Original scientific paper - Izvorni znanstveni rad UDK: 637.143

Changes in selected oxysterols in powdered foodstuffs

doi: 10.15567/mljekarstvo.2016.0107

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Received - Prispjelo: 15.01.2015.
Accepted - Prihvaćeno: 14.12.2015.

Abstract

The present research consisted of producing 3 types of powdered concentrates: the dairy, the egg and the dairy-egg (produced from a blend of raw liquid milk and liquid eggs). The dairy-egg powder was produced in order to facilitate formulation of dry food mixes. Powders were vacuum-packaged and packaged in air atmosphere and stored for 24 months. Changes in contents of selected oxysterols (determined by gas chromatography) were recorded. Regardless of the packaging type, the predominant oxysterol in the dairy-egg powder was α-epoxy-C (7.679 and 5.600 μg/g powder, respectively).

Key words: oxysterols, powdered foodstuffs, food concentrates

Introduction

Cholesterol oxidation products (COPs, oxysterols) found in the human organism are of exo- and endogenous origin as well. COPs can be absorbed with food and/or formed in vivo by the enzymatic or non-enzymatic (free-radical) pathways. Whether free or embedded in lipoproteins, they are excreted with bile or are trapped by cells, where they affect cholesterol metabolism and influence the composition and functions of biological membranes (Wielkoszyński, 2003).

Food technologists are interested in oxysterols since they origin from processes of animal food production and storage and cause the deterioration of nutritive value of foodstuffs. Nutritionists, physiologists and biochemists focus on oxysterols due to the fact that they are readily absorbed from the alimentary tract and exhibit a wide spectrum of biological activity, including e.g. cytotoxicity, inhibition of DNA synthesis, inhibition of cholesterol synthesis, the effect on the structure and functioning of cell membranes (Leonarduzzi et al., 2002; Lordan et al., 2009; Poli et al., 2009; Otaegui-Arrazola et al., 2010; Olkkonen et al. 2012). In their study Mazur et al. (2011) showed that oxysterols contained in the diet affect hepatocyte morphology and the toxic effect of COPs consists mainly in the growth inhibition of these cells.

Cholesterol oxidation products found most frequently in food include 7-ketocholesterol (7-keto-C), 7α-hydroxycholesterol (7α-OH-C), 7β-hydroxycholesterol(7β-H-C), α-epoxycholesterol (α-epoxy-C) and β-epoxycholesterol (β-epoxy-C) (Bösinger et al., 1993; Hur et al., 2007). The process of their formation is presented in Fig. 1.

Animal origin foodstuffs, such as milk, eggs, meat and their processed products, are sources of cholesterol in our diet. Before food is consumed it frequently undergoes various processes such as high temperature heating or accidental irradiation during storage. Such actions may initiate cholesterol oxidation (Mazalli and Bragagnolo, 2007; Saldanha and Bragagnolo, 2008; Boselli et al., 2012; Cardenia et Al., 2012; Ansorena et al., 2013). Total COPs in food amounts on average to 1 % cholesterol content, but its level may be as high as over 10 %. The primary sources of COPs in our

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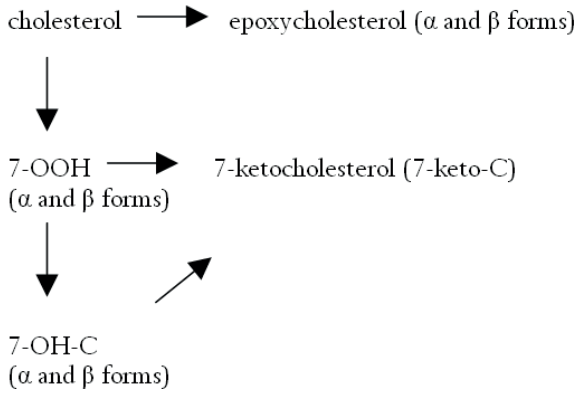

Figure 1. Formation of selected cholesterol oxidation products during auto-oxidation (Tai et al., 1999; Cardenia, 2013)

diet include cholesterol-rich products such as meat, eggs and dairy products (Sampaio et al., 2006; Vicente and Torres, 2007).

There are numerous literature sources which focus on the problem of oxysterol content in dairy and egg powders (Sieber, 2005; Obara et al., 2006; Mazalli and Bragagnolo 2007), while there are no publications presenting analyses of products being mixtures of milk and eggs.

Further, there is no product on the market that would be a dry mixture of milk, egg powder and yolk. The concentrate could be used as an intermediate product for cakes, ice-cream, desserts, sausages and/or other foods whose preparation requires using the above mentioned ingredients. The growing popularity of pancakes and the availability of the pancake dough concentrate on the market brought about the production of a mixture of milk and egg of exactly the same proportions as in dough. Mixing raw milk and eggs and their subsequent drying allows a homogenous blend. The egg powder is usually characterised by low solubility, wetting and flow-ability causing the dosing problems. In order to improve egg powder properties, raw milk and egg were mixed and spray-dried.

The aim of this study was to produce dairy-egg concentrate and to determine changes in the contents of five oxysterols found most commonly in food. Another objective of this work was to compare the amount of oxysterols obtained in the dairy-egg powder with the amount of oxysterols which would be obtained after mixing equal quantities of dry and separately kept milk and egg powder.

Materials and methods

This study implied production of 3 types of powders - dairy, egg and dairy-egg, produced by blending raw liquid milk and liquid eggs at a proportion typical of pancake batter (1 dm^3 milk - 8 medium eggs). Components were homogenized (18MPa/10 min), pasteurised (60 °C/30 min) and subsequently spray dried at 170/75 °C. The experiment was carried out at a dairy, using industrial equipment. As a result, 6 kg of each powder were produced and subsequently packaged into plastic bags in air atmosphere or under vacuum, without access of light. Samples (100 g packages) were collectively stored at 20 °C±1 °C for 24 months in a carton.

Dairy, egg and dairy-egg powders had the following contents of cholesterol and fat (values in parentheses): 0.5 mg/g powder (26.5 %), 13.8 mg/g (42.9 %) and 4.4 mg/g (34.8 %).

Cholesterol oxidation products were determined according to Przygoński et al. (2000) and Chudy et al. (2015a). Silylated oxychole sterols were separated and quantified on an Agilent model 6890 gas chromatograph using a DB-5MS capillary column (30 m × 0.25 mm × 0.25 mm; J&W Scientific, Folsom, California, USA). Hydrogen was used as a carrier gas at a flow rate of 1.5 mL/min. The amount of COP was calculated using an internal standard 19-hydroxycholesterol. Changes in contents of 5 oxysterols were recorded: 7α-OH-C, 7β-OH-C, 7-keto-C, α-epoxy-C and β-epoxy-C.

Values describing the changes of oxysterols contents are arithmetic means of 9 measurements. Three samples of each powder were evaluated per treatment and each treatment was completed in triplicate. The means and standard deviations were calculated using Microsoft Excel spreadsheet from the Microsoft Office package. Significant differences between data were verified using Duncan's test.

Results and discussion

Oxysterols analysed in this study were grouped in accordance with their synthesis pathways. Table 1 presented changes in 7α-OH-C, 7β-OH-C and 7-keto-C (the first group of oxysterols), Table 2 - α-epoxy-C and β-epoxy-C (the second group of oxysterols) in whole milk powder, powdered eggs and in powder mix.

The type of the analysed powder had a statistically significant effect on the contents of oxysterols classified to the first and second group. The application of vacuum packaging system resulted in a delay of the oxysterols' formation.

Rose-Sallin et al. (1995) detected the presence of the first group of oxysterols in whole milk powder after 12-month storage in the amounts very similar to that obtained in the present study, i.e. 1.83 μg/g powder. Caboni et al. (2005) examined egg powder stored at 20 ºC for 12 months and also found cholesterol oxidation products from groups 1 and 2 to be the dominant oxysterols. Pie et al. (1990) investigated commercially available egg powders and recorded higher contents of the two oxysterol groups in comparison to the results obtained in the present study, even after 24 months of storage. The amounts of oxysterols from the first group ranged from 67.04 to 107.22 μg/g powder, while the levels of oxysterols from the second group were 79.25-95.75 μg/g powder.

In milk powder produced within the present study and packaged in air atmosphere, the initially dominant oxysterol was α-epoxy-C, while during the successive months of storage 7α-OH-C was detected in highest amounts. The dominant oxysterols found in vacuum-packaged powder were alternately α-epoxy-C and 7α-OH-C (except for the third month, when β-epoxy-C predominated). In a study by Rose-Sallin et al. (1995) in milk powder 7α-OH-C and 7β-OH-C were formed in greatest amounts. In a study by Nourooz-Zadeh and Appelqvist (1988) immediately after production and in a study by Angulo et al. (1997) after 6 months of storage 7α-OH-C was the dominant oxysterol in milk powder.

Egg powder contained the highest levels (over 1 μg/g powder) of 7β-OH-C, 7α-OH-C and β-epoxy-C after the completion of production which was in agreement with results previously observed by Caboni et al. (2005). 7β-OH-C was the oxysterol produced in highest amounts in both studies in the final period of storage.

In dairy-egg powder packaged in air atmosphere and in vacuum the dominant oxysterol after 24 months was α-epoxy-C, which corresponded to results obtained by Missler et al. (1985) who also studied egg and milk mixtures.

Scopesi et al. (2001) reported the maximum content of 7-keto-C in dairy formulas for children to be at 0.7 μg/g fat (±0.3), since the same amount of 7-keto-C is naturally found in breast milk. However, the examined infant formulas contained frequently 3.6 μg of 7-keto-C /g fat, exceeding the reported limit. In the present study the content of 7-keto-C did not exceed 0.7 μg/g fat neither in milk powder nor in the dairy-egg powder. However, the egg powder starting from 6 months of storage contained an elevated level of this oxysterol. Caboni et al. (2005) found the content of 7-keto-C immediately after production to be 9.0 μg/g fat (4.1 μg/g powder), while after 1 year of storage it increased up to 7.5 μg/g powder. The content of 7-keto-C in the product was most frequently below 2.0 ppm product, except for beef (≤3.5 ppm) and whole egg powder (≤4.6 ppm) (Lercker and Rodriguez-Estrada, 2002).

The amounts of five oxysterols in dairy-egg powder were at the average level, when compared to reference samples i.e. milk powder and egg powder. Thus, the cholesterol contained in the dairy-egg powder proved to be more stable in comparison to the cholesterol contained in pure milk and/or egg powders. In order to obtain 1 g of the mix of milk powder and egg powder of the same proportions as in the experimental dairy-egg mixture, it would be required to combine approximately 0.6 g of pure milk powder and 0.4 g of pure egg powder. Calculating the sum of oxysterols that would arise from milk powder and egg powder produced separately, the obtained value of the sum of 5 oxysterols was much higher than that detected in the experimental dairy-egg powder. As it can be concluded from the experiment, the interactions among the ingredients of the stored mixture affected the amount and the type of oxysterols.

The previous research conducted by Chudy et al. (2015a) and Chudy et al. (2015b) also prove that the content of fatty acids, i.e. the amount of the oxidation prone multi-saturated fatty acids, the non-enzymatic browning reaction and the initial cholesterol content affect the oxidation of cholesterol in dairy-egg mixes.

Table 1. Effect of the type of powder, the packaging system and the storage time on changes in contents of 7α-OH-C, 7β-OH-C, 7-keto-C

Type of powder	Packaging system	Time (months)	7α-OH-C ± SD (μg/g powder)	7β-OH-C ± SD (μg/g powder)	7-keto-C ± SD (μg/g powder)
Milk powder	in air atmosphere	0	0.19 ± 0.00^{A}	0.12 ± 0.01^{B}	0.12 ± 0.01^{C}
		3	0.49 ± 0.03^{D}	0.26 ± 0.02^{D}	0.12 ± 0.01^{C}
		6	$0.84\pm0.05^{G,H}$	0.26 ± 0.02^{D}	0.07 ± 0.00^{B}
		12	0.81 ± 0.05^{G}	0.42 ± 0.02^{F}	0.44 ± 0.02^{G}
		24	2.19 ± 0.17^{I}	0.45 ± 0.03^{F}	0.55 ± 0.04^{H}
	in vacuum	0	0.19 ± 0.00^{A}	0.12 ± 0.01^{B}	0.12 ± 0.01^{C}
		3	0.23 ± 0.02^{B}	$0.15\pm0.02^{B,C}$	0.07 ± 0.00^{B}
		6	0.66 ± 0.04^{E}	0.06 ± 0.00^{A}	0.26 ± 0.02^{E}
		12	0.44 ± 0.03^{D}	0.34 ± 0.02^{E}	0.52 ± 0.02^{H}
		24	$0.75\pm0.04^{F,G}$	$0.13\pm0.01^{B,C}$	0.53 ± 0.02^{H}
Egg powder	in air atmosphere	0	1.67 ± 0.06^{I}	1.08 ± 0.08^{H}	$0.22\pm0.02^{D,E}$
		3	$2.36\pm0.14^{K,L}$	$1.61\pm0.12^{I,J}$	$0.25\pm0.02^{D,E}$
		6	6.52 ± 0.32^{P}	6.87 ± 0.36^{M}	3.33 ± 0.29^{N}
		12	5.23 ± 0.30^{O}	6.77 ± 0.41^{M}	8.42 ± 0.48^{R}
		24	19.97 ± 0.95^{S}	21.52 ± 1.51^{O}	6.48 ± 0.19^{P}
	in vacuum	0	1.67 ± 0.09^{I}	1.08 ± 0.08^{H}	$0.22\pm0.02^{D,E}$
		3	2.16 ± 0.16^{K}	1.40 ± 0.11^{I}	0.02 ± 0.00^{A}
		6	2.10 ± 0.12^{K}	2.42 ± 0.15^{K}	5.47 ± 0.10^{O}
		12	4.38 ± 0.24^{N}	4.65 ± 0.30^{L}	2.02 ± 0.06^{M}
		24	9.38 ± 0.47^{R}	18.40 ± 0.87^{N}	1.22 ± 0.05^{L}
Dairy-egg powder	in air atmosphere	0	0.30 ± 0.02^{C}	0.15 ± 0.01^{C}	0.07 ± 0.00^{B}
		3	0.96 ± 0.07^{H}	0.92 ± 0.06^{G}	0.84 ± 0.04^{J}
		6	1.85 ± 0.09^{J}	0.40 ± 0.02^{F}	1.32 ± 0.06^{L}
		12	2.62 ± 0.16^{L}	1.74 ± 0.08^{J}	1.01 ± 0.09^{K}
		24	5.61 ± 0.33^{O}	6.92 ± 0.55^{M}	$0.88\pm0.06^{J,K}$
	in vacuum	0	0.30 ± 0.02^{C}	0.15 ± 0.01^{C}	0.07 ± 0.00^{B}
		3	$0.70\pm0.05^{E,F}$	0.22 ± 0.02^{D}	0.11 ± 0.01^{C}
		6	0.50 ± 0.03^{D}	0.25 ± 0.02^{D}	0.32 ± 0.02^{F}
		12	3.06 ± 0.18^{M}	0.81 ± 0.05^{G}	0.21 ± 0.02^{D}
		24	3.48 ± 0.25^{M}	4.74 ± 0.29^{L}	0.68 ± 0.04^{I}

^{A,B} - different superscript letters confirm statistical significance of difference in columns, $p<0.05$
SD - standard deviation

Table 2. Effect of the type of powder, the packaging system and the storage time on changes in contents of α-epoxy-C and β-epoxy-C

Type of powder	Packaging system	Time (months)	α-epoxy-C ± SD (μg/g powder)	β-epoxy-C ± SD (μg/g powder)
Milk powder	in air atmosphere	0	0.35±0.01^{D}	0.00±0.00^{A}
		3	0.89±0.03^{H}	0.03±0.03^{A}
		6	0.66±0.03^{F}	0.44±0.03^{F}
		12	0.77±0.04^{G}	0.34±0.02^{D}
		24	1.11±0.08^{I}	0.33±0.02^{D}
	in vacuum	0	0.35±0.01^{D}	0.00±0.00^{A}
		3	0.12±0.01^{A}	0.40±0.03^{E,F}
		6	0.72±0.06^{F,G}	0.12±0.01^{B}
		12	0.97±0.06^{I}	0.32±0.02^{D}
		24	1.05±0.04^{I}	0.42±0.02^{E,F}
Egg powder	in air atmosphere	0	0.77±0.05^{G}	1.07±0.06^{H}
		3	0.37±0.02^{D}	1.77±0.11^{J}
		6	2.90±0.19^{K}	5.40±0.20^{M}
		12	3.28±0.23^{L}	2.90±0.18^{L}
		24	13.93±0.80^{P}	19.24±1.12^{O}
	in vacuum	0	0.77±0.05^{G}	1.07±0.07^{H}
		3	0.28±0.02^{C}	1.03±0.07^{H}
		6	0.75±0.05^{G}	1.81±0.09^{J}
		12	0.73±0.04^{F,G}	2.73±0.11^{L}
		24	4.79±0.52^{M}	10.57±0.67^{N}
Dairy-egg powder	in air atmosphere	0	0.17±0.01^{B}	0.37±0.03^{D,E}
		3	1.03±0.04^{I}	0.18±0.01^{C}
		6	1.28±0.04^{J}	0.86±0.05^{G}
		12	4.26±0.34^{M}	0.76±0.05^{G}
		24	7.68±0.41^{O}	2.55±0.19^{K,L}
	in vacuum	0	0.17±0.01^{B}	0.37±0.03^{D,E}
		3	0.28±0.02^{C}	1.29±0.10^{I}
		6	0.49±0.03^{E}	1.07±0.05^{H}
		12	4.13±0.41^{M}	0.40±0.03^{E,F}
		24	5.60±0.49^{N}	2.46±0.12^{K}

*Denotations as in Table 1

Conclusions

Regarding the powders packaged in air atmosphere, milk powder contained the highest amounts of 7α-OH-C oxysterol, while 7β-OH-C was predominant in egg powder and α-epoxy-C in the dairy-egg mixture. The content of 7-keto-C in milk and milk-egg powders did not exceed 0.7 μg/g fat throughout the entire storage period. Lower amounts of each analysed oxysterol were detected in all vacuum-packaged powders. The sum of five researched oxysterols in the experimental mixture was lower than the sum of oxysterols in milk and egg powders separately.

Promjene na odabranim oksisterolima u praškastoj hrani

Sažetak

U sklopu ovog istraživanja proizvedene su 3 vrste koncentrata u prahu: mliječni, od jaja i mješoviti od jaja i mlijeka (proizveden sušenjem smjese sirovog tekućeg mlijeka i sirovih jaja). Mješoviti prah od jaja i mlijeka proizveden je kako bi se omogućilo formuliranje suhih, praškastih prehrambenih mješavina. Prehrambeni prahovi su vakumirani i pakirani u normalnoj atmosferi (zrak) te čuvani 24 mjeseca tijekom kojih su praćene promjene sadržaja odabranih oksisterola (određivano metodom plinske kromatografije). Bez obzira na vrstu pakiranja, u mješovitom prahu sastavljenom od mlijeka i jaja najzastupljeniji oksisterol bio je α-epoksi-C (odnosno: 7,679 i 5,600 μg/g praha).

Ključne riječi: oksisteroli, hrana u prahu, prehrambeni koncentrati

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