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Effect of dairy animal species and of the type of starter cultures on the cholesterol content of manufactured fermented milks

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ABSTRACT

The aim of the research was to estimate the influence of type of starter cultures (yoghurt, kefir and butter) on the cholesterol content in fermented milk produced from cow's, goat's and ewe's milk. It was established that cow's milk fat was the richest in cholesterol; on the contrary, ewe's milk cholesterol level was the lowest as calculated per g of fat. Goat's milk contained the smallest amount of cholesterol calculated per 100 g of milk.

In freshly fermented milks (yoghurt, kefir, cultured milk), obtained from the milk of three animal species, a statistically significant lowering of cholesterol, in comparison to pasteurised cow's, goat's and ewe's milk, was observed. However, a significant influence of the starter culture kind and chilled storage time of fermented milks on cholesterol content was not observed in the whole range of products obtained from each animal species' milk.

1. Introduction

The content of cholesterol in milk and dairy products is influenced by different factors; one of them is the animal species which produces milk. According to Sankhla and Yadava (1981), the richest in cholesterol is goat's milk fat and the poorest ewe's fat (2.6 and 1.9 mg per g of fat, respectively). Research of Park et al. (2007) supports the above opinion (3.42 and 2.88 mg per g of fat, respectively). Pantulu et al. (1975) and Ahmad et al. (2013) reported even higher levels of cholesterol for goat's milk (5.1 mg per g of fat) concluding that it was present in a greater amount in comparison to cow's and buffalo's milk (3.96 and 3.34 mg per g of fat, respectively).

According to Goudjil et al. (2003) the ewe's milk fat contains from 209 to 441 mg of cholesterol per 100 g of fat. Fletouris et al. (1998) report that the richest in cholesterol is ewe's milk (at average 21.7 mg per 100 g), in the middle is the goat's milk (14.4 mg per 100 g) and the poorest is cow's milk (12.2 mg per 100 g). Talpur et al. (2008) and Sieber and Eyer (2004) consider that the more fat is present in milk products the more cholesterol they contain. The content of fat in ewe's milk is higher than in cow's and goat's what implies the higher cholesterol levels present in ewe's milk.

The cholesterol content in goat's milk, according to different authors, ranges from 2 to 24 mg per 100 ml of milk (Bernacka and Simińska, 2005; Citek et al. 1997; Collins et al., 2003; Kay et al., 2004; Slacanac et al., 2012). However, other authors, among them Bitman and Wood (1990) showed higher amounts of that component in goat's milk: from 14.30 to 16.00 mg per 100 ml of milk. Barłowska et al. (2011), citing after other authors, stated that cholesterol content in different animal species milk was: for buffalo—from 8.89 to 10.24; for ewe 14.23–30.00 (Goudjil et al., 2003); for cow—from 25.60 to 31.40; for camel—from 31.3 to 37.10; for goat—from 16.9 to 18.09 mg per 100 ml of milk.

There is a lack of information in the literature about what the range of cholesterol content is in fermented milks obtained from milk of different animal species.

The results of many authors revealed the positive role of some lactic acid bacteria species in lowering of the level of cholesterol in cow's milk and in dairy products. Vujicic et al. (1992) showed that kefir culture containing streptococci and lactobacilli, acetic acid bacteria and yeasts, after milk inoculation and 24-h incubation, reduced the level of cholesterol by 16%, and after the next whole day of ageing by further 43%. In total, in the 48-h kefirs the level of cholesterol was 41% of its initial value in milk. According to Stepaniak and Fetliński (2004), kefir cultures assimilate from 40 to 84% of the cholesterol content in milk. Akalin et al. (2004) also showed the reduction of cholesterol content in yoghurt obtained from cow's milk inoculated with *Streptococcus thermophilus* and

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Lactobacillus delbrueckii subsp. *bulgaricus*. Juśkiewicz and Panfil-Kuncewicz (2003) established the changes in cholesterol content in milk influenced by yoghurt bacteria and concluded that the bacteria decreased the level of cholesterol in a range of 19.8–22.2%.

Fujishiro et al. (2002), Lv et al. (2002) believe that many microorganisms produce enzymes which disrupt cholesterol *i.e.* cholesterol reductase and/or cholesterol oxidase, which cause the lowering of cholesterol levels in fermented milk products. Peptidoglycan, which is present in bacteria cell-walls, is responsible for the phenomenon of cholesterol binding by lactic acid bacteria. Thanks to its structure and chemical properties, peptidoglycan plays a fundamental role in cholesterol binding (Kimoto et al., 2002; Dambekodi and Gilliland 1998; Naverd et al., 1990). Research conducted by Hosono and Tono-Oka (1995) and Usman and Hosono (1999) revealed that peptidoglycan isolated from bacteria contained from 28 to 34% of the total amount of cholesterol, which was found in cells and/or in cell-walls of bacteria. Ziarno et al. (2006) found that the cell-walls of dead milk fermenting bacteria also have the ability to bind cholesterol, but the amount of cholesterol bound by alive bacteria cells was much higher than that by dead cells.

The aim of the research was to estimate the influence of commercial starter cultures (yoghurt, kefir and butter) on the cholesterol content in fermented milk produced from cow's, goat's and ewe's milk.

2. Materials and methods

2.1. Materials

The research material was cow's, goat's and ewe's milk obtained from bulk tanks at private milk farms. The cow's milk was milked from Polish Holstein-Friesian black–white variety cows at a private farm at Jura Krakowsko-Częstochowska. The goat's milk originated from the Alpina and Anglo-Nubian cross-bread goats from a goatshed at Mszana in Beskid Niski. Ewe's milk originated from Polish Mountain sheep, grazed near Nowy Targ. The milk samples were obtained during the spring season. Lactation stage of animals 3rd–4th month after calving. The animals were grazed; water supplied *ad libitum*. All animals were hand milked and milk was directly sampled from bulk, cooled tanks. The sampling was performed in three series with three replicates in each. All analyses were conducted up to two hours after milking.

Each sample of raw cow's, goat's and ewe's milk was divided into three parts, all parts were pasteurised (in 0,5 l glass bottles; 3 bottles filled up to 400 ml per each sampling part) at 95 °C for 10 min, then cooled to proper (inoculation) temperature. The first of three parts of each milk sample was cooled to 45 °C and inoculated with 2% (quantity converted into the batch starter culture) addition of DVS YC-180 starter by Christian Hansen (Denmark), composed of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, then it was incubated for about 6 h—until the pH of 4.6–4.8 was obtained. By the method described above the cow's, goat's and ewe's yoghurt was obtained.

The rest (2 parts) of milk samples were cooled to 22 °C, and the half of them (cow's, goat's and ewe's) were inoculated with DVS D starter (Rhodia Food Biolacta Ltd., Danisco Group, Poland), composed of: *Lactobacillus* sp., *Lactococcus* sp., *Candida kefir*, *Kluveromyces fragilis*; incubated at 22 °C during 12 h—to obtain the cow's, goat's and ewe's kefir. The last of three samples of the 3 animals species' milk were inoculated with a 2% (quantity converted into the batch starter culture) addition of CHN-11 starter by Christian Hansen, composed of *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *diacetylactis*, *Leuconostoc mesenteroides* ssp. *cremoris*; incubated at 22 °C for 12 h—to obtain the cultured cow's, goat's and ewe's milk.

All fermented milk samples were stored in 200 ml glass jars (before sterilized with seals) at 4 °C for 14 days. The freshly made and 14 day products were subjected to analyses.

2.2. Analyses of milk and fermented milks

In all the samples of pasteurised cow's, goat's and ewe's milk the subsequent analyses were performed:

- total solids by the drying method [%] (AOAC, 1990)
- total fat by the volumetric Gerber's method [%] (AOAC, 1990)
- density by the lactodensimeter [g/cm^3] (AOAC, 1990)
- titratable acidity by the Soxhlet–Henkl method [°SH] (AOAC, 1990)
- active acidity by pH-meter (AOAC, 1990)
- cholesterol content by the enzymatic method with cholesterol oxidase according to Grossmann et al. (1976) and enzymatic method with spectrophotometrical measurements described by R-Biopharm (Boehringer Mannheim/R-Biopharma Enzymatische Bioanalytik, Germany). The absorbance of the examined samples and the control sample was measured at the wavelength of 405 nm using the Helios Gamma and Delta Spectro-Lab spectrophotometer (Thermo Electric Corporation, USA).

In raw, fresh milk of cow, goat and ewe and in fermented milks the amount of cholesterol was estimated by the above described method and the results were calculated per g of fat and per 100 g of product.

2.3. Statistical analysis

For each series of analysis conducted, a minimum of three repetitions were performed and the results are shown as the arithmetic mean with standard error (±SE). Statistical analysis was performed using Statistica v. 8.0 (licensed version). A single-factor analysis of variance (ANOVA) and double-factor analysis of variance (MANOVA) with post hoc new multiple bias Duncan test at signifi-

Table 1
Characteristics of raw milk of three animal species.

Properties	Milk		
	Cow mean ± SE	Goat mean ± SE	Ewe mean ± SE
Total solids content [%]	11.34^b ± 0.63	11.56^b ± 0.22	17.8a^a ± 0.32
Total fat content [%]	3.70^b ± 0.61	3.52^b ± 0.10	6.57^a ± 0.34
Density [g/cm^3]	1.0270^b ± 0.0002	1.0283^b ± 0.0006	1.0362^a ± 0.0003
Titratable acidity [°SH]	6.50^b ± 0.10	5.47^a ± 0.23	6.93^b ± 0.01
pH	6.82^b ± 0.05	6.93^b ± 0.01	6.75^a ± 0.01
Cholesterol content [mg per g of fat]	3.52^c ± 0.08	2.69^a ± 0.05	2.28^b ± 0.06
Cholesterol content [mg per 100 g of milk]	12.84^a ± 0.65	9.46^b ± 0.20	14.99^c ± 0.78

*—Means marked with different letter in rows differ statistically at $p < 0.05$.