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# The effect of water activity on cholesterol oxidation in spray- and freeze-dried egg powders

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#### Abstract

The aim of the paper was to estimate the effect of water activity in spray- and freeze-dried whole egg and egg yolk powders on cholesterol oxidation during 3-months of storage at room temperature.

Egg powders were divided into three groups. The first group of powders was not moisturized, the second and the third groups were moisturized to 8% and 12% of water content. The samples of powders placed in plastic bags without venting were stored for 3 months in darkness at room temperature. Directly after moisturizing of egg powders, the chemical composition (water, proteins, lipid) was measured. In fresh and stored powders the contents of cholesterol and oxysterols were estimated. Water activity was higher in egg yolk powders than in whole egg powders. Egg yolk powders contain less hydrophylic proteins and more hydrophobic lipids than whole egg powders. During storage the highest oxysterol accumulation appeared in egg powders with the lowest water activity; oxysterols accumulation was higher in whole egg powders than in egg yolk powders, and in spray-dried powders it was higher than in freeze-dried ones. Five oxysterols were identified and quantified. In not moisturized spray-dried whole egg powders these oxysterols were (as follows): 5α,6α-epoxycholesterol (5α,6α-EP) > 7α-hydroxycholesterol (7α-HC) > 5β,6β-epoxycholesterol (5 $\beta$ ,6 $\beta$ -EP) > 7-ketocholesterol (7-KC) > 7 $\beta$ -hydroxycholesterol (7 $\beta$ -HC). During storage of powders, 5,6-epoxycholesterols (5,6-EP) were produced in the highest amounts.

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### 1. Introduction

Water in foods may function as a substrate or medium of many reactions. Water significantly influences lipid oxidation processes in food products, but its role in these processes may be different. It can either stimulate or inhibit lipid oxidation reactions (Duckworth, 1975). The inhibition of lipid oxidation in food may be caused by lowering oxygen diffusion to the sites of oxidation, by lowering catalytic properties of metal ions as a result of

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their chelation and also by the effect of binding of hydroperoxides. On the other hand, water can promote lipid oxidation through lowering viscosity and facilitating the movement of the molecules. In food with very low water activity its increase causes the inhibition of the rate of lipid oxidation. The rate of lipid oxidation reaches the minimum when water content corresponds to the monomolecular layer (Smith, Shirazi, & Mulligan, 2002).

The cholesterol oxidation process probably undergoes the same free radical mechanism as the lipid oxidation (Karpiński et al., 1997; Smith, 1981; Tai, Chen, & Chen, 1999). Cholesterol oxidation products are present in many food products of animal origin, as well as in

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<span id="page-1-0"></span>foods fried in deep fat. The presence of oxysterols – the main cholesterol oxidation products in egg powders – has been deeply investigated (Fontana, Antoniazzi, Ciavatta, Trivellone, & Cimino, 1993; Guardiola, Codony, Miskin, Rafecas, & Boatella, 1995; Guardiola et al., 1997; Lai, Gray, Partidge, & Flegal, 1996; Li, Cherian, & Sim, 1996; Morgan & Armstrong, 1989; Morgan & Armstrong, 1992; Nourooz-Zadeh & Appelqvist, 1987). The amounts of oxysterols in egg powders can vary from 8 to 311 mg/kg of powder (Lercker & Rodriguez-Estrada, 2000). One hundred cholesterol oxidation products have been identified (Hwang, 1991). The most popular oxysterols in egg powders are: 7a-hydroxycholesterol (7 $\alpha$ -HC), 7 $\beta$ -hydroxycholesterol (7 $\beta$ -HC), the products of its dehydrogenation: 7-ketocholesterol (7- KC), also:  $5\alpha, 6\alpha$ -epoxycholesterol ( $5\alpha, 6\alpha$ -EP),  $5\beta, 6\beta$ epoxycholesterol  $(5\beta, 6\beta - EP)$  and the product of its hydratation – cholestanetriol (CT), as well as 20 hydroxycholesterol (20-HC) and 25-hydroxycholesterol (25-HC).

The main factor affecting oxysterols appearing in egg powders is the drying process. Thus cholesterol oxidation products are either not detected in freeze-dried egg (Fontana et al., 1993), or their presence is minimal (Morgan & Armstrong, 1992). The concentration of cholesterol oxides in egg powders increases with time and temperature of storage – the longer the time and the higher the temperature of storage – the bigger the amounts of oxysterols produced (Huber, Pike, & Huber, 1995; Li et al., 1996). The presence of oxygen and light also promote oxysterol formation (Guardiola et al., 1997).

Considerable attention has been devoted to the study of cholesterol oxidation resulting from biological activities of its oxidation products associated with human diseases. Research provides evidence that some cholesterol oxides are toxic and may facilitate the development of coronary artery disease and certain cancers (Björkhem & Diczfalusy, 2002; Linseisen & Wolfram, 1998).

So far no research focusing on influence of water content on cholesterol oxidation in egg powders during storage has been carried out. The aim of this study was to assess the effect of water content on accumulation of oxysterols in spray-dried and freeze-dried whole egg and egg yolk powders during 3 months of storage at room temperature.

# 2. Materials

The samples of spray-dried whole egg and egg yolk powders were obtained from ''Ovopol'' Factory (Nowa So'l, Poland). Spray-drying conditions were as follows: indirect heating, with the inlet air temperature between 120 and 170  $\degree$ C, and the outlet air temperature between 52 and 77 °C. Freeze-dried whole egg and egg yolk powders were prepared in our laboratory, using freezdryer OE-950 (LaborMIM, Esztergom, Hungary). Egg yolk powder was freeze-dried after the yolk having been accurately separated from the albumen. The desweetening stage was omitted during the production of both, spray- and freeze-dried powders.

The egg powders were divided into three experimental groups (Tables 1 and 2):  $I$  – not moisturised egg powders,  $II - egg$  powders moisturised to 7–8% of water content,  $III - egg$  powders moisturised to  $11-12\%$  of water content.

Table 1

Chemical composition and water activity of spray-dried and freeze-dried whole egg powders  $(n = 3)$ 

| Components and water activity | Freeze-dried powders |                  |                  | Spray-dried powders |                  |                  |  |
|-------------------------------|----------------------|------------------|------------------|---------------------|------------------|------------------|--|
|                               | Not moisturized      | Moisturized      |                  | Not moisturized     | Moisturized      |                  |  |
|                               | K                    | Н                |                  | K                   |                  | П                |  |
| Water, %                      | $1.37 \pm 0.31$      | $8.10 \pm 0.11$  | $11.89 \pm 0.22$ | $4.05 \pm 0.19$     | $8.61 \pm 0.11$  | $12.14 \pm 0.32$ |  |
| Proteins, %                   | $49.81 \pm 0.63$     | $48.96 \pm 0.46$ | $48.05 \pm 0.26$ | $50.44 \pm 0.05$    | $47.90 \pm 0.04$ | $45.61 \pm 1.81$ |  |
| Lipid, $\%$                   | $45.10 \pm 2.49$     | $43.37 \pm 2.91$ | $41.53 \pm 3.03$ | $43.53 \pm 2.74$    | $41.67 \pm 2.02$ | $0.50 \pm 2.09$  |  |
| $a_{\rm w}$                   | $0.17 \pm 0.01$      | $0.47 \pm 0.01$  | $0.64 \pm 0.01$  | $0.32 \pm 0.01$     | $0.51 \pm 0.02$  | $0.66 \pm 0.01$  |  |

Table 2

Chemical composition and water activity of spray-dried and freeze-dried yolk egg powders  $(n = 3)$ 

| Components and water activity | Freeze-dried powders |                  |                  | Spray-dried powders |                  |                  |  |
|-------------------------------|----------------------|------------------|------------------|---------------------|------------------|------------------|--|
|                               | Not moisturized      | Moisturized      |                  | Not moisturized     | Moisturized      |                  |  |
|                               |                      |                  |                  |                     |                  |                  |  |
| Water, $\%$                   | $1.67 \pm 0.21$      | $7.97 \pm 0.15$  | $11.99 \pm 0.47$ | $2.78 \pm 0.24$     | $8.28 \pm 0.20$  | $11.77 \pm 0.32$ |  |
| Proteins, %                   | $32.50 \pm 0.13$     | $30.92 \pm 1.15$ | $30.28 \pm 0.57$ | $33.66 \pm 0.09$    | $32.78 \pm 0.01$ | $31.72 \pm 1.14$ |  |
| Lipid, $\%$                   | $63.38 \pm 1.72$     | $61.06 \pm 1.00$ | $59.77 \pm 1.02$ | $60.19 \pm 1.19$    | $58.98 \pm 1.00$ | $57.22 \pm 1.34$ |  |
| $a_{\rm w}$                   | $0.26 \pm 0.01$      | $0.67 \pm 0.01$  | $0.93 \pm 0.02$  | $0.33 \pm 0.01$     | $0.66 \pm 0.01$  | $0.87 \pm 0.01$  |  |

The moisturising procedure was as follows: samples weighting 30–40 g were placed in a vacuum chamber (10 kPa), with water layer at the bottom, then they were moisturised to the desired water content. Low pressure fastened water absorption by the egg powders. Moisturised powders were sealed in plastic films and stored for 3 months in 20  $\degree$ C. Freshly moisturised samples were tested for chemical composition and water activity. The total cholesterol and oxysterols levels were assessed on the first day, and again after 3 months of storage of powders at room temperature.

# 3. Methods

The subsequent chemical components were measured: water content by the drying method, the protein content by the Dumas method with UNIT B-324 apparatus (Büchi, Flawil, Switzerland), the lipid content by refractometry with  $\alpha$ -bromiumnaphtalen. The results are expressed as a percentage of the dry mass of the powders.

Water activity was determined from sorption isotherms of egg powders executed by the Labuza method (Labuza, 1983; Labuza, Kaaname, & Chen, 1985).

The cholesterol level was estimated in lipid phase by gas chromatography AOAC Official Method, with modification of Thompson and Merola (1993). Lipid extraction was executed by the Folch method (Folch, Lees, & Stanley, 1957). Cholestan was used as the internal standard. The extracted lipid was saponified with potassium methylate in methanol, then extracted one more time with hexane, and finally transformed into trimethylchlorinesilyl (TMCS) ether.

The TMCS cholesterol derivative was analysed by GC capillary on a HP 5890 s II (Hewlett–Packard, Palo Alto, CA) equipped with a flame ionisation detector and the HP-1 column (25 m  $\times$  0.20 mm) 0.11 µm. The injection (24:1) was performed at 300  $\degree$ C, and column temperature was programmed from 250  $\degree$ C (4 min hold) to 300 °C (5 min hold) at 5 °C/min. The detector temperature was held at  $310\text{ °C}$ . Helium was used as the carrier gas at a constant inlet pressure of 100 kPa. The cholesterol was identified by the retention time, according to a GC-FID chromatogram of the standard mixture, and quantited by the CHEMSTATION computer program according to the internal standard quantity.

Oxysterols were identified and quantited in lipid phase by gas chromatography method of Schmarr, Gross, and Shibamoto (1966). Lipid extraction was executed by the Folch method (Folch et al., 1957). 6-ketocholesterol was used as the internal standard. For transesterification, a 10% sodium methylate in methanol was used. The transesterified lipids were fractionated on Amino-Phase SPE Cartidges from Supelco (Bellefonte, PA, USA). Oxysterols subjected in the polar fraction

were transformed into trimethylsilyl ethers (TMS ethers) and then injected into the gas chromatograph.

The TMS oxysterols derivatives were analysed by GC capillary on a HP 5890 s II (Hewlett–Packard, Palo Alto, CA) equipped with a flame-ionisation detector and the BPX-5 column (30 m  $\times$  0.25 mm) 0.25 µm. Spiltless injection (0.5 min spiltless time) was performed at 290  $\degree$ C, and the column temperature was programmed from 110 °C (3 min hold) to 230 °C (5 min hold) at 30  $\rm{C/min}$  and then to 310  $\rm{C}$  (5 min hold) at 3  $\rm{C/min}$ . The detector temperature was held at  $325$  °C. Helium was used as the carrier gas at a constant inlet pressure of 150 kPa. Oxysterols were identified by the retention time, according to a GC-FID chromatogram of the standard mixture, and quantited by the CHEMSTA-TION computer program, according to the internal standard quantity.

The results were analyzed by STATISTICA version 5 ([Table 5](#page-4-0)).

# 4. Results and discussion

The chemical composition of egg powders is presented in [Tables 1 and 2](#page-1-0).

Freeze-dried egg powders contained significantly less water and more proteins and lipid than spray-dried powders [\(Tables 1 and 2\)](#page-1-0). The increase of water content in powders through moisturizing caused the decrease of protein and lipid content and was approximately proportionate to the level of the diminishing dry mass of powders. The decrease of protein and lipid content in moisturised powders was probably caused by the increase of water content in powders.

Despite similar water content in moisturized whole egg and yolk powders, water activity in whole egg powders was significantly lower than water activity in egg yolk powders. Whole egg powders contain more hydrophylic proteins and less hydrophobic lipids than egg yolk powders. Water activity in spray- and freeze-dried egg powders was similar – in powders of similar water content. The method of drying had little effect on the level of bounding of the added water, both in whole egg powders and in egg yolk powders.

The cholesterol content in whole egg powders ranged from 1.22 to 1.64 g of cholesterol/100 g of powder, whereas in egg yolk powders from 1.64 to 2.10 g of cholesterol/100 g of powder. The cholesterol content in analyzed egg powders was lower than the data given by Guardiola et al. (1995) and Li et al. (1996).

Cholesterol and products of its oxidation occur in lipid phase of egg powder. Lipid content in analyzed powders was different and depended both on the type of egg powders, the method of drying and the extent of powders moisturization. Considering the effect of the examined factors (type of egg powders, method of drying,



<span id="page-3-0"></span>Table 3Changes in the content of cholesterol and products of its oxidation in whole egg powders. Mean value and standard error  $(n = 3)$ 

| Compound<br>(mg/100 g)<br>of fat) | Water content (freeze-dried powders) <sup>a</sup> |                   |                  |                  |                  | Water content (spray-dried powders) <sup>a</sup> |                  |                  |                  |                  |                  |                  |
|-----------------------------------|---|-------------------|------------------|------------------|------------------|--|------------------|------------------|------------------|------------------|------------------|------------------|
|                                   |   |                   |                  |                  |                  |  |                  |                  |                  |                  |                  |                  |
|                                   |   |                   | $^{\circ}$       |                  |                  |  | $0^{\circ}$      |                  |                  |                  |                  |                  |
| Cholesterol                       | $3359 \pm 7$                                      | $3296 \pm 3$      | $3067 \pm 14$    | $3092 \pm 29$    | $3377 \pm 10$    | $2934 \pm 4$                                     | $3769 \pm 10$    | $3346 \pm 13$    | $3080 \pm 67$    | $3056 \pm 19$    | $3103 \pm 28$    | $3121 \pm 24$    |
| Total oxysterols                  | $54.33 \pm 2.29$                                  | $149.55 \pm 6.96$ | $45.53 \pm 4.16$ | $91.34 \pm 0.65$ | $76.31 \pm 0.03$ | $107.11 \pm 0.75$                                | $71.37 \pm 6.97$ | $11671 + 033$    | $59.60 \pm 3.84$ | $91.95 \pm 0.12$ | $86.80 \pm 0.69$ | $92.74 + 0.87$   |
| $5\alpha$ , $6\alpha$ -EP         | $12.15 \pm 0.26$                                  | $52.60 \pm 3.69$  | $1975 + 257$     | $28.60 \pm 2.25$ | $33.15 \pm 0.26$ | $35.10 \pm 3.93$                                 | $29.70 \pm 4.39$ | $4740 + 427$     | $26.05 \pm 0.72$ | $42.65 \pm 2.68$ | $44.35 \pm 3.09$ | $41\,70 + 0\,58$ |
| $5\beta, 6\beta$ -EP              | $12.40 \pm 1.93$                                  | $34.85 \pm 1.82$  | $6.58 \pm 0.51$  | $22.40 \pm 0.46$ | $15.85 \pm 0.51$ | $22.15 \pm 2.11$                                 | $13.20 \pm 0.98$ | $27.00 \pm 2.48$ | $9.30 \pm 0.98$  | $16.15 \pm 0.38$ | $10.45 \pm 0.38$ | $1430 + 0.06$    |
| $7\alpha$ -HC                     | $13.13 \pm 0.36$                                  | $23.85 + 0.55$    | $9.31 \pm 1.33$  | $13.60 \pm 0.52$ | $1115 + 015$     | $19.05 + 1.76$                                   | $13.65 \pm 0.84$ | $1530 + 012$     | $11.25 \pm 1.13$ | $13.00 + 0.06$   | $15.35 \pm 1.88$ | $1430 + 046$     |
| $7B-HC$                           | $450 + 0.26$                                      | $2545 + 049$      | $2.15 \pm 0.37$  | $17.65 \pm 0.26$ | $4.81 \pm 0.61$  | $2010 + 115$                                     | $5.49 \pm 0.15$  | $15.25 \pm 0.55$ | $5.35 \pm 0.39$  | $12.70 \pm 0.40$ | $727 + 025$      | $1420 + 0.58$    |
| $7-KC$                            | $12.15 \pm 0.03$                                  | $12.80 \pm 0.40$  | $7.75 \pm 0.12$  | $9.09 \pm 0.87$  | $11.35 \pm 0.03$ | $10.71 \pm 0.80$                                 | $9.33 \pm 0.91$  | $11.76 \pm 0.13$ | $7.65 \pm 0.61$  | $7.45 \pm 0.13$  | $9.39 \pm 0.11$  | $824 + 017$      |

 $^a$  C – control, not moisturized egg powders; I – moisturized egg powders, water content 7–8%; II – moisturized egg powders, water content 11–12%.<br><sup>b</sup> Time of storage (months).





 $\alpha$  C – control, not moisturized egg powders; I – moisturized egg powders, water content 7–8%; II – moisturized egg powders, water content 11–12%.

<sup>b</sup> Time of storage (months).

<span id="page-4-0"></span>water content, storage in room temperature) on cholesterol oxidation, the levels of cholesterol and oxysterols were shown in respect to constant quantity (100 g) of lipid in egg powders.

The cholesterol content in fresh egg powders ([Tables](#page-3-0) [3 and 4](#page-3-0)) ranged from 3.07 g/100 g of lipid (freeze-dried whole egg powders moisturized to 8% of water content) to 3.77 g/100 g of lipid (spray-dried whole egg powders not moisturized). Generally, cholesterol content in moisturized powders had lower values than in not moisturized ones. During 3 months of storage of egg powders, the reduction of cholesterol content was observed. Lower cholesterol content in moisturized powders and in stored powders can be the consequence of decomposition of cholesterol during the process of moisturizing and storage of powders. Little differences in cholesterol content in spray- and freeze-dried, whole egg and egg yolk powders can be influenced by natural variability of cholesterol content in output material (eggs).

The investigating processes of cholesterol decomposition and changes in oxysterols content were estimated. The differences in total oxysterol content in analyzed egg powders, as well as changes in the total level of oxysterols during 3 month storage at room temperature, are shown in [Tables 3 and 4.](#page-3-0)

The total oxysterol content in whole egg powders, independently of drying method and other analyzed factors, was higher than in egg yolk powder. Similar results were obtained by Nourooz-Zadeh and Appelqvist (1987).

The total level of oxysterols was higher in spray-dried powders than in freeze-dried ones. According to Morgan and Armstrong (1989) oxysterols are present in freezedried egg yolk powders, although their concentration is much lower than in spray-dried powders. Measurable amounts of oxysterols (2–111 ppm) in spray-dried whole egg powders were estimated by many authors (Fontana et al., 1993; Guardiola et al., 1995; Guardiola et al., 1997; Lai et al., 1996; Nourooz-Zadeh & Appelqvist, 1987). The results gathered in our research are in agreement with the data given above. During the drying process, the growth of total oxysterol level was significantly higher in whole egg powder than in yolk egg powder.

During 3 months of storage of egg powders at room temperature, a significant increase of the total oxysterol content in whole egg powders, as well as in egg yolk powders, in spray- and freeze-dried powders was observed. The growth of oxysterol concentration in food products during their storage was shown by all investigators working with cholesterol decomposition in food (Guardiola et al., 1997; Larkenson, Dutta, & Hansson, 2000; Lercker & Rodriguez-Estrada, 2000; Smith, 1996; Tai, Chen, & Chen, 2000).

Water content in powders had a highly significant influence on oxysterol accumulation during storage of both types of powders. The highest accumulation of oxysterols was found in egg powders with the lowest water content, both in whole egg powder and in egg yolk powder. During storage, accumulation of oxysterols in egg powder moisturized to 8% and 12% of water content was much lower than in not moisturized powders.

In analyzed egg powders, changes in the level of five oxysterols were estimated. In not moisturized whole egg spray-dried powders it was (as follows):  $5\alpha$ ,  $6\alpha$ -EP >  $7\alpha$ - $HC > 5\beta, 6\beta$ -EP > 7-KC > 7 $\beta$ -HC. The content of all the

Table 5

Mean square analysis of variance content of cholesterol, total oxysterol and oxysterols in egg powders depending on: type of powder, method of drying, water content and time of storage

| Source of variation            | Degree of freedom | Variables    |                 |               |              |            |                        |                      |
|--------------------------------|-------------------|--------------|-----------------|---------------|--------------|------------|------------------------|----------------------|
|                                |                   | Cholesterol  | Total oxysterol | $7\alpha$ -HC | $7\beta$ -HC | 7-KC       | $5\alpha, 6\alpha$ -EP | $5\beta, 6\beta$ -EP |
| Type of powder $-A$            |                   | 137419.0**   | 9568.63**       | 290.526**     | 292.175**    | 84.43501** | $1001.617**$           | 515.6063**           |
| Method of drying $- B$         |                   | 64350.8**    | 1526.70**       | $30.1476**$   | 0.001        | 7.78151**  | $1145.170**$           | 9.9124               |
| Water content $- C$            |                   | 378501.1**   | 3279.38**       | $20.154**$    | $46.823**$   | 67.83721** | $646.169**$            | 279.1416**           |
| Time of storage $- D$          |                   | 357223.8**   | 24458.64**      | 186.6312**    | 1466.743**   | 2.82031*   | 6470.384**             | 713.8809**           |
| Interactions:                  |                   |              |                 |               |              |            |                        |                      |
| $A \times B$                   |                   | 38.3         | $1816.39**$     | $112.500**$   | $102.937**$  | 97.86005** | 3.491                  | 187.8407**           |
| $A \times C$                   |                   | 133857.4**   | $225.11*$       | 57.7201**     | $7.644**$    | 7.77305**  | 119.455**              | 24.5019*             |
| $B \times C$                   |                   | 263865.9**   | 551.99**        | 20.8551**     | $4.580**$    | 10.38604** | $113.607**$            | 56.0218**            |
| $A \times D$                   |                   | 2032.0       | 587.99**        | $17.6715**$   | 234.073**    | 11.52000** | 476.093**              | 489.9232**           |
| $B \times D$                   |                   | 24920.3**    | 64.50           | 56.3391**     | $33.103**$   | 0.25205    | 54.523                 | 13.6373              |
| $C \times D$                   |                   | $14033.6***$ | 2991.74**       | 62.9591**     | $10.479**$   | 1.48655    | 1182.689**             | 106.9809**           |
| $A \times B \times C$          | 2                 | 157327.6**   | 699.69**        | 25.6666**     | $11.119**$   | 8.23284**  | $200.629**$            | 2.6108               |
| $A \times B \times D$          |                   | 37606.5**    | 2952.77**       | 49.5013**     | 191.329**    | 0.48511    | 451.276**              | 110.1499**           |
| $A \times C \times D$          | 2                 | 188652.4**   | 87.02           | 15.4191**     | $7.511**$    | 3.21911**  | 27.076                 | 35.5192**            |
| $B \times C \times D$          |                   | 39365.4**    | 35.58           | 0.3830        | 0.296        | 3.58921**  | 5.029                  | 33.6818**            |
| $A \times B \times C \times D$ |                   | 116081.4**   | $724.37**$      | 18.3059**     | $6.081**$    | 1.23579    | 273.803**              | 24.5615*             |
| Error                          | 48                | 1163.031     | 58.13388        | 1.941377      | 0.679424     | 0.526185   | 16.37531               | 5.006462             |

\*  $F$  values significant for  $P \le 0.05$ .

\*\*  $F$  values significant for  $P \le 0.01$ .

mentioned oxysterols was significantly higher in whole egg powders than in egg yolk powders.

Considering the effect of the drying method of powders, no significant changes were observed in the level of subsequent oxysterols:  $7\alpha$ -HC,  $5\beta$ ,  $6\beta$ -EP,  $7\beta$ -HC and 7-KC. Spray-dried egg powders contained significantly more  $5\alpha$ ,  $6\alpha$ -EP than freeze-dried egg powders.

The levels of  $5\beta, 6\beta$ -EP,  $7\beta$ -HC and  $7$ -KC were significantly higher in not moisturized powders than in moisturized powders. There were no significant differences in 7a-HC content in not moisturized and moisturized egg powders.

5a,6a-EP content accumulated in the highest amounts in egg powders was also dependent on the level of water. The lowest concentration of this oxysterol was observed in egg powders moisturized to 8% of water content.

During 3 months of storage in room temperature, the significant growth of all oxysterols (except 7-KC) was observed. Among the researched compounds the biggest increase during storage was shown in 7b-HC. Probably during storage of powders,  $7\alpha$ -HC isomer undergoes transformation into 7b-HC isomer, which is more stable thermally. This conversion of both isomers in egg powders was observed by Lai et al. (1996).

Before storage, 5a,6a-EP constituted 37% of total oxysterols in egg powders. After 3 months of storage, the  $5\alpha$ ,  $6\alpha$ -EP content increased to 47% of total oxysterols. The results of Sander, Addis, Park, and Smith (1989) showed that during powders storage,  $\alpha$ -epoxysterols were the main accumulating products of cholesterol oxidation in egg powders. Similar results were obtained in this research.

In analyzed powders, also traces of 19-HC, 20-HC and 25-HC were detected. This is the evidence that the reactions of oxidation in a side chain of cholesterol also took place in the examined material.

# 5. Conclusions

After 3 months' of storage, at room temperature, the accumulation of cholesterol oxidation products was the highest in egg powders with the lowest water activity. The amounts of produced oxysterols were bigger in stored whole egg powders than in stored egg yolk powders. The cholesterol oxidation products occurred in higher quantities in spray-dried egg powders than in freeze-dried egg powders, after 3 months of storage. Oxysterols presented in the highest amounts in stored egg powders were 5,6-EP isomers.

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