

# A Review of Fatty Acid and Amino Acids Profile from Pasteurized Egg Liquids Produced in Romania

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## Abstract

Egg is a rich source of nutrients, such as high-quality protein (containing the ideal proportion of amino acids necessary for human body development) and a rich variety of vitamins and minerals. Liquid egg products have grown to a large extent in the food industry and beyond, which is an important reason to know their quality. For the analysis was used fresh pasteurized yolk and white wrapped bag in box. The fatty acids were determined by gas chromatographic method and amino acids by high performance liquid chromatography method. The results indicated that 34% of total fatty acids analyzed are saturated fatty acids, the main constituent being palmitic acid (25%) and 66% are unsaturated acids and the main constituent is oleic acid (37%). The rate of essential amino acids which are in pasteurized liquid white represents 46% from total analyzed amino acids and in the case of yolk the share is 44%. From obtained data for fatty acids content our limits are higher for polyunsaturated acids than those indicated by other researchers, and for monounsaturated acids are lower than data obtain by other authors. Regarding the essential amino acids content, the obtained data have higher values than the ones obtain by other authors. A possible explanation for the fluctuations in the profile of fatty acids and amino acids could be the feed distributed to the birds from which the eggs are obtained.

**Keywords:** amino acids, fatty acids, quality, white, yolk

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## Introduction

Nowadays, society is increasingly interested in consuming products that bring welfare and reduce the risk of disease (Lamas et al., 2016). In this context, polyunsaturated fatty acids, in particular omega-3 fatty acids have shown that they have positive effects on health of consumers and in particular have cardiovascular effects (Lands, 2000).

During the past years, the changes in diets and lifestyles resulting from industrialization, urbanization, economic development and market globalization have increased rapidly and particularly in developing countries where major socio-economic changes are occurring. Whereas general improvement in the standard of living has been observed, this has often been accompanied

by unhealthy dietary patterns and insufficient physical activity to maintain an optimal energy balance and a healthy weight. The net result has been increased prevalence of diet-related chronic diseases in all socio-economic groups and which now represent the main cause of deaths and disability worldwide.

The amount and type of fatty acids which we consume influences how the body uses them, and hence their role in some diseases, such as coronary heart disease and some ischemic conditions.

Most of the acids can be produced in the body, but linoleic acid and linolenic acid should be supplied by food.

The egg is such a food, which is considered to be the food with a perfect composition from the whole nature, provides the main source of

phospholipids in daily diet, thus contributing to provision of linoleic acid. In the body, however, linoleic acid and linolenic acid must be supplied by food.

Due to its acceptable price and its embedded properties, it is commonly used both as a standalone food and as an ingredient in food industry. Egg is not just a food rich in fatty acids but it is also a major source of high quality protein, which contains the total essential amino acids for a well functioning of body, as well as numerous vitamins and minerals. In order to increase the eggs' shelf life and for a better utilisation of them (in correlation with consumers preferences and by increasing demand of activity sectors which use eggs as raw materials/auxiliaries), the so-called derived from eggs or egg products, became foods that largely retain the quality of natural product but have a much better preservation than it (Machado *et al.*, 2007; Stadelman and Cotterill, 1995).

Egg contains many functional proteins, and their functional properties are very well known. Egg is an important animal protein, it contains all the essential amino acids needed for human body (Sakanaka *et al.*, 2000, Friedman 1996) and it is suitable for persons at all ages. Egg protein has the 'perfect' amino acids composition and was used as the reference protein for biological evaluation and assessment of amino acids patterns. The nutritional value of egg proteins, which has long been investigated, is the result of ideal balance of indispensable amino acids (Guyot *et al.*, 2013). The main proteins in white and yolk are ovalbumin, ovotransferin, lysozyme, ovomucoid, ovomucin and immunoglobulin Y, the ovalbumin is the most abundant of these proteins, which accounts for more than half of the protein in egg white.

Due to the unique functional properties of proteins, such as gelling and foam formation, white proteins are commonly used in processed products such as confectionery and pastry but also in meat industry (Machado *et al.*, 2007).

So, egg is recognized as an animal product that can be transformed, which is an important reason for knowing the profile of amino acids and fatty acids from pasteurized liquid white and yolk produced in Romania.

The aim of the current paper is to present the profile of fatty acids from pasteurized liquid yolk

and amino acids from pasteurized egg products produced in our country.

### Materials and methods

Analyses were carried out on samples of yolk and pasteurized white, used as raw material in various branches of food industry.

The material subjected to analysis was represented by 3 packing units of 2 kg each.

For analysis of foods for fatty acids information, the AOAC Official method 996.06 is recommended (AOAC, 2005). The procedure involves hydrolysis of food samples using either an acid or a base, followed by ether extraction of the released fat, trans-etherification of extracted fat to FAME and determination of fatty acid profile by capillary GC.

Determination of fatty acids from samples of pasteurized liquid yolk was done by chromatographic method involving the conversion of fatty acids into methyl esters and then separation on a chromatographic column and comparison of the results with standard chromatogram, as well as their percentage quantisation. Preparation of methyl esters was performed in according with ISO 5508:2002. The results are expressed as percentage of methyl esters of fatty acids.

As far as the determination of amino acids is concerned, this was done by liquid chromatography which involved the breakdown of amino acid from protein molecule by acid hydrolysis. Amino acids are determined after orthophaladehyde samples and 338  $\mu\text{m}$  detection. The method was performed in according with SR EN ISO 13903:2005, concentration calculation was done by plotting the peak area at calibration curve. Results of analyses were processed by statistically calculating position and variance estimators (arithmetic mean, standard deviation of mean  $S$  and variation coefficient  $V\%$ ).

### Results and discussion

It can be seen that the sum of saturated fatty acids was  $34.42 \pm 0.08$  g/100 g, less than the sum of monounsaturated fatty acids. Palmitic acid (C16:0) is the main constituent of these saturated fatty acids having a value of 24.54 g/100 g, followed by stearic acid with a value of 9.34 g/100 g.

The sum of monounsaturated fatty acids was  $41.40 \pm 0.09$  g/100 g, the main constituent acid being oleic acid with a value of 37.57 g/100 g, the average value obtained was less than variation

limits found in literature, especially for liquid yolk which ranged from 40.77-53.21 g/100 g (Badr et al., 2006; Kovalcuks et al., 2014). In the case of polyunsaturated acids, linoleic acid was the main component, its value being 15.63 g/100 g, which is within the limits found in literature (13.65-16.81 g/100 g) (Tesedo et al., 2006; Badr et al., 2006).

The fact that yolk contributes to the health of consumers is evidenced by the rate of omega-6 / omega-3 fatty acids to 16.45 g/100 g.

Approximately 20% of total lipids are fatty acids, of which 90% are n-6 PUFA and 10% n-3 PUFA. The n-6 / n-3 rate is 9:1, so to improve this ratio, some supplements rich in n-3 are used because it has been shown that a 5:1 ratio can reduce the risk of thrombosis, inflammation of vascular wall and myocardial arrhythmia at humans (Lands, 2000; Leaf et al., 2000).

Research over the past decade has elucidated the importance of omega-3 or n-3 fatty acids in human and animal health. The current consumption of n-3 fatty acids in many Western countries is less than the recommended values (Report of the National Cholesterol Education Program, 1988; Simopoulos, 2000).

Animal products contribute more than 70% of SFA in a typical diet. It is widely accepted that the current diets are low in n-3 PUFA and high in SFA. The reason for this low consumption of PUFA may be due to recent changes in dietary habits and to management and feeding practices of domestic animals.

The results of our study on the fatty acid profile show differences from the studied literature due to genetics, age, feeding program, and also to the level and type of dietary lipids.

**Table 1.** The content of yolk in fatty acids

Fatty acid (%)	$\bar{X} \pm s_{\bar{x}}$	Limits of variation in literature	
		Liquid pasteurized yolk	Raw yolk
C14:0	0.29 ± 0.01	0.09 <sup>1</sup> -0.48 <sup>2</sup>	0.36 <sup>3</sup> -0.57 <sup>4</sup>
C15:0	0.06 ± 0.01	0.08 <sup>1</sup> -0.09 <sup>1</sup>	
C16:0	24.54 ± 0.08	22.27 <sup>1</sup> -25.09 <sup>2</sup>	25.1 <sup>3</sup> -31.01 <sup>4</sup>
C17:0	0.18 ± 0.01	0.19 <sup>1</sup> -0.21 <sup>2</sup>	0.35 <sup>4</sup>
C18:0	9.34 ± 0.09	6.10 <sup>1</sup> -9.19 <sup>2</sup>	7.29 <sup>4</sup> -8.37 <sup>3</sup>
<b>Σ SFA</b>	<b>34.42±0.08</b>	<b>28.80<sup>1</sup>-35.08<sup>2</sup></b>	<b>33.8<sup>3</sup>-39.22<sup>4</sup></b>
C14:1	0.06 ± 0.01	0.03 <sup>1</sup> -0.09 <sup>2</sup>	
C15:1	0.10 ± 0.01		
C16:1	3.36 ± 0.01	0.28 <sup>1</sup> -3.76 <sup>2</sup>	3.23 <sup>3</sup>
C17:1	0.08 ± 0.01	0.11 <sup>1</sup> -0.12 <sup>1</sup>	
C18:1n9	37.57 ± 0.05	40.77 <sup>2</sup> -53.21 <sup>1</sup>	41.29 <sup>4</sup> -46.7 <sup>3</sup>
C22:1n9	0.09 ± 0.01	0.03 <sup>1</sup>	
C24:1n9	0.29 ± 0.01	0.02 <sup>1</sup>	
<b>Σ MUFA</b>	<b>41.40±0.09</b>	<b>45.13<sup>2</sup>-53.91<sup>1</sup></b>	<b>46.95<sup>4</sup>-50<sup>3</sup></b>
C18:2n6	15.63 ± 0.07	13.65 <sup>1</sup> -16.81 <sup>2</sup>	12.43 <sup>3</sup> -13.1 <sup>4</sup>
C20:2n6	0.17 ± 0.01	0.01 <sup>1</sup>	
C20:3n6	0.20 ± 0.01	0.15 <sup>1</sup> -0.19 <sup>1</sup>	
C20:4n6	0.95 ± 0.08	0.07 <sup>1</sup> -1.77 <sup>2</sup>	1.17 <sup>4</sup> -1.83 <sup>3</sup>
<b>Σ n-6 PUFA</b>	<b>16.95 ± 0.15</b>	<b>13.88<sup>1</sup>-18.78<sup>2</sup></b>	<b>13.6<sup>4</sup>-14.93<sup>3</sup></b>
C18:3n3	0.27 ± 0.02	0.99 <sup>2</sup> -1.77 <sup>1</sup>	
C22:5n3	0.09 ± 0.01	0.05 <sup>1</sup> -0.06 <sup>1</sup>	
C22:6n3	0.67 ± 0.01	0.95 <sup>1</sup> -1.02 <sup>1</sup>	0.85 <sup>3</sup>
<b>Σ n-3 PUFA</b>	<b>1.03 ± 0.04</b>	<b>1.99<sup>1</sup>-2.85<sup>1</sup></b>	
<b>Ω6/Ω3</b>	<b>16.45</b>	<b>6.59<sup>1</sup>-6.97<sup>2</sup></b>	<b>16<sup>3</sup>-17.58<sup>4</sup></b>

<sup>1</sup>Kovalcuks, 2014, <sup>2</sup>Badr, 2006, <sup>3</sup>Samman, 2008, <sup>4</sup>Tesedo, 2006

**Table 2.** The amino acids content of yolk

Amino acids (g/100 g)	$\bar{X} \pm s_{\bar{x}}$	Limits of variation in literature	
		Liquid pasteurized yolk	Raw yolk
Valine	2.53 ± 0.02	0.53 <sup>3</sup> -0.96 <sup>4</sup>	0.15 <sup>1</sup> -1.50 <sup>2</sup>
Isoleucine	1.87 ± 0.01	0.43 <sup>3</sup> -0.87 <sup>4</sup>	0.14 <sup>1</sup> -1.29 <sup>2</sup>
Leucine	2.94 ± 0.02	1.33 <sup>3</sup> -1.39 <sup>4</sup>	0.24 <sup>1</sup> -2.12 <sup>2</sup>
Lysine	2.72 ± 0.01	1.16 <sup>3</sup> -1.21 <sup>4</sup>	0.22 <sup>1</sup> -1.94 <sup>2</sup>
Methionine	0.45 ± 0.01	0.18 <sup>3</sup> -0.37 <sup>4</sup>	0.37 <sup>1</sup> -0.88 <sup>2</sup>
Threonine	1.79 ± 0.02	0.45 <sup>3</sup> -0.85 <sup>4</sup>	0.14 <sup>1</sup> -1.42 <sup>2</sup>
Phenylalanine	1.65 ± 0.02	0.31 <sup>3</sup> -0.68 <sup>4</sup>	0.11 <sup>1</sup> -1.10 <sup>2</sup>
<b>Total essential amino acids</b>	<b>13.95</b>	<b>4.39<sup>3</sup>-6.33<sup>4</sup></b>	<b>1.37<sup>1</sup>-10.25<sup>2</sup></b>
Alanine	1.91 ± 0.01	0.80 <sup>3</sup> -0.83 <sup>4</sup>	0.14 <sup>1</sup> -2.30 <sup>2</sup>
Arginine	2.74 ± 0.01	1.07 <sup>3</sup>	0.19 <sup>1</sup> -3.14 <sup>2</sup>
Asparagic acid	3.07 ± 0.01	1.48 <sup>3</sup> -1.55 <sup>4</sup>	0.62 <sup>1</sup> -3.38 <sup>2</sup>
Glycine	0.76 ± 0.01	0.47 <sup>3</sup>	0.08 <sup>1</sup> -0.76 <sup>2</sup>
Glutamic acid	4.31 ± 0.02	0.85 <sup>3</sup> -1.91 <sup>4</sup>	0.35 <sup>1</sup> -4.86 <sup>2</sup>
Serine	2.53 ± 0.01	1.28 <sup>3</sup> -1.32 <sup>4</sup>	0.23 <sup>1</sup> -3.00 <sup>2</sup>
Tyrosine	1.63 ± 0.01	0.67 <sup>4</sup>	0.12 <sup>1</sup> -2.24 <sup>2</sup>
Cysteine	0.33 ± 0.01	0.27 <sup>3</sup>	0.05 <sup>1</sup> -0.81 <sup>2</sup>
<b>Total non-essential amino acids</b>	<b>17.28</b>	<b>6.89<sup>3</sup>-8.09<sup>4</sup></b>	<b>1.78<sup>1</sup>-20.49<sup>2</sup></b>
<b>Total</b>	<b>31.23</b>	<b>11.28<sup>3</sup>-14.42<sup>4</sup></b>	<b>3.15<sup>1</sup>-30.74<sup>2</sup></b>

<sup>1</sup>Nys, 2011, <sup>2</sup>Harniakova, 2007, <sup>3</sup>Badr, 2006, <sup>4</sup>Darvish, 2012

Amino acid analysis from pasteurized liquid yolk for the fresh product are presented in correlation with the variation limits found in literature for both pasteurized liquid yolk and raw yolk. Analysis of essential amino acids indicates a total mean value of 13.95 g/100 g with a major constituent of leucine which had a mean value of 2.94±0.02 g/100 g and at the opposite was the average content in methionine which was 0.45±0.01 g/100 g. The variation limits found in literature for total essential amino acids ranged from 4.39-6.33 g/100 g for pasteurized liquid yolk and between 1.37-10.25 g/100 g for raw yolk. The total non-essential amino acids determined were 17.28 g/100 g, mainly being glutamic acid with an average of 4.31±0.02 g/100 g and the lowest intake had cysteine whose mean value was 0.33±0.01 g/100 g.

Other authors such as Badr *et al.*, 2006, Nys *et al.*, 2011 and Darvish *et al.*, 2012 showed variations in amino acid variation in pasteurized liquid yolk ranging from 11.28-14.42 g/100 g.

Data on amino acid content highlights the same as in Harnikova *et al.* 2007 their fluctuations

are due to the enzyme content of the feed administered.

White proteins are rich in essential amino acids and possess excellent nutritional properties are considered reference proteins (Vacaru-Opriş *et al.*, 2000).

We analysed the amino acids in the pasteurized liquid white, expressed for the fresh product. Analysis of essential amino acids indicates a total mean value of 35.94 g/100 g with a major constituent of leucine whose mean value was 7.41±0.01 g/100 g and at opposite was the average methionine content of 1.61±0.01 g/100 g. The variation limits found in literature for total essential amino acids were between 30.5-32.87 g/100 g for pasteurized liquid white and between 43.76-45.33 g/100 g for raw white. The total non-essential amino acids determined were 42.07 g/100 g, mainly glutamic acid with an average of 11.77±0.03 g/100 g, and the lowest intake had cysteine with an average value of 1.11±0.03 g/100 g. According to Sosulski and Imafidon (1990) the highest individual amino acids content in egg was lysine (509 mg/g) whereas the lowest was

**Table 3.** The amino acids content of white

Amino acids	$\bar{X} \pm s_{\bar{x}}$	Limits of variation in literature	
		Liquid pasteurized white	Raw white
Valine	7.23 ± 0.02	6.25 <sup>1</sup>	7.20 <sup>2</sup> -7.35 <sup>2</sup>
Isoleucine	4.67 ± 0.02	4.31 <sup>1</sup>	5.60 <sup>2</sup> -6.28 <sup>2</sup>
Leucine	7.41 ± 0.01	7.46 <sup>1</sup>	8.8 <sup>2</sup> -9.17 <sup>2</sup>
Lysine	5.71 ± 0.04	0.8 <sup>1</sup> -2.37 <sup>1</sup>	6.6 <sup>2</sup> -6.83 <sup>2</sup>
Methionine	1.61 ± 0.01	2.98 <sup>1</sup>	4.13 <sup>2</sup>
Threonine	4.20 ± 0.01	4.36 <sup>1</sup>	4.70 <sup>2</sup> -4.84 <sup>2</sup>
Phenylalanine	5.11 ± 0.02	4.34 <sup>1</sup>	6.73 <sup>2</sup>
<b>Total essential amino acids</b>	<b>35.94</b>	<b>30.5<sup>1</sup>-32.87<sup>1</sup></b>	<b>43.76<sup>2</sup>-45.33<sup>2</sup></b>
Alanine	4.91 ± 0.01	0.7 <sup>1</sup> -7.62 <sup>1</sup>	6.94 <sup>2</sup>
Arginine	5.45 ± 0.02	3.71 <sup>1</sup>	6.21 <sup>2</sup>
Asparagic acid	7.74 ± 0.01	8.96 <sup>1</sup> -12.20 <sup>1</sup>	10.08 <sup>2</sup>
Glycine	2.17 ± 0.01	5.38 <sup>1</sup>	3.85 <sup>2</sup>
Glutamic acid	11.77 ± 0.03	10.41 <sup>1</sup>	15.10 <sup>2</sup>
Serine	5.33 ± 0.01	7.4 <sup>1</sup> -7.98 <sup>1</sup>	7.60 <sup>2</sup>
Tyrosine	3.59 ± 0.02	2.63 <sup>1</sup>	3.97 <sup>2</sup>
Cysteine	1.11 ± 0.01	2.37 <sup>1</sup>	2.77 <sup>2</sup>
<b>Total non-essential amino acids</b>	<b>42.07</b>	<b>41.56<sup>1</sup>-52.3<sup>1</sup></b>	<b>56.52<sup>2</sup></b>
<b>Total</b>	<b>78.01</b>	<b>72.06<sup>1</sup>-85.17<sup>1</sup></b>	<b>100.28<sup>2</sup>-101.85<sup>2</sup></b>

<sup>1</sup>Badr, 2006, <sup>2</sup>Nys, 2011

cysteine (128 mg/g). Other authors such as Badr et al., 2006 and Darvish et al., 2012 provide variation values for pasteurized liquid white of 41.56-52.3 g/100 g.

### Conclusions

The results obtained for pasteurized liquid yolk recorded lower values of saturates and monounsaturated fatty acids compared to crude fatty acids from Badr et al., 2006 and Tessedo et al., 2006 (34.42 vs. 35.08 and 39.22g/100 g), but also higher values of polyunsaturated fatty acids (Samman et al., 2009, Kovalcuks et al., 2014, 16.95 vs. 14.93 and 13.88 g/100 g) due to pasteurization temperatures affecting the structure of omega 3 fatty acids. The fatty acids profile of processed eggs depends on their origin and the treatment received before they reach the market.

The data obtained for amino acids content from pasteurized liquid yolk indicates an average value of 31.23 g/100 g being similar to the limits found in literature (Nys et al., 2011, Darvish, 2012).

Concluding, the data obtained for amino acids content from pasteurized liquid white indicates an average value of 78.01 g/100 g, which strengthens

the idea of standard protein used in food pyramid due to the major benefits brought by consumption of this protein in human nutrition.

The recommendations made on the basis of the study are to introduce the eggs into the daily diet because they provide vitamins, minerals and amino acids essential to the human body and at the same time contain two essential acids that cannot be synthesized by the human body (18:2n6 and 18:3n3).

It can be said, based on this study, that egg preservation processes do not irreversibly affect their quality and the unique properties they hold, fact which is so important to modern consumer but also to industry.

### References

1. Badr HM (2006). Effect of gamma radiation and cold storage on chemical and organoleptic properties and microbiological status of liquid egg white and yolk. *Food Chemistry*, 97:285-293.
2. Darvishi H, Khoshtaghza MH, Zarein M, Azadbakht M, (2012). Ohmic processing of liquid whole egg, white egg and yolk. *Agric. Eng. Int.: CIGR Journal*, 5(1):210-216.
3. Friedman M, (1996). Food browning and its prevention: an overview. *J. Agric. Food Chem.*, 44(3):631-653.

4. Guyot N, Jan S, Rehault-Godbert S, Nys Y, Gautier M, Baron F, (2013). Antibacterial activity of egg white: influence of physical-chemical conditions. *World's Poultry Science Journal*, Volume 69, Supplement.
5. Horniakowa E, Michálková J, (2007). Effect of diet on amino acid profile of egg yolk. *Pol. J. Food Nutr. Sci.* Vol. 57,4(B): 235-238.
6. Kovalcuks A, Duma M, (2014). Solvent extraction of egg oil from liquid egg yolk. *Foodbalt 9<sup>th</sup> Baltic Conference on Food Science and Technology "Food for Consumer Well-Being"*:253-256.
7. Lamas A, Anton X, Miranda JM, Roca-Saavedra P, Cardelle-Cobas A, Rodriguez JA, Franco CM, Cepeda A, (2016). Technological development of functional egg products by an addition of n-3 polyunsaturated-fatty-acid-enriched oil, *CyTA. Journal of Food*, 14(2):289-295.
8. Lands WEM (2000). Lipid mediators in a paradigm shift: balance between  $\omega$ -6 and  $\omega$ -3. In: Sim JS, Nakai S, Guenter W. *Egg nutrition and biotechnology* (p. 35-52). CABI Publishing, Wallingford, Oxon., UK.
9. Leaf A, Kang JX, Xiao YF, (2000). Antiarrhythmic effects of n-3 polyunsaturated fatty acids. In: Sim JS, Nakai S, Guenter W. *Egg nutrition and biotechnology* (p. 25-33). CABI Publishing, Wallingford, Oxon., UK.
10. Machado FF, Coimbra JS, Rojas EEG, Minim LA, Oliveira FC, Rita de Cássia SS (2007). Solubility and density of egg white proteins: Effect of pH and saline concentration. *LWT-food Science and Technology*, 40(7):1304-1307.
11. Nys et al., (2011). Improving the safety and quality of eggs and eggs products. Woodhead Publishing Limited, U.K (Chapter 11).
12. Sakanaka S, Kitahata K, Mitsuya T, Gutierrez MA, Juneja LR, (2000). Protein quality determination of delipidated egg-yolk. *Journal of Food Composition and Analysis*, 13(5): 773-781.
13. Sammam S, Kung FP, Carter L, Foster M, Ahmad Z, Phuyal J, Petocz P, (2009). Fatty acid composition of certified organic, conventional and omega-3 eggs. *Food chemistry*, 116:911-914.
14. Stadelman WJ, Cotterill OJ, (1995). *Egg science and technology*. The Haworth Press Inc., New York.
15. Simopoulos AP, (2000). Human requirement for n-3 polyunsaturated fatty acids. *Poult. Sci.*, 79(7):961-70.
16. Sosulski FW, Imafidon GI, (1990). Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. *J. Agric. Food Chem.* 38(6):1351-1356.
17. Tesedo J, Barrado E, Sanz A, Tesedo A, De la Rosa F, (2006). Fatty acid profiles of processed chicken egg yolks. *Journal of Agricultural and food chemistry*, 54(17):6255-6260.
18. Vacaru-Opriş I. et al., (2000) and (2007 republished). *Tratat de Avicultura*. vol. I, Editura Ceres, Bucureşti.
19. ISO 13903:2005(en). *Animal feeding stuffs — Determination of amino acids content*.
20. SR EN ISO 5508:2002. *Oils and fats of vegetable and animal origin. Analysis by gas chromatography of methyl esters of fatty acids*.
21. Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. The Expert Panel. *Arch Intern Med.* 1988 Jan, 148(1):36-69.