A Research on Determination of Lecithin in Eggs by Applying Microwave Digestion Techniques and Spectrophotometry

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ABSTRACT: *A method to quick detect concentration of lecithin in eggs, namely microwave digestion spectrophotometry, was established in this research. The homogenate of eggs was treated with absolute ethanol to eliminate phosphate protein in eggs which could possibly affect concentration of lecithin examined. A sample then received a new way of pre-treatment, called microwave digestion, before UV-Vis spectrometry was applied to examine the concentration of phosphate at 400nm. The linear equation was A = 0.08628X (μg), the corresponding coefficient of correlation was 0.9998,the detection limit of phosphorous was 0.2μg (n=11). The content of lecithin in eggs was then obtained. According to the result, the recovery of 90% was secured; therefore the conclusion of high degree of accuracy was reached.*

KEY WORDS: *Spectrophotometry, Microwave digestion, Phosphatidylcholine.*

INTRODUCTION

Lecithin is one of the most common natural phospholipids[1], that is mainly composed by three compounds, PhosphatidylCholine (PC), PhosphatidylEthanolamine (PE) and PhosphatidylInositol (PI)[2]. As an important constituent of biological membranes, lecithin exists in plant as well as in many animal tissues and organs, such as brain cell, sperm, and egg yolk. Lecithin acts as a vehicle for fatty acid transportation which plays an important role in embryonic development, slows down the procedure of memory decay, prevents alzheimer's disease and protects human body against atherosclerosis, hypertension, thrombosis and ulcers[3]. Due to its valuable physicochemical, biological and physiological property, lecithin is widely used in the pharmaceutical[4], food[5] and cosmetics[6] industry.

Usually lecithin is designated its main component PC. Fig.1 is the molecular structure of PC. Study shows various techniques have been developed to monitoring PC quantitatively. The phosphatidylcholine in hydration oil leftover was determined qualitatively and quantitatively by HPLC with Lichorosorb si-60 chromatographic column, flow-phase of chloroform-methanol-water and evaporate-scattering detector [7]. Normal-phase high performance liquid chromatography with evaporative light scattering detection was applied to determine phosphatidylcholine in egg-yolk [8]. High-resolution High-field proton Nuclear Magnetic Resonance spectroscopy (^{1}H-NMR) as the analytical tool was used to analyze the platelet lipid profiles to evaluate the coronary artery disease [9]. However, the applications of many of those techniques are limited due to their complicated

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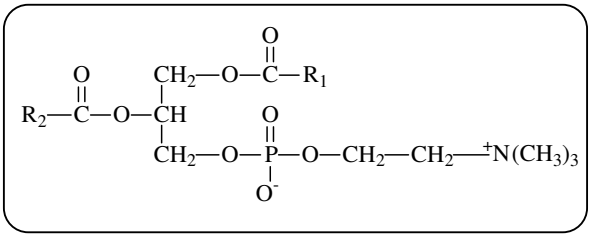

Fig. 1: Molecular structure of PC.

operating procedures as well as high equipment and maintenance cost. UV-Vis spectrophotometry is therefore wildly used simply because of its cheep and easy to operate. Study suggests the sample preparation procedure can carried out as follows, the content of lecithin in functional food is extracted by acetone and determined by ultraviolet spectrophotometry [10], and phosphatidylcholine content in two kinds of commercial lecithin capsules is extracted by acetone first and then measured by UV-Vis spectrophotometry [11]. From the experiment, the recovery reached a poor result by using the sample prepared following the above procedure. Therefore, a simple and promising method of sample preparation is desired.

The aim of this work was to establish a new method of determining the lecithin content in eggs. As low reagent consumption and short experimental time, microwave digestion was considered as a novel sample pretreatment method. The results from applying microwave digestion/UV-Vis spectrohotometry to determine PC in eggs shows a great consistence with the results from applying a traditional method [12].

EXPERIMENTAL SECTION

Materials

Eggs were provided by Quality Inspection Center for Poultry Products- Ministry of Agriculture (Yangzhou).

MARS microwave digestion (CEM company, USA), Lambda UV-Vis spectrometer (PE company, USA), rotary evaporator (Buchi company, Switzerland);

Ethanol (AR), nitric acid (AR), hydrogenperoxide (AR), phosphatidylcholine (sigma, USA), water (purified with Milli-Q Academic);

vanadium ammonium molybdate: 1.25g of ammonium metavanadate was firstly dissolved in 200mL of hot water, waited it to cool down, then added 250mL of nitric acid to form solution 1; 25.0g of ammonium molybdate was mixed with 400mL of hot water, waited it to cool down, marked as solution 2; Once solution 1 and 2 was prepared, mixed them together and filled it up with water until it reached 1000mL. The vanadium ammonium molybdate solution was then stored in darkness. Discharge it when precipitation appeared.

Phosphorus standard solution (1mg/mL): potassium dihydrogen phosphate (GR) was maintained at 105℃ for 2 hours. Exactly 0.439g of potassium dihydrogen phosphate was weighted and dissolved in water and filled it up with water until it reached 100mL

Phosphorus work solution: phosphorus standard solution was serially diluted to 50μg/mL.

Methods

Mechanism

Phosphorus in eggs is not only bonded with fat but also with protein. According to its chemical property, lecithin can be dissolved in ethanol, therefore, protein and lecithin in eggs can be separated by ethanol treatment, which eliminates the disturbance of phosphorus-protein. Lecithin extracted from eggs was digested in microwave digestion system to form phosphorus acid, vanadium ammonium molybdate was then added into phosphorus acid to form complex $(NH)PO_4NH_4VO_3$ $16MoO_3$. The complex was determined at wavelength of 400nm, from that the concentration of phosphorus was obtained, and the content of lecithin was 25 times of the phosphorus concentration.

Sample preparation

2 or 3 eggs (hen-eggs, duck-eggs, pigeon-eggs) were chosen arbitrarily and the eggs were mixed homogeneously in a homogenizer. 10.0g of homogenate was weighted and placed in a 50mL centrifuge tube; 30mL of ethanol was added into it. The mixture was kept stirring for 5 minutes to ensure its uniformity and then centrifuged. The supernatant was transferred to a 250mL round bottom flask. Residuals in the tube were treated with ethanol twice as described previously and the extract solution was collected in a flask. The solution was dried with rotary evaporator and the sample was stored at temperature of 4℃.

0.300g sample was weighted in a PolyTetraFluoroEthylene (PTFE) digestion vessel, 5.0mL of concentrated HNO_3 and 1.0mL of 30 % (w/v) H_2O_2

Table 1: Digestion procedures of Automatic Pressure Microwave Digestion System.

Step	Time/min	Power/W	Temperature/℃
1	1	800	120
2	5	800	160
3	15	800	190

Table 2: Boiling points of acids.

	Nitric acid	Perchloric acid	Hydrochloride acid	Sulfuric acid
Boiling point ℃	120	203	110	340

was added. The mixture was kept still for 5 minutes and digested in microwave digestion (see Table 1 for the digestion procedure). Once the sample solution cooled, transferred it into a 50mL flask, and filled it up with water until it reached 50mL and stored for determination.

Sample determination

10.0mL of sample solution was transferred into a 50mL flask, 10mL of vanadium ammonium molybdate solution was added and filled it up with water until it reached 50mL Solution was kept still for 10 minutes at room temperature and determined at a wavelength, λ_{max}, of 400nm. The sample blank was prepared as state above without sample solution.

RESULTS AND DISCUSSION

Selection of digestion system

A commonly used organic sample digestion system is usually a mixture, mainly including a mixture of nitric acid, hydrogen peroxide and hydrofluoric acid, a mixture of nitric acid and hydrogen peroxide, a mixture of nitric acid, hydrochloride acid and hydrogen peroxide, as well as a mixture of hydrochloride acid and nitric acid. The boiling points of those acids were shown in Table 2. Study shows that perchloric acid mixing with organic sample could cause explosion under high pressure; the boiling point of sulfuric acid was too high that PTFE digestion vessel can not stand. It could form insoluble compounds and cause damage on digestion vessel at high temperature and pressure. Therefore, those two systems should be avoided in this case. Hydrochloric acid system was also eliminated due to its poor oxidative property. Hydrogen peroxide could reduce the amount of nitrogenous gas which formed in the digestion process. It also could accelerate the decomposition of organic samples under high pressure and temperature. In this case, nitric acid and hydrogen peroxide system was the only choice.

Optimization of the addition of nitric acid and hydrogen peroxide

An orthogonal test is a high-efficiency and economical experiment design method. It accurately evaluates dominate influential factors in each experiment as well as the degree of interaction between those factors. Research suggested that the amount of nitric acid and hydrogen peroxide utilized was the two factors affecting the digestion performance in this experiment. The orthogonal test was carried out at different amount of nitric acid and hydrogen peroxide (see Table 2 for the results from the orthogonal test).

The calculation formula of lecithin content is:

$$c(\%) = \frac{25 \times X \times 5}{m \times 10^6} \times 100$$

X——lecithin content calculated according to the linear equation (μg);

m——— sample mass (g);

5——— time for sample dilution;

25———transformation coefficient of phosphorus to lecithin

From Table 3, it can be seen that $R_{HNO3} > R_{H2O2}$, the amount of HNO_3 was the dominate factor; for HNO_3, $k_3 > k_4 > k_2 > k_1$, for H_2O_2, $k_3 > k_4 > k_2 > k_1$, so the optimum system was 5.0mL HNO_3 and 2.5mL H_2O_2.

Interferences

The effects of diverse ions on the determinations of analytes were investigated. With a relative error of less than ±5%, the tolerance limits for the foreign substance were listed in Table 4 (The concentration of phosphorus in solution is 6.0 ng/mL).

Analytical parameters

0,1.0, 2.0, 4.0, 6.0, 8.0, 10.0mL of (at phosphorus content of 0, 50, 100, 200, 300, 400, 500μg) phosphorus standard solution was transferred into a 50mL flask respectively, then 10mL of vanadium ammonium molybdate solution was added and filled up with water up to 50mL. The solution was kept still at room temperature

Table 3: Results from the orthogonal test.

Run	HNO_3/mL	H_2O_2/mL	Lecithin content/%
1	1（3.0）	1（1.0）	2.57
2	2（4.0）	2（2.0）	2.69
3	3（5.0）	3（2.5）	2.93
4	4（6.0）	4（3.0）	2.81
5	1	2	2.59
6	2	3	2.78
7	3	4	2.89
8	4	1	2.86
9	1	3	2.65
10	2	4	2.76
11	3	1	2.92
12	4	2	2.90
13	1	4	2.70
14	2	1	2.62
15	3	2	2.94
16	4	3	2.84
$^{*}K_1$	10.51	10.97	--
K_2	10.67	11.12	--
K_3	11.68	11.2	--
K_4	11.41	11.16	--
$^{**}k_1$	2.63	2.74	--
k_2	2.71	2.78	--
k_3	2.92	2.80	--
k_4	2.85	2.79	--
$^{***}R$	0.29	0.06	--

$^{*}K_i$ means of the sum of results which listed in line i;

$^{**}k_i$ means of the average value of K_i, it indicates the optimum group of the two factors

$^{***}R$ means of the range of the two factors.

Table 4: Tolerance of Interference Ions.

Foreign ions	Tolerance ratio in mass
Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}	1000
Fe(II), Fe(III), Cu(II)	500
Mn(II), Cr(VI)	100

Table 5: Results of determination of lecithin content.

Sample	Average lecithin content/%	RSD/%	Added/%	Determination value/%	Recovery/%
Hen-eggs	2.94	1.63	0.50	3.40	92.0
Duck-eggs	3.81	1.76	0.50	4.29	96.0
Pigeon-eggs	4.68	1.54	0.50	5.13	90%

for 10 minutes and determined at the wavelength of 400nm. The sample blank was prepared as stated previously without sample solution. The linear equation was $A = 0.08628X$ (μg), the corresponding coefficient of correlation was 0.9998, the detection limit of phosphorus was 0.2 μg (n=11).

Precision and accuracy of the method

To assess the performance of the microwave digestion and to validate its accuracy, several recovery experiments were carried out. Three commercially available samples (hen-eggs, duck-eggs, pigeon-eggs) were chosen (see Table 5 for the results of determination of lecithin content). The average recovery level was satisfactory ranging between 90.0% and 96.0%. The precision was also appropriated for the analysis of lecithin in eggs.

CONCLUTIONS

A new method of Microwave Digestion-Spectrophotometry was established to determine the concentration of lecithin in eggs. The pre-treatment of the sample was optimized and the most efficient digestion system was confirmed: 5.0mL HNO_3 and 2.5mL H_2O_2. UV-Vis spectrometry was applied to determine the concentration of phosphate at 400nm. The results showed that the method reached a promising accuracy and precision. Compared with the traditional methods, the Microwave digestion-Spectrophotometry has its great advantages on less reagents usage and short time consumption. Therefore, it was an environmental friendly, effective and practical method.

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REFERENCES

[1] Li Y., "Study of Analyses and Refine Method of Lecithin in Soybean", Zhejiang University, Hangzhou, (2004).

[2] Kuligowski J., Quintás G., Garrigues S., M. de la Guardia, Determination of Lecithin and Soybean Oil in Dietary Supplements Using Partial Least Squares-Fourier Transform Infrared Spectroscopy, *Talanta*, **77**, p. 229 (2008).

[3] Campanella L., Magrì A.L., Sorbo A., Tomassetti M., Determination of Lecithin Drug Specialties and Diet Integrators; by Means of First or Second Derivative Enzymatic-Spectrophotometric Analysis, *J. Pharm. Biomed. Anal.,* **29**, p. 1135 (2002).

[4] Campanella L., Pacifici F., Sammartino M.P., Tomassetti M., Analysis of Lecithin in Pharmaceutical Products and Diet Integrators Using a New Biosensor Operating Directly in Non Aqueous Solvent, *J. Pharm. Biomed. Anal.*, **18**(4-5), p. 597 (1998).

[5] Heshmati A., Khodadadi I., Reduction of Cholesterol in Beef Suet Using Lecithin, *J. Food Composition and Analysis*, **22**(7-8), p. 684 (2009).

[6] Pardeike J., Hommoss A., Müller R.H., Lipid Nanoparticles (SLN, NLC) in Cosmetic and Pharmaceutical Dermal Products, *Int. J. Pharm.*, **366**(1-2), p. 170 (2009).

[7] Zhou H., Xia H.T., Lian G.X., Jing P., Determination of Phosphatidylcholine by High Performanceliquid Chromatography in the Soybean Mixed Phospholipid, *J. Qiqihar University*, **18**(4), p. 33 (2002).

[8] Gong Y., Wang Q., Yang Y., Ding M.Y., Determination of Egg-Yolk Phosphatidylcholine by Normal-phase High Performance Liquid Chromatography with Evaporative Light Scattering Detection, *Chinese Journal of Chromatography*, **24**(4), p. 373 (2006).

[9] Noula C., Bonzom P., Brown A., Gibbons W.A., Martin J., Nicolaou A., ^{1}H-NMR Lipid Profiles of Human Blood Platelets; Links with Coronary Artery Disease, Biochim. *Biophys. Acta.*, **1487**(1), p. 15 (2000).

[10] Chen W.T., Zhang D.Q., Zhang B.L., Zhou H.J., Determination of Lecithin in Functional Food by UV Spectrophotometry, *J. The Chinese Cereals and Oils Association*, **21**(3), p. 189 (2006).

[11] Guan M., Li Y., Zhao S.X., Determination of Phosphatidylcholine in Lecithin Capsule by UV, *China Brewing*, (23), p. 95 (2008).

[12] Ning Z.X., "Food Composition Analysis Manual[M]", China Light Industry Press, Beijjing, p.171 (1997).