EFFECT OF THE PHOSVITIN ISOLATION METOD ON YIELD OF THE PROCESS

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Abstract
The aim of the present study was to compare two NaCl extraction methods of hen egg yolk phosvitin isolation, which differ in the use of organic solvents. The study was conducted on eggs from hens of Lohmann line and Green-leg breed, which were divided into 4 groups : I – eggs from Lohmann line fed without supplementation, II – eggs from Lohmann line fed with the addition of humokarbowit and humobentofet, III – eggs from Green-leg breed fed without supplementation, IV – eggs from Green-leg breed fed with the addition of humokarbowit and humobentofet. In the study, the yield of the phosvitin was examined and the following chemical components of yolk were analysed: dry matter, free fat and protein content.
The results of the experiment showed that the breed of hens is responsible for yield of phosvitin isolation from hen egg yolk. Green-leg breed had the highest yield of phosvitin isolation in comparison to Lohmann line. The highest yield of phosvitin isolation was recorded in groups fed with addition of humokarbowit and humobentofet (II, IV). The type of used method of phosvitin isolation from hen egg yolk had no significant effect on the yield of the process.
Key words: phosvitin, isolation method, egg yolk protein

Introducion
The avian egg is an encapsulated material indispensable for the development of the embryo. The chicken embryo develops into a chick by utilizing only the materials inside the shell at an adequate temperature. The protein, lipids, carbohydrate, vitamins (except vitamin C), and minerals contained in the egg are all necessary and sufficient for developing the chicken's body (Gutierrez *et al.,* 1997).
Egg proteins have an ideal balance of amino acids. The protein value of whole egg protein is considered to be 100. The World Health Organization, approved egg protein as a standard for measuring the nutrition quality of other food proteins (FAO/WHO/UNU, 1985).
Egg yolk is considered a potentially important source of energy because more than 65% of the contents of dry yolk is lipids. Egg yolk contains triglycerides, phospholipids, and sterols. Hen eggs are a rich source of linoleic acid, which is essential in human nutrition (Gutierrez *et al.,* 1997).
Egg yolk phosphatidylcholine contains important quantities of ω3 fatty acids as the non polar part. Choline, from the polar part is an important nutrient in brain development, liver function and cancer prevention (Gutierrez *et al.* 1997). Consumption of phosphatidylcholine increases plasma and brain choline level and accelerates neuronal acetylcholine synthesis. It has been demonstrated that consumption of yolk phospholipids tends to alleviate the symptoms of Alzheimer disease (Juneja 1997).
Hen egg yolk phosvitin represents about 11% of yolk protein (Ito *et al.,* 1962). Phosvitin has a molecular weight of about 35 kDa and contains 217 amino acid residues, of which 123 residues are serine residues (Byrne *et al.,* 1984, Clark *et al.,* 1971). Of the 123 serine residues, 118 are phosphorylated and 5 are "free" (Clarc *et al.,* 1985). Phosvitin, due to its peculiar physical and chemical characteristics, possesses a variety of functional and biological properties (Anton *et al.,* 2007).

Material and Methods
The study was conducted on two types of eggs from Lohmann line and Green-leg breed. The eggs were divided into 4 groups, depending on the line of hens and the feeding method. Two groups of hens were fed without supplementation and the remaining were fed with addition of humokarbowit and humobentofet. Humokarbowit and humobentofet contain polyunsaturated fat acids and their formula is exclusive.

The four groups were:
I – eggs from Lohmann line fed without supplementation,
II – eggs from Lohmann line fed with the addition of humokarbowit and humobentofet,
III – eggs from Green-leg breed fed without supplementation,
IV – eggs from Green-leg breed fed with the addition of humokarbowit and humobentofet.
In the study, the following chemical components of yolk were analysed: dry matter by thermal drying method in 105 °C according to PN-ISO 1442:2000, free fat by Soxlet's method according to Polish standards of PN-ISO 1444:2000 and protein content by Kjeldahl's method, using a Kjeltec™ 2300 according to Polish standards of PN 75/A-0418.
The isolation of phosvitin was prepared with 15 eggs from each group.
The first method of isolation of hen egg yolk phosvitin was modification of the Losso and Nakai method (1994). Fifteen egg yolks were diluted with 0.5 l of cold distilled water at pH 5.0 and stirred at 4 °C for 1 h. The precipitated material was collected by centrifugation at 10,000g for 20 min at 4 °C. The pellet was dissolved in 200 ml distilled water, stirred for 1 h and centrifuged at 10,000 for 20 min at 4°C. The pellet was extracted with 400 ml of hexan: etanol (3:1, v/v) at 4 °C for 3 h and centrifuged. The resulting cake was dried with acetone and extracted with 200 ml of 1.74 M NaCl overnight at 4 °C. Next, the suspension was centrifuged at 10,000 g for 20 min at 4 °C and the supernatant was dialyzed against distilled water for 24 h at 4 °C and freeze dried.
The second method of isolation of hen egg yolk phosvitin was modification of the Pangborn method (1950). Fresh egg yolk from 15 eggs was diluted with 200 ml of 0.9 NaCl solution at pH 5.5 and centrifuged at 10,000 g for 1.5h. The granules were extracted in 100 ml of acetone, stirred for 1h and centrifuged at 10,000 g for 10 min at 4 °C. The precipitated material was extracted in 100 ml acetone, stirred for 1 h and centrifuged at 10.000 g for 10 min at 4 °C. Next, the pellet was extracted twice in 100 ml of etanol, stirred for 1 h and centrifuged (10.000 g, 10 min, 4 °C). The resulting cake was dried with acetone and extracted with 100 ml of 1,74 NaCl overnight at 4 °C and the supernatant was dialyzed against distilled water for 24 h at 4 °C and freeze dried.
The data were analyzed statistically, using 6.0 software. Two-way analysis of variance was used to test the effect of diet and breed on the variable examined. Significant differences between the mean values were determined using Duncan's test ($\alpha = 0.05$).

Results and Discussion

In the study, the following chemical components of yolk were examined: dry matter, free fat and protein content (49.57%, 16.19% and 30.16%) in research groups.
The results obtained in the study showed that the breed of hens and way of feeding was not responsible for dry matter content (Table 1). However, the breed of hens was responsible for protein content. Free fat content depend on the type of hen feeding, but it is significant only at $0.01<P<0.05$.

Table 1

Influence of hen line and the method of hen feeding on chemical components

Chemical components	Variability	F Value	Significance
Dry matter	Feeding stuff	2.118	0.1489
	Hen line	0.901	0.3551
Protein content	Feeding stuff	1.069	0.3038
	Hen line	10.220	0.0019**
Free fat	Feeding stuff	6,736	0.0147*
	Hen line	0.073	0.7922

*** $P \le 0.001$ and ** $0.001<P\le 0.01$ and *$0.01<P<0.05$ meant the significant difference between groups and isolation method.

The measurements of the dry matter content (Table 2) in hen egg yolk showed that the highest content was in eggs from Green-leg breed fed without supplementation. The remaining groups (I, II, IV) had similar content of dry mater and the differences were insignificant.
The highest content of protein (Table 2) was found in egg from both Green-leg breed fed with supplementation and without it. The way of feeding was not responsible for protein content.
Eggs from Green-leg breed fed without supplementation had the highest content of free fat (31.25%) (Table 2). Group I, II and IV had lower content of free fat, but the differences were insignificant.

Table 2

Yolk chemical components

Chemical components	**Research group**			
	I	**II**	**III**	**IV**
Dry matter[%]	49.41 a	49.92 b	49.61 ab	49.34 a
Protein content[%]	16.10 a	16.31 b	16.07 a	16.30 b
Free fat[%]	30.03 ab	31.25 c	30.19 b	29.17 a

a, b, c – different letters in the same row for the same parameter indicate statistically significant differences ($P \leq 0.05$)

The results obtained in the study show that the yield of phosvitin isolation did not depend on research groups and the isolation method (Table 3).

Table3

Influence of research group and isolation method on the yield of phosvitin isolation

Structure	**Variability**	**F Value**	**Significance**
Phosvitin	Group Isolation method	2.006 0.052	0.1369 0.8231

*** $P \leq 0.001$ and ** $0.001 < P \leq 0.01$ and *$0.01 < P < 0.05$ meant the significant difference between groups and isolation method.

Table 4 shows the yield of phosvitin isolation from eggs using Nakai- Losso and Pangborn method.

Table 4

Yield phosvitin isolation method from hen egg yolk [%]

Isolation method	**Research group**			
	I	**II**	**III**	**IV**
Nakai- Losso	2.92 a	3.62 ab	3.55 ab	4.00 b
Pangborn	2.36 a	3.74 ab	3.73 ab	4.73 b

a, b, c – different letters in the same row for the same parameter indicate statistically significant differences ($P \leq 0.05$)

The results of the experiment showed that the highest yield of phosvitin isolation using Nakai-Losso method was in eggs from Green-leg breed fed with supplementation. The lowest yield of phosvitin isolation was recorded in eggs from Lohmann line fed without supplementation. The highest yield of phosvitin isolation by Pangborn method was found in eggs from Green-leg breed fed with supplementation and the lowest was in eggs from Lohmann line fed without supplementation.

Table 5

Comparison of phosvitin isolation method from hen egg yolk [%]

Isolation method	Research group			
	I	II	III	IV
Nakai- Losso	2.92 ab	3.62 ab	3.55 ab	4.00 ab
Pangborn	2.36 a	3.74 ab	3.73 ab	4.73 b

a, b, c – different letters in the same row for the same parameter indicate statistically significant differences ($P \leq 0.05$)

Table 5 shows the yield of phosvitin isolation from all research groups isolated by Nakai-Losso and Pangborn method. The analysis of the results showed that the highest yield of phosvitin isolation was in eggs from Green-leg breed fed with supplementation, isolated using Pangborn method. The lowest yield of phosvitin isolation was recorded in eggs from Lohman line fed without supplementation, also isolated using Pangborn method.

Conclusions

1. The results of the experiment showed that the breed of hens is responsible for the yield of phosvitin isolation from hen egg yolk. The eggs from Green-leg breed had the highest yield of phosvitin isolation in comparison to Lohmann line.
2. Feeding with addition of humokarbowit and humobentofet, which contains polyunsaturated fat acids, is responsible for yield of phosvitin isolation.
3. The results obtained in the study show that isolation method was not responsible for the yield of phosvitin.

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References

1. Anton M., Castellani O., Guerin-Dubiard C. (2007) *Bioactive Egg Compounds.* Springer 4:17, pp. 23.
2. Byrne B.M., van het Schip A.D., van de Klundert J.A.M., Amberg A.C., Gruber M., Geert A.B. (1984) Amino acid sequence of phosvitin derived from the nucleotide sequence of part of the chicken vitellogenin gene. Biochem 23, pp. 4275–4279
3. Clarc, R.C., Joubert F.J., (1971) The composition of cyanogens bromide cleavage production from hen's egg phosvitin. FEBS Lett. 13, pp. 225.
4. Clarc, R.C., (1985) The primary structure of avian phosvitins. Contributions through the Edman degradation of methylmercaptovitins prepared from the constituent phosphoproteins. Inter J Biochem. 17, pp. 983
5. Damodaran S., Xu S., (1996) The role of electrostatic forces in Anomalous Adsorption Behavior of Phosvitin at the air/water interface, Jurnal of Colloid and Interface Science 178, pp. 426–435.
6. FAO/WHO/UNU., Energy and protein requirements, Reports of the Joint FAO/WHO/UNU Expert Consultation, Geneva, Switzerland, 1985
7. Gutierrez M.A., Takahashi H., Juneja L.R. (1997) *Nutritive evaluation of hen eggs*. In: Yamamoto T., Juneja L.R., Hatta H., Kim M. (eds) Hen eggs, their basic and applied science. CRC Press, New York, pp 25–35.
8. Ito Y., Fujii, T. (1962) Chemical composition of the egg yolk lipoproteins. J *Biochem* 52: pp. 22–222
9. Juneja L.R. (1997) *Egg yolk lipids*. In: Yamamoto T., Juneja R.L., Hatta H., Kim M. (eds) Hen eggs, their basic and applied science. CRC Press, New York, pp 73–98.
10. Losso J.H., Nakai S.A.(1994) Simple procedure for the isolation of phosvitin from chicken egg yolk. In J. S. Sim, S. Nakai (eds.). Egg used and processing technologies: New developments (pp. 50-157). Oxon, UK: Cab International.
11. Pangborn M.C.,(1950) A simplified purification of lecithin, J. Biol. Cem., 188, pp. 471
12. Ragiel A. (2007), Initial steps of phosvitin isolation procedure from the chicken egg yolk, Master's thesis, Wroclaw University of Environmental and Life Sciences.