

## Total lipids, cholesterol and fatty acids composition of ostrich eggs: a methodological approach

Lipídios totais, colesterol e composição de ácidos graxos de ovos de avestruz: uma abordagem metodológica

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### ABSTRACT

The present study compared the total lipids and lipid fractions contents of ostrich eggs with of chicken eggs, both *in natura*, and the methodologies used for these determinations were assessed. Total lipids were quantified by Folch and Soxhlet technique. Cholesterol contents was quantified by colorimetric method and using a C18 HPLC column, mobile phase composed of acetonitrile: isopropanol (80:20), and the detection level was adjusted to 210 nm. Fatty acids were determined by gas chromatography. No significant differences in the results were found in determining cholesterol by both methods. *In natura* ostrich egg yolk presents higher percentage of mono and polyunsaturated fatty acids and lower percentage of saturated fatty acids when compared to *in natura* chicken egg yolk. Although ostrich egg yolk presented higher lipid contents, the amount of cholesterol was similar in both egg types. *In natura* ostrich egg yolk contains highest rate of monounsaturated, polyunsaturated and saturated, which could be considered as the best nutritional quality fat.

**Key words.** egg yolk, lipids, ostrich egg.

### RESUMO

Neste estudo os valores de lipídios totais e das frações lipídicas dos ovos de avestruzes foram comparados com os dos ovos de galinha *in natura*. Os lipídios totais foram quantificados pela técnica de Folch e Soxhlet. O colesterol foi quantificado pelo método colorimétrico e por HPLC utilizando-se coluna de C18, fase móvel de acetonitrila:isopropanol (80:20) e detecção fixada em 210 nm. Os ácidos graxos foram determinados por cromatografia gasosa. Diferenças significativas foram observadas entre os resultados obtidos pelos métodos de extração de lipídios totais da gema e do ovo integral de avestruz. Não houve diferença significativa nos resultados obtidos pelos dois métodos de determinação de colesterol. A gema *in natura* de ovo de avestruz apresenta maior percentual de ácidos graxos mono e poli-insaturados e menor percentual de ácidos graxos saturados, quando comparados a gema de ovo de galinha *in natura*. Apesar da gema de ovo de avestruz apresentar maior conteúdo lipídico, a quantidade de colesterol entre os dois tipos de ovos foi semelhante. A gema de ovo de avestruz *in natura* contém uma melhor proporção de ácidos graxos mono, poli e saturados, que pode ser considerada como gordura de melhor qualidade nutricional.

**Palavras-chave.** gema de ovo, lipídios, ovos de avestruz.

## INTRODUCTION

Egg is considered one of the most complete foods, which biological and nutritional value has been evaluated and attested by several researchers. Egg is a source of proteins known for their nutrition, biological and technological potential<sup>1</sup>. It contains liposoluble vitamins, A, D, E and K, besides vitamins from the vitamin B complex such as thiamine, riboflavin, niacin, pyridoxine and cyanocobalamin. Among minerals, iron, calcium, potassium, sodium, phosphorous and zinc, among others, are found. Egg is an important source of cholesterol and unsaturated fatty acids, mainly oleic acid.

For more than 40 years, publications have informed about the relationship between the consumption of eggs and the risk of coronary diseases. This information is based on three observations: 1. eggs are rich in dietary cholesterol; 2. the dietary cholesterol increases the serum cholesterol and 3. high serum cholesterol induces the appearance of coronary diseases. The high consumption of eggs or any cholesterol-rich foods by diabetic individuals, cancer carriers, and those with hypercholesterolemia can unchain coronary diseases<sup>2</sup>. However, data on healthy population have demonstrated that the consumption of the egg is not associated with the high cholesterol level. In recent researches, was reported that the excessive ingestion of saturated fat is the main responsible for the appearance of cardiovascular diseases and that although dietary cholesterol has influenced the atherogenic and anti-atherogenic cholesterol fractions, these effects are minimum and mainly, they do not affect the proportion between LDL and HDL cholesterol.

The highest concentration of lipids in eggs is in the yolk, among its components, lipoproteins, vitellin and vitellinine can be mentioned; a water-soluble fraction, livetin, and finally phosphovitin. Fats from egg yolk are strongly emulsified, being represented by triacylglycerols and cholesterol.

According to Brum<sup>3</sup>, lipids extraction is an important determination in biochemical, physiological and nutritional studies of the most several types of foods and, therefore, it should be accomplished with accuracy. Some samples request special care in obtaining the lipid fraction due to factors such as the co-extraction of non-lipid components and undesired oxidation, which could influence the final quality of the lipid fraction.

Egg is a cheap and nutritious food part of the human diet in all social levels, since it is widely used in food industries and in several food preparations. In countries such as Spain and Italy, infertile ostrich eggs (not incubated) are widely used in bakeries, pastry shops and food industries, once each egg weighs between 1.3 and 1.7kg with identical flavor and similar chemical and physical properties as chicken eggs and an ostrich egg is equivalent to approximately 24 chicken eggs.

The objective of this work was to assess the nutritional value of ostrich eggs when the total lipids and lipid fractions of these eggs are compared to *in natura* chicken eggs, as well as to evaluate the methodologies specifically used for these determinations, searching for more data on this type of sample.

## MATERIAL AND METHODS

### Material

Eggs used in this experiment were provided by Coovestruz-PB (Ostrich Breeders Association of the state of Paraíba), being collected using sterile gloves, conditioned in individual sterile sacks and transported to the laboratory in styrofoam and under cooling. Chicken eggs used in this experiment are label “*Dagama*”, produced at the *Pedra de Fogo* region - Paraíba - Brazil, classified as intermediate (in relation to the size and weight), which were purchased in local supermarkets of João Pessoa - Paraíba - Brazil.

### Methods

#### Total lipids

The extraction of lipids linked to proteins and carbohydrates was performed according to methodology proposed by Folch et al<sup>4</sup> using the polar solvents, chloroform: methanol (2:1 v/v), as well as Soxhlet for the determination of total lipids.

#### Fatty acids composition of ostrich and chicken egg yolk Preparation of the methylic esters

The samples consisted of *in natura* ostrich and chicken egg yolks. The method consisted of weighing 0.2g of ostrich egg yolk with the addition of 3.0 mL of hydroxide of methanol potassium to 5.0 N (as hydrolyzing agent). Heat until ebullition and leave to rest until the emergence of a single phase. Later, 7 mL of esterification solution was added (methylic alcohol, ammonium chloride and sulfuric acid),

maintaining the mixture in reflux for 4 minutes more. Soon afterwards, it was transferred into a separation funnel, with the addition of 25 mL of distilled water and 12.5 mL of ethyl ether, shaking gently. This procedure was performed when the separation of the two phases was observed. The lipid fraction was washed three times with 25 mL of distilled water, decanting and discarding the aqueous phase. Finally, the organic phase was filtered with anhydrous sodium sulfate to keep the excess of water.

### Identification and quantification of the fatty acids methylic esters

The identification and quantification of the methylic esters were performed at the Flavor Laboratory of the Paraíba Federal University CT-UFPB through gas chromatography connected to a mass spectrophotometer model Saturn 2000 - Varian CG/MS.

Were transferred 990µL of the hexane extract from samples into chromatographic flasks, with 10µL of hexane for the chromatographic analyses. 1µL of this solution was injected into a gas chromatograph equip with capillary column CP Sil - 8 low bleed of 30 m of length x 0.25 mm of internal diameter x 0,25 µm of thickness of the film of 5% phenyl - 95% dimethylpolysiloxane.

The operational conditions of the chromatograph were: temperature of 120°C for injector and detector; initial temperature of 120°C for the column for 1 minute, with increase of 8°C per minute, remaining at 210°C for 10 minutes, with heating ratio of 5°C per minute up to 250°C, remaining at this temperature for 8 minutes. The running time was 38 minutes and 25 seconds. Helium was used as dragging gas, with a flow of 0.1mL min<sup>-1</sup>.

The fatty acids characterization occurred comparing the spectrum of masses obtained with data from literature using specific *software* installed in the computer coupled the CG-MS device. Based on values of the total area of peaks, which corresponds to 100%, the percentage of fatty acids could be quantified in function of the relative area of each peak.

### Ostrich and chicken egg yolk cholesterol determination

Figure 1 represents the flowchart of methods used in this study to determine cholesterol of *in natura* ostrich and chicken egg yolks. For both methods, the total lipids were extracted with chloroform: methanol (2:1), according to Folch et al<sup>4</sup>.

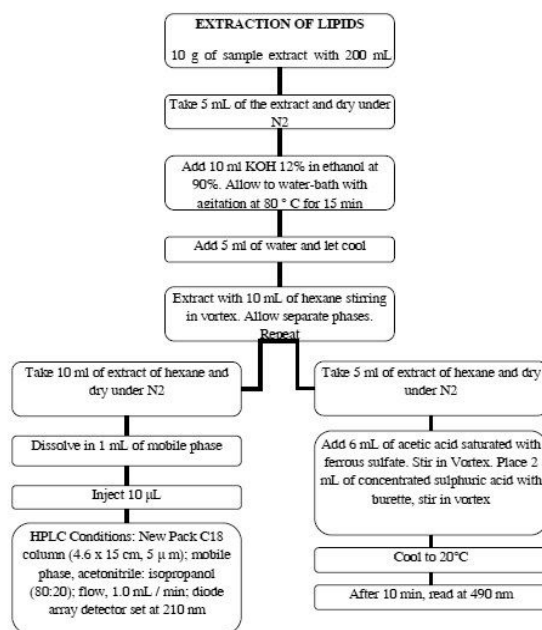


Figure 1. Flowchart for cholesterol determination through HPLC and colorimetry

### Cholesterol determination through colorimetry method

Cholesterol was determined through colorimetry method using *in natura* ostrich and chicken egg yolk as samples according to methodology described by Bragagnolo e Rodriguez-Amaya<sup>5</sup>. Were weighed 0.25 g of sample in test tubes with twist cap of 70 mL and 10 mL of 2% KOH in absolute ethanol was added. Later, the tubes were put in water bath at 50°C with agitation for 2 hours. Soon afterwards, 5 mL of distilled water was added and cooled. For the extraction of the unsaponifiable matter, 10 mL of hexane mL was added stirring in vortex for one minute. After separation, the entire hexane phase was transferred into a tube of 50 mL with twist on lid. The extraction was repeated twice. The last stage was the color reaction that was performed with tubes involved in aluminum paper, without incidence of light. 5mL of the hexanic extract were taken to dry in nitrogen and water-bath at 55°C, with the addition of acetic acid, being later saturated in FeSO<sub>4</sub> and titled with concentrated sulfuric acid.

The reading was performed in UV-vis scanning spectrophotometer label Quimis model Q798U2VS after 10 minutes at wavelength of 490nm. A calibration curve for cholesterol (from 0.5 to 5 mL) was performed before the entire process with the sample. The result is presented as the cholesterol content in mg/g sample according to the following formula:

$$\text{mg/g of cholesterol} = \frac{C \times D \times V \times 100}{A \times P \times v}$$

C - cholesterol concentration

A - Bracket of the hexane extract for the color reaction (5mL)

D - dilution of the sample

P - weigh of the sample

V - real volume of the hexane extract

v - ideal volume of the hexane extract

### Cholesterol determination through HPLC

For the cholesterol determination through HPLC, a liquid chromatograph label Varian Pro Star composed by isocratic pump with manual injector Reody, with sampling loop of 20µl and coupled to diode array detector (DDA) was used. The column chromatograph used was: New column Pack C18 with 15 x 4.6cm and 5µm of particle diameter.

Standard cholesterol in hexane solutions was prepared after drying and resuspension in acetonitrile, and their concentrations were determined by means of the absorbance measured in the HPLC. The standard solutions were conditioned in flasks wrapped up with aluminum paper and stored at -10°C. The manipulation of the standards and samples during the extraction was performed in environment with low brightness.

The extracts were filtered in membranes Millipore Fluoropore 0.5 µm to be soon later injected in the chromatograph column. The separation of the cholesterol column from the reverse phase was performed through an isocratic system.

As mobile phase, the acetonitrile/isopropanol (80:20) mixture was used at the flow rate of 1ml/min, being necessary 10 min of chromatographic operation and 5min of reconditioning of the column between injections. Wavelength of 210nm was used for the cholesterol detection.

For identification, the comparison of the retention times obtained standards at the same chromatograph conditions was used, and the absorption spectra obtained in the DAD. The quantification was accomplished according to external standardization method.

### Statistical analysis

The results of the research were submitted to statistical tests with the aid of the statistical package SPSS - Statistical Package for Social the Science version 11.0.

The results of the cholesterol analysis and the comparison between methods for the determination of lipids of white egg presented normal distribution, and the *t* Student test was applied for difference of averages at the level of significance of 5%. The data of the comparison between methods for lipid determination in yolk and whole egg did not present normal distribution; therefore, the test Mann-Whitney U was applied for difference of averages at the level of significance of 5%.

## RESULTS AND DISCUSSION

### Total lipids - Comparison of results obtained through Folch and Soxhlet methods

There were significant differences ( $p < 0.05$ ) between results obtained using different methods for the extraction of lipids in yolk and whole ostrich egg. The percentage of lipids from the ostrich egg yolk was  $38.48 \pm 1.07$  for Folch method and  $50.8 \pm 0.76$  Soxhlet method, while for the whole egg, the percentage of lipids was  $11.50 \pm 0.44$  by Folch method and  $34.50 \pm 1.32$  by Soxhlet method. No significant difference was observed in the percentage of lipids from ostrich egg white determined by the methods of Folch and Soxhlet which were respectively  $0.30 \pm 0.04$  and  $0.34 \pm 0.04$ . However, for the extraction of lipid in white egg, similarity was observed in the lipid quantification, what is explained because egg white presents only traces of lipids substances.

A difference of more than 20% could be observed in results obtained with the Soxhlet method in relation to the Folch method, in the quantification of lipids in yolk and whole egg. This evident difference could be mainly due to particularities of the different samples and to the choice of the solvents used (polarity of solvents).

The method of Folch et al<sup>4</sup> is known as a cold extraction method when compared with the Soxhlet method, this method has demonstrated to be very efficient in the determination of total lipids in foods rich in phospholipids such as eggs. Solvents chloroform and methanol used in the Folch method are more polar than n-hexane, used in the Soxhlet method and hence, polar and non-polar lipids are efficiently extracted<sup>3</sup>. The liposoluble vitamins and pigments are unsaponifiable and, therefore they cannot be extracted through the Folch method.

Solvent n-hexane used in the Soxhlet method, due to its non-polar characteristic, extracts not only lipids (triglycerides, phospholipids and cholesterol), but also non-polar substances<sup>3</sup> such as liposoluble vitamins and pigments, what could overestimate the lipid fraction in the egg fat, once this is the source of all liposoluble vitamins (A, D, E, K), besides containing many carotenoid pigments in the yolk that grant its characteristic color.

Moreover, in the Soxhlet method, where the sample is submitted to heat for more than 4 consecutive hours, lipid oxidation could occur, once this is favored by factors such as the water content, the time and the exposition temperature, the exposition degree of the sample to the light, among other factors, leading to the formation of compounds of higher molecular weight (peroxides, aldehydes, oxidized acids, etc.).

### Fatty acids

*In natura* ostrich egg yolk presents higher percentile of mono and polyunsaturated fatty acids and lower percentile of saturated fatty acids when compared to *in natura* chicken egg yolk (Table 1).

The fatty acids identified in this study were the same as those identified by Mazalli<sup>6</sup> in *in natura* and dehydrated chicken egg yolks. The main fatty acids identified in this study were the same as those identified by Di Meo et al<sup>7</sup> in *in natura* of ostrich egg yolks. Among the fatty acids identified in the samples, one can highlight the saturated fatty acids (SFA): tetradecanoic, pentadecanoic, hexadecanoic, octadecanoic, monounsaturated fatty acids (MUFA): hexadecenoic and octadecenoic and polyunsaturated fatty acids (PUFA): octadecadienoic, docosahexaenoic and eicosapentanoic.

**Table 1.** Main fatty acids present in chicken and ostrich egg yolk

Ostrich egg	Yolk	
	Chicken <i>in natura</i>	Ostrich <i>in natura</i>
<b>Total saturated fatty acids - SFA (%)</b>	26.94% <sup>a</sup>	25.61% <sup>b</sup>
Tetradecanoic	0.27% <sup>a</sup>	0.20% <sup>b</sup>
Pentadecanoic	10.27% <sup>b</sup>	10.78% <sup>b</sup>
Hexadecanoic	9.33% <sup>a</sup>	8.34% <sup>b</sup>
Octadecanoic	7.07% <sup>a</sup>	6.29% <sup>b</sup>
<b>Total monounsaturated fatty acids - MUFA (%)</b>	52.64% <sup>a</sup>	53.98% <sup>b</sup>
Hexadecenoic	0.33% <sup>b</sup>	1.03% <sup>a</sup>
Octadecenoic	52.31% <sup>b</sup>	52.95% <sup>a</sup>
<b>Total polyunsaturated fatty acids - PUFA (%)</b>	18.42% <sup>b</sup>	19.41% <sup>a</sup>
Octadecadienoic	13.93% <sup>b</sup>	15.31% <sup>a</sup>
Docosahexaenoic	0.30% <sup>b</sup>	0.90% <sup>a</sup>
Eicosapentanoic	1.30% <sup>b</sup>	1.75% <sup>a</sup>
Other fatty acids	2.85%	1.45%
<b><math>\omega</math>-6/ <math>\omega</math>-3 Ratio</b>	8.49	5.77

\*Averages followed by same letters in same row means no statistic difference ( $p > 0.05$ )

\*\*For lipids present in whole egg yolk, the Mann-Whitney U test was applied, and for egg white, the T-Student test was applied

The fatty acids composition of fat from yolk can be modified by diet, and the amount of dietary unsaturated fatty acids can modify the proportions of fatty acids present in yolk, what can explain the difference between the percentile of fatty acids present in chicken egg yolk when compared to ostrich egg yolk. It was observed that

only the results of polyunsaturated fatty acids were similar to those obtained by Sussi et al<sup>8</sup> in a study conducted in Italy in which ostrich egg yolks presented 36.26% of saturated of fatty acids, 47.61% of monounsaturated fatty acids and 15.45% of polyunsaturated fatty acids. Among the unsaturated fatty acids, the highest percentile observed

was of linoleic acid, and in the study conducted by Sussi et al<sup>8</sup> and considering monounsaturated fatty acids; the highest percentile found was of oleic acid.

In the last decades, studies have reported that diets with appropriate amounts of polyunsaturated fatty acids omega 3 (PUFAs  $\omega$  -3) and omega 6 (PUFAs  $\omega$  -6) play important role in the prevention and treatment of several diseases.

According to documents published, the relationship between  $\omega$ -6:  $\omega$ -3 from 10 to 5:1 is considered satisfactory. In this context, values presented by *in natura* chicken egg yolk and *in natura* ostrich egg yolk are within this range.

### Cholesterol - Comparison of the results obtained through both colorimeter and HPLC methods

The amount of cholesterol present in the *in natura* chicken egg yolk assessed in this study through the colorimeter method is of  $13.23 \pm 2.22$  mg/g, similar to that found in ostrich egg yolk, also determined through colorimetry, which is  $12.70 \pm 1.47$  mg/g.

Moreover, no statistic difference between the cholesterol contents found in chicken egg yolk obtained through HPLC, which was of  $12.71 \pm 1.54$  and the cholesterol contents found in ostrich egg yolk obtained through HPLC, which was of  $12.17 \pm 1.36$  mg/g was observed.

With the aid of the statistical analysis, it was observed that the results of both methods (colorimetry and HPLC) were equivalent for both *in natura* chicken egg yolk and ostrich egg yolk samples. In a similar study conducted by Bragagnolo e Rodriguez-Amaya<sup>9</sup> in meats, equivalence was also verified between cholesterol contents by both methods. However, the same authors have suggested that the HPLC method showed better repeatability, because the repeatability of the colorimetry method depended on the rigorous control of the reaction conditions.

The cholesterol level in the organism of birds is more dependent on its endogenous synthesis than on its dietary contribution. However, the place and the cholesterol synthesis vary with the species, age and feeding. Approximately two thirds of the synthesis occurs in the liver, 25% in the carcass and 6% in the intestine and in the skin.

The results obtained are close to those reported by Bragagnolo e Rodriguez-Amaya<sup>5</sup>, who also performed this determination through the colorimetry method and presented a variation range in the cholesterol value of chicken eggs between 10.33 and 18.86 mg/g yolk. The results obtained for the amount of cholesterol in the ostrich egg yolk corroborate with that found by Horbanczuk et

al<sup>10</sup>, which was of 13 mg/g yolk. Therefore, the amount of cholesterol present in the ostrich egg yolk is similar that found in the chicken egg yolk.

The most efficient method for the total lipids quantification in eggs is that developed by Folch et al<sup>4</sup>. The colorimetry method used to determine cholesterol is cheaper; however it can present many other variables, requiring rigorous control of the conditions and stages accomplished in this method. The HPLC method is more expensive but presents no other variables for its performance; however it requires qualified technicians for the use of its apparatus.

### CONCLUSIONS

Although ostrich egg yolk presents higher lipid content, the amount of cholesterol between the two types of eggs was similar. However, in terms of fatty acids, *in natura* ostrich egg yolks presented higher percentile of mono and polyunsaturated fatty acids and lower percentile of saturated fatty acids, being a fat of better nutritional quality.

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