THE CONTENT OF CHOLESTEROL OF ESTONIAN YOGHURT AND ICE CREAM

K. Kolk, I. Linnik, R. Pällin, T. Püssa

Estonian University of Life Sciences

ABSTRACT. *The people with elevated risk of coronary heart disease are advised to control their nutritional habits and limit the consumption of milk products, to decrease the amount of cholesterol in their diet. Although the recents results of investigation show that some milk products rather decrease than increase the blood cholesterol level, many people are reluctant to consume milk products because the content of cholesterol is not known. The article presents the results of measurement of the content of cholesterol of 8 yoghurts and 10 ice creams produced in Estonia, by gas chromatography. The concentration of cholesterol was also calculated by the fat percentage of the products. The experimentally determined contents of cholesterol were found in good agreement with the calculated values in case of yoghurts, while in ice cream the experimental values were lower, due to vegetable fats containing in the ice creams. Based on the linear correlation between fat percentage and cholesterol content of yoghurt, the cholesterol content of milk fat – 393 mg/100 g was found. Accordingly, the content of milk fat of ice creams can be determined by the concentration of cholesterol.*

Keywords: *cholesterol, nutrition, milk products, milk fat, gas chromatography.*

Introduction

The literature about the correlation between human health and nutrition, is abundant, but often contraversial. As a result, certain tendencies and customs occur, which are placed in doubt in due time. One of those is the myth about milk products as a harmful source of cholesterol. Cholesterol as a lipid is insoluble in water and it is transported as a constituent of lipoproteins. Among them there are high density lipoproteins (HDL) which are called "good cholesterol", and low density lipoproteins (LDL), so-called "bad cholesterol". LDL can deposit a plaque on the inner lining of arterial walls, in particular of coronary arteries, what may cause ateriosclerosis. On the other hand, HDL help to prevent the accumulation of cholesterol in the blood, therefore high HDL level is associated with decreased risk coronary artery disease.

Nowadays, the dependence of the blood cholesterol level on the amount of food cholesterol has not been conclusively proven. Most of the food cholesterol decomposes in the liver, while the body durably synthesizes cholesterol which is needed for many purposes.

As far as the role of dietary cholesterol has not been elucidated, the people with elevated risk of coronary heart disease are advised to control their nutritional habits and limit the consumption of milk products. Although recents results of investigation show that some milk products rather decrease than increase the blood cholesterol level, many people are reluctant to consume milk products because the content of cholesterol is usually not shown on the package. The objective of this paper was the determination of cholesterol in respect to fat content in a number of yoghurts and ice creams produced in Estonia.

Material and methodics

The ice cream was warmed on the water bath and mixed to remove the air. Yoghurt was mixed to maintain homogeneous consistency. Both fat and cholesterol contents were determined in samples prepared in this manner. All tests were repeated for three times.

Determination of fat

A 5 g sample of yoghurt or 2 g sample of ice cream was weighed with 0.01 g accuracy into a milk butyrometer. 16 ml of sulphuric acid (H_2SO_4 d = 1.6 g/cm^3) and 1 ml of isoamyl alcohol were added. Butyrometer was capped tightly and turned up and down repeatedly, to let the acid mingle and to have the proteins dissolved entirely. The butyrometer then was immersed in a 65±2 ºC water bath for 5 minutes. Following heating, the butyrometer(s) were centrifuged for 5 minutes at 1000 rpm. After removing from the centrifuge, the lower level of the fat column in the butyrometer was set at the start point of the scale by means of the cap. The read was taken by the lower meniscus of the scale.

Determination of cholesterol

Cholesterol was determined by capillary gas chromatography, using the method of direct saponification for the sample preparation (Fletouris *et al*., 1998).

Materials and appliances: reagent-grade hexame (Reachim), methanol, KOH (CHEMAPOL). Centrifuge 5810 R. Saponification solution was prepared by dissolving 5.6 g KOH in methanol, diluting to 200 ml with the solvent, cholesterol (Sigma Chemical Co). Cholesterol standard solution 0.98 µg/µl (24.9 mg of cholesterol was solved in hexane and diluted to 25 ml), Eppendorf centrifuge 5810 R, centrifuge tubes with caps 15 ml, thermostate water bath (temperature 80±0.5 ^{o}C).

A 5 g sample of yoghurt or 2 g sample of ice cream was weighed with 0.01 g accuracy into a dry centrifuge tube to which 5 ml of methanolic KOH solution were also added. The tube was capped tightly, and its contents were vortexed for 15 s. The lower half of the tube was then immersed in a 80 °C bath and kept there for 15 min, for the hydrolysis of fat and cholesterol esthers and for re-esterification. Every 5 min the tube was removed from the bath, to vortex for 15 s. Following heating, the tube was cooled with tap water, 1 ml of water was added to improve the extraction of the cholesterol, and then 5 ml of hexane was also added. The contents were vortexed vigorously for 1 min and then centrifuged for 1 min at 1400 rpm, or maintained, capped, at the room temterature round-the-clock, to separate the hexane phase. An aliquot of the upper phase was then transferred into the autosampler vial pending Gas Chromatographic analysis.

The samples for the calibration curve were prepared in the same manner described previously, while instead of the yoghurt or ice cream 50, 100 and 200 µl of cholesterol standard solution (0.98 µg/µl) respectively, was used.

To check the retention time in the samples of milk products, 50 or 100 µl of cholesterol standard solution was added to the sample of yoghurt.

The prepared samples were analysed in the Food Hygiene laboratory of the Estonian University of Life Sciences, on the Gas Chromatograph Agilent 19091 J-413 HP-5, in 5% phenylmethyl silica capillary column (30.0 m × 310 µm × 25 µm). The injection volume was 1 µl of sample with a split ratio 50:1. The temperature of the column was set at 285 °C, flame ionization detector temperature at 300 °C. The flow rates were 2 ml/min.

Results and discussion

Fat content of yoghurts and ice creams

As a rule, on the package, the content of proteins, carbohydrates and fat content is shown by the producer. No information is presented about the cholesterol. In this paper, the content of fat in yoghurts and ice creams was determined experimentally, to set against the producer's data, and to find out whether the information on the package allows to estimate the possible amount of cholesterol.

In tables 1 and 2 experimentally determined fat contents and those read from the package are presented, for 8 yoghurt sorts and 10 ice cream sorts, produced in Estonia.

The results show that that experimental fat percentage differs, to an extent, from the producer's number. In case of ice creams, the difference is bigger than in case of yoghurts. Probably these differences are caused by the difficulty of taking even samples of products which contain various additonal ingredients, such as fruits, berries, jams, cereals, flavorings *etc*. Yet, the results are equal to the limit and the content of cholesterol can be compared with the producer's data about the content of fat, as well as the experimental results of this work.

Table 1. The content of fat and cholesterol of yoghurts

Name of product, company	Fat % on package	Fat % determined	Content of cholesterol mg/100 g, determined
Peach yoghurt, Tere	1.5	1.32	5.53±0.1
AB apple-cinnamon bioyoghurt, Tere	1.5	1.45	8.03±0.5
Gefilus wild strawberry yoghurt, Valio	2.0	1.76	8.7±1.16
Jou! Banana yoghurt, Alma	2.0	2.2	9.2±1.2
Wild strawberry yoghurt, Alma	2.0	2.31	8.1±0.87
Apple-pear-grain yoghurt, AS Põlva Piim	2.2	2.53	9.51±0.2
"Hellus" pasha bioyoghurt, Meieri	3.0	2.2	7.8±1.88
Wildberry cream-yoghurt, Tere	6.0	5.6	22.1±1.59

Table 2. The content of fat and cholesterol of ice creams

Name of product, company	Fat % on package	Fat % determined	Content of cholesterol mg/100 g determined
Top$_s$ Bio-yoghurt ice cream with wildberry filling, Balbiino	4.73	5.94	20.01±0.89
Top$_s$ Toffee ice cream, Balbiino	8.18	9.68	22.2±0.31
Vanilla ice cream with toffee, Balbiino	9.09	10.12	16.3±0.48
Top$_s$ Blueberry ice cream, Balbiino	9.64	12.43	29.1±0.24
Blueberry-raspberry ice cream, Balbiino	10.18	11.33	24.8±0.06
Wildberry ice cream, Premia	10.36	12.54	27.1±1.58
Vanilla ice cream with chocolate cream, Balbiino	10.55	12.1	23.5±1.02
Top$_s$ Vanilla ice cream, Balbiino	11.27	13.97	29.1±0.87
Vanilla-chocolate ice cream with cranberry jam, Premia	11.82	10.78	32.8±0.42
Fruit-flavoured ice cream with chips, Premia	13.64	10.23	29.6±1.97

Cholesterol content of yoghurts and ice creams

The concentration of cholesterol was measured by gas gromatography, the results of measurements are shown in tables 1 and 2, for yoghurts and ice creams, respectively.

The results of the research show that the ice creams produced in Estonia contain *ca* 16–29 mg/100 g of cholesterol, which is less than the data found by Fletouris – 46,7 mg/100 g (Fletouris *et al.*, 1998).

This brings to the conclusion that the Estonian ice cream is not a hazardous source of cholesterol. Nutrition experts suggest as 24 hour consumption 300 mg of cholesterol. So, a 100 g pack of ice cream covers as a maximum 10% of suggested daily limit. The limitation of the consumption is mainly meant for the people with arteriosclerosis risk. In general, the human food must contain a certain amount of cholesterol, to maintain normal work of the system of blood lipoproteins. For example, in Germany, the average daily intake of cholesterol with food, is 450–500 mg, of which 50–60% will absorb (Kokassaar, 1996).

Correlation between the content of fat and cholesterol

As far as the milk cholesterol is wholly located in the fat, the linear correlation must apply between the content of milk fat and cholesterol.

By the method of regressioon analysis [Excel, Date Analys] the coefficient of linearity ***a*** was determined,

$$y = a \cdot x \qquad (1)$$

where **x** is the content of milk fat of the product, g/100 g,
y is the content of cholesteron of the product, mg/100 g.

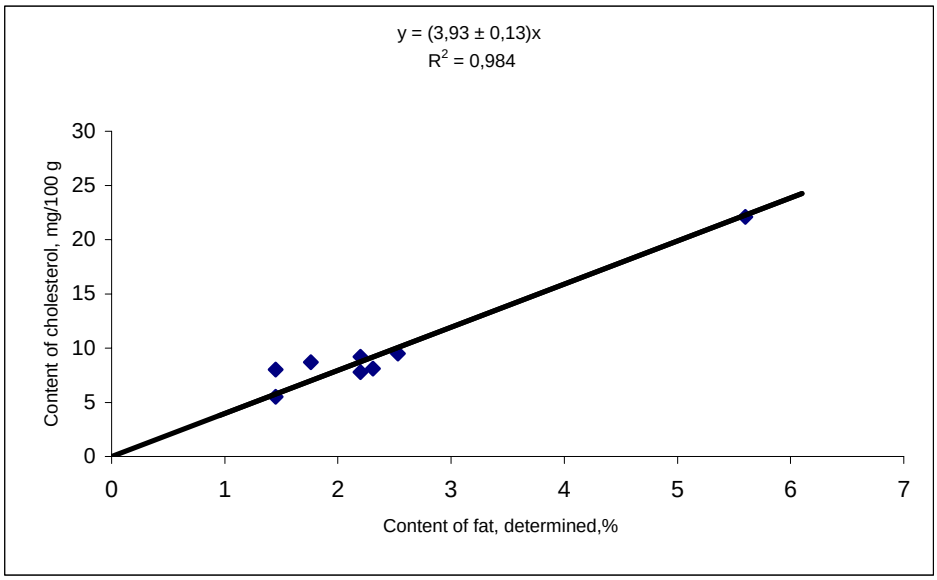

Figure 1. Correlation between the content of fat, determined experimentally, and cholesterol, of yoghurts

With all the data, for both yoghurts and ice creams, applied, the coefficient ***a*** was 3.62±0.29, and the correlation coefficient R = 0.902.

When the data of yoghurts and ice creams were dealt with separately, for the yoghurts good correlation ($y = (3.93\pm0.13)x$, $R^2 = 0.984$) was observed between the content of cholesterol and experimental results of fat percentage (Figure 1) and adequate correlation was found in case of the fat percentage shown on the package ($y=3.79x$, $R^2 = 0.836$).

The good correlation shows that one can calculate the content of cholesterol of yoghurts on the basis of their fat percentages. It also brings to the conclusion that the only fat in the yoghurts must be milk fat, other fats have not been added.

According to the linear correlation the between the content of milk fat and cholesterol, the content of cholesterol of pure milk fat can be calculated, and found to be 393 mg/100 g (Figure 1).

This content is in acceptable agreement with the data found in the literature, according to which the mean content of cholesterol of whole milk is 13 mg/100g (Laht, 2001). In all, milk fat may contain 0.2–0.4%, *i.e*. 200–400 mg/100 g of sterols, mainly cholesterol (Laht, Olkonen, 2001). Walstra *et al*. (1999) gives the same value, 0.013% (13 mg/100 g) of the content of cholesterol of milk, by that the content of sterols is from 0.25% (250 mg/100 g), to 0.32%, *i.e.* 320 mg/100 g. The analysis of 1142 German milk fats led to a mean cholesterol content of 265.6±20.0 mg/100 g, (range: 204.4 to 382.5), which is lower result than the one of present research (Precht, 2001).

In case of ice creams, the correlation between the fat and cholesterol content was inadequate: $R^2 = 0.0172$, Figure 2.

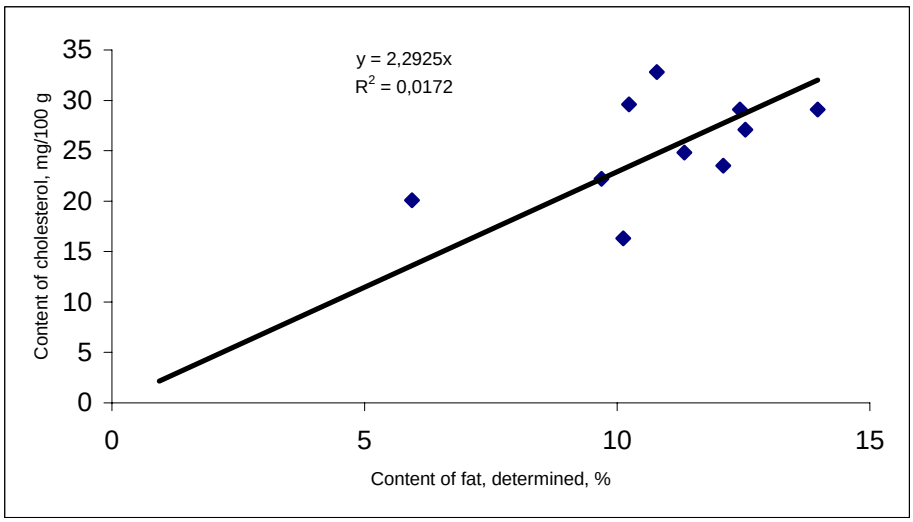

Figure 2. Correlation between the content of fat and cholesterol, of ice creams

Consequently, the content of cholesterol of ice creams cannot be calculated directly on the basis of their fat percentage. The low correlation points at the presence of other, low cholesterol fats, in particular vegetable fats, beside the milk fat in the composition of ice creams.

Milk fat and other fats in ice creams

Ice creams produced in Estonia contain *ca* 16–29 mg/100 g of cholesterol. Given that the content of cholesterol of pure milk fat is 393 mg/100 ml (equation 1, Figure 1) the amount of milk fat in ice creams can be calculated, and compared with the amount of other, vegetable fats. The contents of milk fat and other fats, and the ratios of both types of fats, of the ice creams are presented in the Table 3.

The ice creams under the research, contained approximately 6–16% of fat, by that the content of milk fat of them was around 4–8% and the content of the cholesterol-free fats was 1–6%. To analyse the fat ingredient as a whole, in different ice creams, 15–60% of the fat is not milk fat.

In all likelihood, most of the other fat is vegetable fat that has been added to the composition, but the content of fat can be increased also by the additives, such as in Balbiino ice creams with toffee (60% of other fats) or chocolate cream (51%).

Table 3. The ratio of milk fat and other fats in ice creams

Name of product, company	Concentration of milk fat in ice cream %	Concentration of other fat in ice cream %	Concentration of milk fat in fat as a whole %
Top$_s$ Bio-yoghurt ice cream with wildberry filling, Balbiino	5.1	0.8	86
Top$_s$ Toffee ice cream, Balbiino	5.6	4.1	58
Vanilla ice cream with toffee Balbiino	4.1	6.0	40
Top$_s$ Blueberry ice cream, Balbiino	7.4	5.0	60
Blueberry-raspberry ice cream, Balbiino	6.3	5.0	56
Wildberry ice cream, Premia	6.9	5.7	55
Top$_s$ Bio-yoghurt ice cream with wildberry filling, Balbiino	6.0	6.2	50
Top$_s$ Vanilla ice cream, Balbiino	7.4	6.2	53
Vanilla-chocolate ice cream with cranberry jam, Premia	8.3	2.5	77
Fruit-flavoured ice cream with chips, Premia	7.5	2.8	73

Summary and conclusions

The contents of fat and cholesterol of 8 yoghurt sorts and 10 ice cream sorts, produced in Estonia, were determined in the research. The contents of cholesterol were determined by gas-chromatography, using direct saponification of samples.

The determined fat contents were equal to the limits of the producer for the fat content.

Between the contents of cholesterol and fat, good correlation was obtained, in case of yoghurts. This shows that a) it is possible to estimate the content of cholesterol of yoghurts on the basis of their fat percentages, and b) the only fat in the yoghurts is milk fat.

In case of ice creams, the correlation between the contents of cholesterol and fat was low. This can be explained with the recipe of ice creams, in which some part of the milk fat is replaced by vegetable fats, which do not contain any cholesterol. Therefore, the content of cholesterol of ice creams cannot be calculated from the data about the fat.

Based on the linear correlation between fat percentage and cholesterol content of yoghurt, the cholesterol content of milk fat, 393 mg/100 g was found. Accordingly, the content of milk fat of ice creams can be determined by the concentration of cholesterol.

The ratio of milk fat and other fats was calculated for ice creams. It was found that ice creams contain 4–8% of milk fat and 1–6% of other, mainly vegetable fats. In other words, 15–60% of the fat in the ice cream, is not milk fat.

The content of cholesterol of yoghurts and ice creams, produced in Estonia, is not high (ca 10 mg/100 g in yoghurts and ca 20 mg/100 g in ice creams) and it is safe to consume these milk products. Furthermore, due to the added fruits and berries, the yoghurts and ice creams have favourable effect in excretion cholesterol from the body, and act as antioxidants.

References

Fletouris, D. J., Botsoglou, N. A., Psomas, I. E., Mantis, A. I. 1998. Rapid determination of cholesterol in milk and milk products by direct saponification and capillary gas chromatigraphy. – J. Dairy Sci., 81:2833–2840.

Kokassaar, U., Vihalemm, T., Zilmer, M., Pulges, A. 1996. Inimtoidu loomulikud ja sünteetilised komponendid. – Tartu, 50 lk.

Laht, T-M, Olkonen, A. 2001. Piima koostis ja füüsikalis-keemilised omadused. – Piimanduse käsiraamat (koostaja A. Olkonen). – EPMÜ Loomakasvatusinstituut, lk 109.

Laht, T-M. 2001. Piim ja piimatooted toiduna. – Piimanduse käsiraamat (koostaja A. Olkonen). EPMÜ Loomakasvatusinstituut, lk 81–83, 90–101.

Precht, D. 2001. Cholesterol content in European bovine milk fats. – Nahrung. Feb, 45(1):2–8. Bundestanstalt für Milchforschung, Institut für Chemie und Physik, Hermann-Weigmann-Strasse 1, D-24103 Kiel, Germany.

Walstra, P., Geurts, T. J., Noomen, A., Jellema, A., van Boeckel, M. A. J. S. 1999. Dairy Technology Marcel Dekker, Inc., 50 pp.