glycerol backbone, two fatty acid chains, a phosphate group, and a polar head group. This unique structure allows phospholipid to form the lipid bilayer that constitutes the cell membrane, providing structural integrity and regulating the movement of molecules into and out of the cell. The lipid bilayer acts as a barrier that separates the internal cellular environment from the external surroundings (Alberts et al., 2014).

Phospholipids play a crucial role in cell metabolism and possess unique structures and properties that are essential for the normal functioning of cells(Rudra Pratap Singh, H.V. Gangadharappa, & Mruthunjaya, 2017).

Phospholipids play a crucial role in brain cells and nerve tissues, constituting approximately 30% of the brain's weight, and they are essential for facilitating the transmission of neuronal information within the brain(Ali AH et al., 2017).

Phospholipids also contribute to membrane fluidity and flexibility which are essential for various cellular processes such as cell division and migration (Van Meer et al., 2008).

Furthermore, phospholipids serve as precursors for important signaling molecules such as diacylglycerol molecules which are involved in intracellular signaling pathways (Nishizuka,1995)

2.5 Natural sources of phospholipid

Soybeans: "Soybeans are a rich source of phospholipids, particularly phosphatidylcholine and phosphatidylethanolamine" (Yan et al., 2018). These phospholipids have been shown to have numerous health benefits, including improving liver function and reducing inflammation. These phospholipids are extracted from soybeans to produce soy lecithin which is widely used as an emulsifier in food products. Studies have shown that soy lecithin can contribute to the intake of essential phospholipids in the diet (Rouser et al., 1970)

Another source is sunflower seeds which are rich in phosphatidylcholine, and it is important in maintaining the structural integrity of cell membranes. This makes it a valuable source of this important component (Garcia et al., 2005)

Krill oil: "Krill oil is a supplement that contains high levels of phospholipids, primarily phosphatidylcholine and phosphatidylserine" (Ulven et al., 2011). These phospholipids have been shown to have anti-inflammatory effects and may have benefits for heart health. Milk: "Milk is a good source of phospholipids, particularly sphingomyelin" (Le et al., 2018). Sphingomyelin has been shown to have a protective effect on the gastrointestinal tract and may have benefits for brain health.

2.6 Extraction methods

2.6.1 Organic solvent extraction

Solvent extraction is a method to separate and extract lecithin by using the difference in the selectivity of the solvent to the components of phospholipids(Patil, Galge, & Thorat, 2010).Lecithin extraction from eggs has been a long-standing practice, with solvent extraction being one of the earliest methods used(Zhao, Li, Liu, & Chen, 2023). There are two main methods for solvent extraction: single organic solvent extraction and mixed organic solvent extraction.

In single organic solvent extraction, ethanol is the preferred solvent for extracting lecithin from fresh egg yolks(Zhao et al., 2023). Under optimal process conditions, the extraction rate can reach as high as 93.38% (Hui-hui & Lu-hong, 2014). This method is relatively simple and requires minimal equipment, but it can be time-consuming and may not yield high purity lecithin(Zhao et al., 2023).

Mixed organic solvent extraction, on the other hand, takes advantage of lecithin's solubility in ethanol but insolubility in acetone. By using a combination of ethanol and acetone, the method can extract lecithin more efficiently and with higher purity(Zhao et al., 2023). However, this method requires more equipment and expertise, and the process can be more complex and time-consuming (Hui-hui & Lu-hong, 2014).

2.6.2 Super-critical extraction

Supercritical extraction is an innovative technology that separates substances based on their differences in properties during the supercritical state. This technology can extract lecithin with exceptionally high purity (95-98%) from egg yolk powder(Hui-hui& Lu-hong, 2014). The reason for this high purity is that phospholipids are insoluble in supercritical carbon dioxide, which requires the addition of an entrainer to separate phospholipids. supercritical carbon dioxide is a state of matter where the temperature and pressure are elevated beyond the critical point of a substance(Zhao et al., 2023). In this state, the substance exhibits properties of both liquids and gases, allowing for unique separation properties. By adjusting the temperature and pressure conditions, substances with different properties can be separated from one another(Zhao et al., 2023).In the case of lecithin extraction from egg yolk powder, the supercritical carbon dioxide is used to selectively extract the lecithin from the other components in the egg yolk(Zhao et al., 2023). The phospholipids, which are insoluble in supercritical carbon dioxide, are separated from the lecithin using an entrainer, such as ethanol or acetone, the resulting lecithin is then collected and purified, resulting in a high-purity product(Zhao et al., 2023).

2.6.3 Subcritical extraction

Subcritical extraction refers to a fluid extraction technology that utilizes a thermodynamic state that is at the edge of a supercritical state, meaning it is above the critical pressure and below the critical temperature(Zhao et al., 2023). This technology is used to extract valuable compounds from plant materials, such as essential oils, flavors, and pharmaceuticals. In subcritical extraction, a solvent, such as carbon dioxide or a hydrocarbon, is used to extract the desired compounds from the plant material, the solvent is heated to a temperature that is below its critical temperature, but above the critical pressure, creating a state known as a "supercritical fluid"(Zhao et al., 2023). This state allows the solvent to exhibit unique properties that are not found in either liquid or gas states, such as high solubility and low viscosity.

Other methods include enzymatic hydrolysis extraction, column lamination method, cryo-precipitation and membrane separation methods(Zhao et al., 2023).

2.7 Analysis of phospholipids

Analytical tools for analyzing phospholipids encompass a range of techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared spectrometer (FT/IR) and gas chromatography (GC)(Patel et al., 2019). These tools enable the separation, identification, and quantification of phospholipids based on their chemical properties and molecular structures. TLC is used for initial separation, while HPLC and GC are employed for quantitative analysis(Vavrušová, 2020). MS and NMR provide detailed structural information, aiding in the identification of phospholipid species(Alexandri et al., 2017). Additionally, these analytical tools can be coupled with various detectors to enhance sensitivity and selectivity in phospholipid analysis(Peterson & Cummings, 2006).Mass spectrometry is one of the most widely used tools for phospholipid analysis due to its high sensitivity, specificity, and ability to provide detailed structural information(Blanksby& Mitchell, 2010). It allows for the identification and quantification of phospholipid species present in complex biological samples(Blanksby& Mitchell, 2010). Mass spectrometry techniques such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are commonly employed for phospholipid analysis(L. Li et al., 2014).In particular, liquid chromatography-mass spectrometry (LC-MS) has become the method of choice for phospholipid analysis due to its ability to separate complex lipid mixtures and provide accurate mass measurements(Cajka& Fiehn, 2014). LC-MS enables the comprehensive profiling of phospholipid species in biological samples, making it a powerful tool for lipidomics research(Li, Yang, Bai, & Liu, 2014).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Sources of eggs

Fresh eggs for both ducks and turkey were purchased from the local farmers in Nagongera town council particularly those of Mahanga cell.

3.2 Chemicals used

Ethanol (96 %) was used in the solvent extraction of phospholipids from chicken eggs as it helps to dissolve the phospholipids, separating them from the egg components. N-Hexane (95 %) was used to further extract lipids from the egg mixture, since it has a high lipid solubility. Cold acetone was then used to precipitate the phospholipids, allowing for their separation from other components in the mixture.

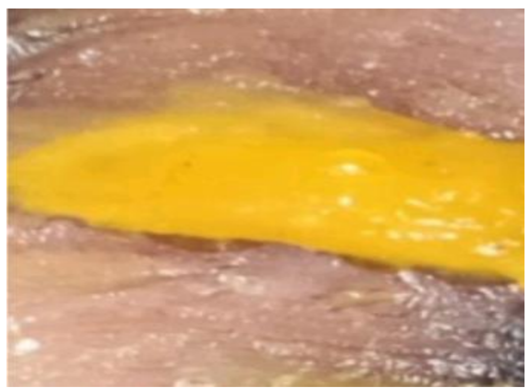
3.3 Methods

The eggs were gathered from two distinct groups of poultry, specifically ducks and turkeys in Tororo district. The eggshells underwent a gentle washing process using distilled water, after which they were delicately cracked open to separate the egg yolk from the egg white. The combined egg yolks were then stored in a container at 0^0 C until they were ready for use.

Egg yolks (5 eggs) were placed in a 100-ml flask with a magnetic stirrer, about 250 ml of ethanol were then added and the mixture stirred for 10 min. The supernatant was then removed and filtered into a round-bottomed flask. The extraction of egg yolk with ethanol was repeated four times. The extracts were then be pooled and the ethanol evaporated under reduced pressure. The residue was then dissolved in hexane (120 ml) and transferred to a flask placed in an ice bath (0^0 C). Next, about 250 ml of cold acetone (0^0 C) was carefully poured into the stirring mixture. Following precipitation of phospholipids, the stirring was stopped and the supernatant decanted off. The precipitate was then carefully washed 5 times with 20-ml portions of cold (0^0 C) acetone and the solvent removed by decantation each time. The yield of phospholipid was then be determined by weighing its mass.

Fourier Transform Infrared Spectrometer (FT/IR) was used to identify the key functional groups present in the isolated phospholipids by detecting specific absorption bands associated with the different chemical bonds present in the different phospholipid fractions. The spectra obtained for both duck and turkey eggs (figure 1) provided valuable information about the specific functional groups present in the isolated phospholipids. Consequently, the different phospholipid fractions present as a complex mixture of phospholipids in the sample were inferred. This confirmed the presence of phospholipids in the sample.

The figure below shows the physical appearance of the phospholipid extracts from both the duck and turkey egg yolk.



Duck Turkey

Figure 1: physical appearance of phospholipid extracts of the two birds.

4 CHAPTER FOUR: RESULTS AND DISCUSSION

Phospholipids are a type of lipids that serve as a crucial component of cell membranes, including those found in the egg yolk. They play a vital role in maintaining the integrity and functionality of cell membranes. Within the egg yolk, which constitutes the yellow part of the egg, various phospholipids such as phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol are present. These phospholipids are essential for the development of the embryo within the egg and also act as a source of nutrients for the growing chick. Furthermore, phospholipids are associated with numerous health benefits, including their contribution to brain health, liver function, and overall cellular function. The primary sources of phospholipids are eggs, soybeans, sunflower seeds, krill oil, and organ meats, with eggs having the highest concentration. This research assumed that different types of eggs, from both ducks and turkeys, have varying phospholipid content. The results presented in Table 1 indicate that duck eggs have a higher phospholipid content compared to turkey eggs.

Sample	Weight of egg yolks (g)	Weight of extract (g)	Percentage yield (%)
Duck eggs	140	15	10.7
Turkey eggs	140	12	8.6

Table 1 shows the weights and percentage yield of phospholipids extracted

4.1 Characterization of phospholipids

Characterizing phospholipids involves a comprehensive examination of their structure, composition, and properties. The methods utilized for analyzing phospholipids encompass a diverse range of techniques, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared spectrometer (FT/IR), and gas chromatography (GC).

The use of these analytical instruments allows for the differentiation, recognition, and measurement of phospholipids based on their chemical characteristics and molecular compositions. Thin-layer chromatography (TLC) is primarily employed to initially separate phospholipids, while high-performance liquid chromatography (HPLC) and gas chromatography (GC) are utilized for quantitative analysis. Mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) offer comprehensive structural details, aiding in the identification of specific phospholipid species. In a particular research endeavor, Fourier Transform Infrared spectrometer (FT/IR) was utilized to detect the primary functional groups present in the phospholipid extracts from ducks and turkey eggs. The FT/IR Spectroscopy analysis revealed specific vibrational frequencies at approximate wave numbers of 3300 cm^{-1}, 2922 cm^{-1}, and 1733 cm^{-1}, as seen in figure 1.

The findings presented in figure 2 and table 2 indicate the presence of common functional groups in the phospholipid extracts, which include; O-H, C=C, and C=O. These functional groups are attributed to the diverse structures of phospholipid extracts such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and lysophosphatidic choline.

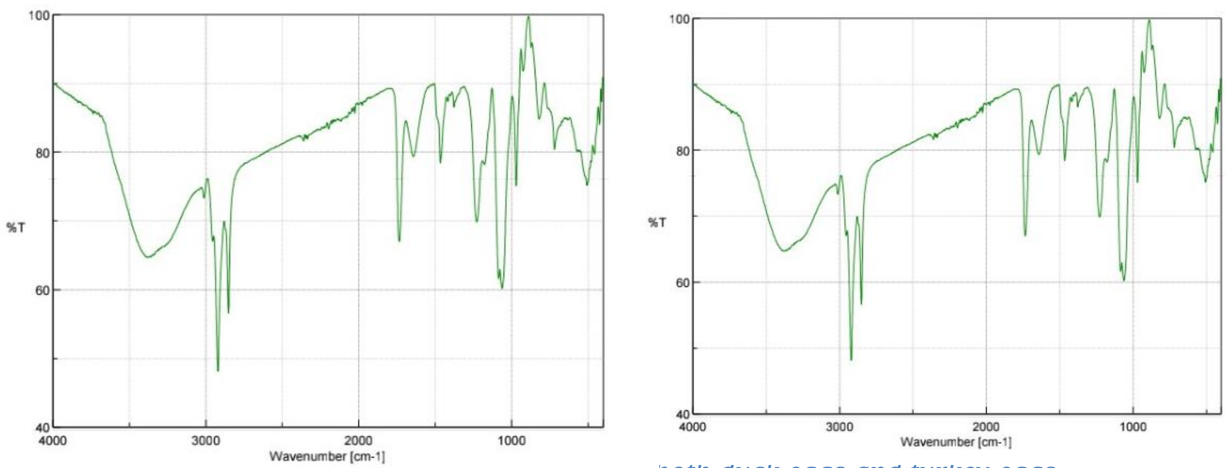


Figure 2 FT/IR chromatograms for both duck eggs and turkey eggs

Wave number (cm^{-1})	Functional group present
3300	O-H
2922	C=C
1733	C=O

Table 2 vibrational frequencies of the different phospholipid fractions

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The findings of this research indicate that ducks and turkeys have a similar phospholipid composition, although ducks have a higher phospholipid content compared to turkeys. This variance can be attributed to disparities in the feeding methods, breed and habitats of these two bird species.

5.2 Recommendation

The phospholipid extracts from the two species, duck eggs and turkey eggs, were analyzed for their key functional groups and percentage yield. However, due to resource constraints, the purity of phospholipids in the two species and other parameters such as composition could not be determined. To obtain a comprehensive understanding of the phospholipid composition in both bird species and identify any differences between duck eggs and turkey eggs, I recommend using a combination of techniques such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). HPLC is a powerful analyal technique that can separate, identify, and quantify the various components within a mixture, including phospholipids. By using HPLC, researchers can determine the types and amounts of phospholipids present in the two bird species and identify any differences in their phospholipid compositions. For a more detailed analysis, conducting a thin layer chromatography (TLC) can assist in determining the precise number of components present in the phospholipids. TLC is a widely utilized technique that enables the separation and identification of individual components within a mixture based on their size and polarity. By comparing the TLC profiles of duck and turkey eggs, valuable insights can be gained regarding the differences in phospholipid composition, potentially leading to the identification of specific species with higher purity phospholipids. Additionally, further research could explore the various factors influencing the phospholipid content of duck and turkey eggs, including breed, diet, and environmental conditions.

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