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ORIGINAL PAPER

THE EFFECT OF SELECTED FACTORS ON YIELD AND PROTEIN AND MINERAL RETENTION IN TRADITIONALLY PRODUCED TVAROG*

Jolanta Król[1], Monika Kedzierska-Matysek[1], Aneta Brodziak[2], Agnieszka Zaborska[1], Anna Litwińczuk[1]

[1]Department of Commodity Science and Processing of Raw Animal Materials
[2]Department of Breeding and Protection of Cattle Genetic Resources
University of Life Sciences in Lublin, Poland

Abstract

Tvarogs, known in Poland as 'white cheese', are highly popular products among consumers. The objective of the study was to determine the degree of retention of protein and selected minerals in tvarogs produced from the milk of cows raised on traditional farms, in relation to a breed (Holstein-Friesian and Simmental), production season (spring/summer and autumn/winter) and the starter cultures used (homo- and heterofermentative). Samples of bulk milk were collected from cows (three each from each breed from the spring/summer and autumn/winter seasons). Tvarogs were produced from milk according to a traditional technology, using two types of starter cultures, i.e. homofermentative (culture 1) and a mixture of homo- and heterofermentative cultures (culture 2). Basic chemical composition and contents of K, Ca, Na, Mg and Zn were determined in the milk, whey and tvarogs. The content of protein and minerals was used to calculate the degree of retention of these nutrients in the experimental tvarogs. The yield and quality of tvarogs is integrally linked to the quality of the milk used to produce them. Higher tvarog yield was obtained using milk from Simmental cows in the summer, which can be linked to higher protein content in this milk. The dry matter of tvarogs produced from the milk of Holstein-Friesians contained significantly more fat and less protein. It should be noted that no significant differences were found in the degree of protein retention from the raw material to the products, while a higher retention index was obtained during the summer season. Higher content of minerals was noted in the milk obtained from the Simmental cows in the spring/summer and in the tvarogs produced from it. However, very low retention indices were found for these minerals. It should be emphasised that both the production season and the cultures used significantly determined the degree of retention. Higher retention indices were obtained in the spring/summer season using culture 1.

Keywords: milk, whey, tvarog, season, elements, degree of retention.

Jolanta Król, PhD DSc, Department of Commodity Science and Processing of Raw Animal Materials, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland, e-mail: jolanta.krol@up.lublin.pl

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INTRODUCTION

Tvarogs, known in Poland as ‘white cheese’, are highly popular products among consumers, owing to the centuries-long tradition, dietary habits, and low prices (Gołąbek 2011, Górska-Warsewicz 2005). They comprise a numerous and varied group of dairy products with an established position on the Polish market. Tvarog owes its high nutritional value mainly due to its content of complete animal protein with very high digestibility, estimated at 96-97%, as well as its relatively low calorific value. Moreover, tvarogs enrich the human diet with easily digestible milk fat, vitamins, and minerals. They are also a potential source of bioactive peptides, which exert a beneficial effect on the human health (Barać et al. 2017). Owing to these qualities, tvarogs are included in diets of healthy people as well as those with various non-pathological (e.g. pregnancy or engaging in sport) or pathological conditions (such as injuries, infections or postoperative states) (Siemianowski, Szpendowski 2014). In recent years, the annual per capita consumption of tvarog in Poland has remained at over 5 kg and is exhibiting an upward trend. According to the Institute of Agricultural and Food Economics – National Research Institute (2017), the mean annual consumption of tvarogs in Polish households in 2015 was 5.16 kg/person. The purchase of tvarogs is estimated to account for about one fifth of expenditures associated with the purchase of dairy products by an average household. The largest quantity is consumed by households of pensioners (on average 0.60 kg/person per month).

Traditionally-produced tvarog contains on average 0.8-0.9% minerals, of which calcium and phosphorus make up the largest share (about 40% of ash). Calcium, known primarily for its role in the formation of bone and teeth, performs numerous other functions in the body; for example, it is involved in the conduction of nerve signals and regulation of the excitability of nerves, muscle contractility, enzyme activation and blood coagulation (Siemianowski, Szpendowski 2014, Zaręba et al. 2012). It should be emphasised that the bioavailability of calcium from dairy products reaches 30-40%, whereas in the case of many plant products it does not usually exceed 10%. This is because dairy products contain substances which increase the bioavailability of calcium, i.e. vitamin D, phosphopeptides generated as a result of casein hydrolysis, lactose, or alkaline amino acids (Siemianowski, Szpendowski 2014, Kusiuk et al. 2009). The low pH of tvarogs also favourably affects the bioavailability of this element. Milk and dairy products are not the best source of magnesium among food products, although the presence of lactose and protein is conducive to assimilation of this element by the human body. Magnesium is an essential macronutrient for the proper development and function of the body; it takes part in protein biosynthesis, nerve conduction, muscle contractility, thermoregulation processes and blood pressure regulation. Tvarog contains smaller quantities of other minerals nutrients, including K, Na and Zn. Potassium and sodium are necessary for the body’s fluid and electrolyte balance. Zinc is responsible for maintaining cell membrane stability, taste and smell perception, alcohol metabolism and immune defence (Śmigielska et al. 2005, Zaręba et al. 2012).

Unfortunately, 50-60% of dry matter components are lost during tvarog production with the whey, which is estimated to contain on average 95% of the whey proteins, 33% of the casein, 96% of the lactose, 8% of the fat and 81% of the minerals from the milk (Bednarski 2001). Recent years have seen dynamic advancements in the processing of milk to produce tvarogs, mainly the introduction of new processing techniques (Siemianowski, Szpendowski 2015). Efforts are made to optimally utilise all valuable nutrients in the milk, particularly protein and minerals, while at the same time increasing production efficiency.

The objective of the study was to determine the degree of retention of protein and selected minerals in tvarogs produced from the milk of cows raised on traditional farms, in relation to a breed (Holstein-Friesian and Simmental), production season (spring/summer and autumn/winter) and the starter cultures used (homo- and heterofermentative).

MATERIAL AND METHODS

Samples of bulk milk were collected from cows of the Holstein-Friesian and Simmental breeds (three each from each breed from the spring/summer and autumn/winter season). The cows were raised in a traditional farming system and their diet was based mainly on feed from permanent grassland, i.e. green forage supplemented with hay and concentrate (cereal meals) in the summer season and haylage and hay in the winter season.

Bulk milk was used to produce tvarogs in laboratory conditions according to a traditional technology, using two types of starter cultures. Following pasteurization the milk was cooled and inoculated with homofermentative cultures (culture 1) and a mixture of homo- and heterofermentative cultures (culture 2). The milk was incubated at 30-32°C for 12 h, until a pH of about 4.6 was attained. The curds obtained were cut and briefly heated and then separated into drip bags made of medical gauze. The whey was allowed to drip for about 2 h. The weight of tvarog obtained and the amount of separated whey were used to calculate the cheese yield, as the quantity of milk used to produce 1 kg of tvarog.

The following were determined in each sample of milk and whey: proximate chemical composition, i.e. content of crude protein, fat and dry matter (with an Infrared Milk Analyzer, Bentley Instruments, USA). Somatic cell count (SSC) was determined (with a Somacount 150 apparatus, Bentley Instruments, USA) to eliminate milk samples with SSC over 400,000/ml. Moisture content in the tvarog samples was determined by drying at 102°C

(PN-73/A-86232), fat content was determined by the Gerber's method (PN-73/A-86232), and protein content - by the Kjeldahl method (PN-EN/ISO 8968-1:2004). The analyses were performed in triplicate.

The content of selected minerals (K, Ca, Na, Mg and Zn) in the study material, i.e. in milk, whey and tvarogs, was determined by flame atomic absorption spectrometry (FAAS), with atomization in a strongly-oxidizing air-acetylene flame, using a Varian AA240FS Fast Sequential Atomic Absorption Spectrometer (Varian, Inc., USA). Samples were prepared according to PN-EN 13804. Milk and whey in the amount of 1 ml each and about 0.5 g of tvarog (within 0.0001 g), together with 1 ml 30% hydrochloric acid and 5 ml 65% suprapure nitric acid, were collected into flasks. The solutions were mineralised under increased pressure in a CEM MARS 5 Xpress microwave digester (CEM Corporation, USA). Then, the mineralised samples were quantitatively transferred using deionised water into 25 cm^3 volumetric flasks. To validate the method, certified reference material NCS ZC 73015 Milk powder was analysed concurrently with the experimental samples, in an identical manner. The content of the elements was read from a calibration curve of the dependency of the absorption on the content of the element.

The content of protein and minerals in the milk and whey was used to calculate the degree of retention of these nutrients in the experimental tvarogs, according to the following equation (Szpendowski et al. 2013):

$$R(\%) = \frac{C_{milk} - C_{whery}}{C_{milk}} \cdot 100,$$

C_{milk} – content of the component in milk,
C_{whey} – content of the component in whey.

Statistical analysis of the results was performed using StatSoft Inc. Statistica v. 13 software.

RESULTS AND DISCUSSION

The raw material obtained from the Polish Holstein-Friesian cows had significantly (at $p \leq 0.05$) higher content of dry matter (by 0.44 p.p), including fat (by 0.23 p.p), than the milk produced by the Simmental cows (Table 1). The milk of the Simmental cows contained much more protein, by 0.15 p.p (at $p \leq 0.01$). Protein content, especially the casein fraction, determines the suitability of milk for processing, particularly for production of cheese, including tvarog (Barłowska et al. 2014), because low protein content in milk significantly reduces cheese yield per unit volume of milk, which thereby increases production costs. By reducing the amount of milk used to produce 1 kg of tvarog by just 0.1 litre, 10 kg more tvarog can be obtained from 100,000 litres of milk, which amounts to 3,000 kg per year (300 days)

Table 1

Chemical composition of raw milk for tvarog production in relation to the factors analysed ($\bar{x} \pm SD$)

Component	Simmental breed			Polish Holstein-Friesian breed			Breed x season interaction
	summer season	winter season	total	summer season	winter season	total	
Protein (%)	$3.59^{B} \pm 0.45$	$3.34^{A} \pm 0.41$	$3.46^{*} \pm 0.44$	3.38 ± 0.40	3.21 ± 0.36	$3.31^{*} \pm 0.38$	*x*
Fat (%)	$3.86^{A} \pm 0.53$	$4.13^{B} \pm 0.49$	$3.98^{**} \pm 0.61$	4.07 ± 0.69	4.37 ± 0.71	$4.21^{**} \pm 0.64$	*x*
Dry matter (%)	$12.48^{a} \pm 0.88$	$12.81^{b} \pm 1.00$	$12.76^{*} \pm 0.92$	12.92 ± 0.99	13.34 ± 1.02	$13.20^{*} \pm 1.04$	*x*
Ca (mg L^{-1})	$1,381.95^{a} \pm 200.3$	$1,616.95^{b} \pm 156.8$	$1,501.65^{*} \pm 178.5$	1309.22 ± 217.1	1469.91 ± 291.5	$1388.54^{*} \pm 312.8$	*x*
Mg (mg L^{-1})	$102.78^{a} \pm 19.5$	$126.37^{b} \pm 20.3$	114.85 ± 20.1	$111.66^{a} \pm 10.3$	$130.97^{b} \pm 15.4$	122.31 ± 13.6	ns
K (mg L^{-1})	$1,562.07 \pm 137.2$	$1,645.25 \pm 195.5$	$1,601.37 \pm 149.3$	$1505.89^{a} \pm 210.4$	$1701.65^{b} \pm 195.5$	1604.58 ± 202.5	ns
Na (mg L^{-1})	565.61 ± 141.2	533.59 ± 99.6	550.48 ± 124.6	503.77 ± 190.5	530.92 ± 187.3	518.23 ± 175.6	ns
Zn (mg L^{-1})	2.55 ± 1.2	3.31 ± 1.7	2.95 ± 1.6	2.88 ± 1.7	3.09 ± 0.9	2.99 ± 1.0	ns

a, *b*, *A*, *B* – differences between seasons; *a*, *b* – differences significant at $P \leq 0.05$; A, B – differences significant at $P \leq 0.01$;
*, ** – differences between breeds; * – differences significant at $P \leq 0.05$; ** – differences significant at $P \leq 0.01$;
x – significant at $P \leq 0.05$; xx – differences significant at $P \leq 0.01$; ns – not significant.

(Chojnowski 2013). The present study indicates that the factors analysed (breed, season, and culture) significantly influenced the yield of tvarog (Table 2). Less milk was used to produce 1 kg of tvarog (4.69 l) when milk from the Simmental breed was used, which can be linked to the higher content of protein in this milk. As much as 0.88 l more milk from Holstein-Friesian cows was required to produce 1 kg of tvarog. The yield of tvarog was also significantly influenced by seasonal changes in the composition of the milk primarily the protein content. Milk obtained from both breeds had higher protein content in the spring/summer season, when the animals were pastured. The lower protein content in the milk from the winter season was reflected in the use of more raw material to produce 1 kg of tvarog (by 1.43 l as compared to the summer season), and in consequence the yield of tvarog from 100 litres of milk was over 5 kg lower. The tvarog variants differed significantly in terms of the content of dry matter components (Table 2). As the yield of tvarog increased (less milk used to produce a unit of product), the content of dry matter components decreased in the tvarogs. At the same time, the water content increased in these tvarogs, which may be explained by the higher protein content in the milk, as proteins bind water and thereby increase the content of water in the weight of the finished product.

It should be noted that no significant differences were observed in the degree of protein retention from the milk to the product, although a higher retention index was obtained in the summer season (Table 3). The value for this parameter for the tvarogs ranged from 85.64% for tvarog H (milk from Holstein-Friesians, winter season, culture 2) to 88.02% for tvarog A (milk from Simmentals, summer season, culture 1). Siemianowski et al. (2013), in an evaluation of tvarogs produced from milk with different concentrations of dry matter, obtained protein retention at a similar level to that observed in the present study. It ranged from 85.90 for tvarogs produced from reconstituted milk containing 25.94% dry matter, including 9.36% protein, to 87.94% for tvarogs made from milk with the lowest content of dry matter (9.54%, including 3.4% protein). A higher level of protein retention in tvarogs can be obtained by thermal processing and the addition of calcium chloride or by ultrafiltration. Increasing the concentration of calcium ions before heating the milk enhances aggregation of whey proteins, their interactions and the formation of complexes with casein (Siemianowski, Szpendowski 2015). Szpendowski et al. (2007) obtained 89.31% protein retention by adding calcium chloride in the amount of 0.05% and pasteurizing the milk at 90°C for 15 s. Adding transglutaminase to milk at the initial stage of processing (before adding starter cultures) also leads to increased protein retention in the product (Mazuknaite et al. 2013) and to 10-15% higher tvarog yield (Bohdziewicz 2010). The use of ultrafiltration in tvarog production enables nearly 100% of the protein in the milk to be utilised in the product (Hinrichs 2001).

During the processing of milk to produce cheese, including tvarogs, an effort is made to utilise the valuable nutrients of the raw material to the

Table 2

Chemical composition and yield of tvarogs in relation to the factors analysed ($\bar{x} \pm SD$)

Factors	Yield of tvarog (milk used to produce 1 kg of cheese)	Protein (%)	Fat in dry matter (%)	Dry matter (%)	Ca (mg L^{-1})	Mg (mg L^{-1})	K (mg L^{-1})	Na (mg L^{-1})	Zn (mg L^{-1})
Breed									
Simmental	$4.69^{A} \pm 0.4$	$12.06^{a} \pm 0.8$	$39.45^{A} \pm 3.3$	28.31 ± 0.6	1368.23 ± 175.1	105.67 ± 9.8	1573.56 ± 118.9	305.87 ± 30.2	4.48 ± 0.5
Holstein--Friesian	$5.57^{B} \pm 0.4$	$13.39^{b} \pm 0.6$	$45.34^{B} \pm 3.9$	29.89 ± 1.2	1310.56 ± 171.9	116.25 ± 1.1	1590.08 ± 116.3	328.74 ± 27.6	4.78 ± 0.4
Season									
Summer	$4.44^{A} \pm 0.3$	$11.31^{A} \pm 0.9$	$38.74^{A} \pm 2.7$	$27.18^{a} \pm 1.6$	1293.42 ± 135.6	113.89 ± 5.6	1558.31 ± 95.6	321.28 ± 33.2	4.74 ± 0.3
Winter	$5.87^{B} \pm 0.5$	$14.36^{B} \pm 1.2$	$43.11^{B} \pm 3.1$	$30.65^{b} \pm 2.0$	1380.64 ± 68.5	111.09 ± 9.2	1560.55 ± 110.3	315.36 ± 26.7	3.90 ± 0.8
Culture									
1	$4.98^{a} \pm 0.3$	$11.21^{a} \pm 0.7$	$38.49^{A} \pm 1.7$	27.21 ± 2.3	$1278.78^{a} \pm 84.7$	113.12 ± 10.8	1565.12 ± 99.6	301.49 ± 36.5	$2.95^{a} \pm 0.5$
2	$5.30^{b} \pm 0.3$	$13.65^{b} \pm 0.9$	$43.38^{B} \pm 3.0$	29.86 ± 1.9	$1410.35^{b} \pm 109.3$	110.32 ± 6.9	1589.32 ± 111.7	335.12 ± 28.6	$4.46^{b} \pm 0.4$

Culture 1 – homofermentative cultures, culture 2 – mixture of homo- and heterofermentative cultures;
a, *b*, *A*, *B* – differences within the factor; *a*, *b* – differences significant at $P \leq 0.05$; *A*, *B* – differences significant at $P \leq 0.01$.

Table 3

Yield of tvarogs and indices of protein and mineral retention from raw material to product ($\bar{x}$ ± SD)

Type of tvarog	Yield of tvarog (milk used to produce 1 kg of cheese)	Retention (%)					
		protein	Ca	Mg	K	Na	Zn
Tvarog A	4.34 ± 0.37	88.02 ± 0.86	24.13 ± 1.53	26.66 ± 4.64	26.20 ± 2.71	12.78 ± 1.22	35.86 ± 4.71
Tvarog B	4.52 ± 0.44	87.12 ± 0.95	20.54 ± 1.87	23.91 ± 4.35	19.31 ± 2.61	13.08 ± 1.31	39.28 ± 4.42
Tvarog C	4.85 ± 0.38	86.32 ± 0.62	20.01 ± 2.31	18.93 ± 3.62	22.45 ± 3.26	12.53 ± 1.29	32.37 ± 3.79
Tvarog D	5.02 ± 0.51	85.74 ± 0.61	18.64 ± 1.61	16.28 ± 2.31	19.49 ± 2.56	11.95 ± 2.31	32.87 ± 4.51
Tvarog E	5.17 ± 0.42	87.84 ± 0.87	26.69 ± 2.19	33.85 ± 3.54	30.93 ± 2.19	18.37 ± 1.50	39.26 ± 5.52
Tvarog F	5.34 ± 0.36	87.56 ± 0.82	24.27 ± 1.56	22.08 ± 3.82	20.42 ± 3.28	15.54 ± 2.16	40.28 ± 4.57
Tvarog G	5.69 ± 0.59	85.96 ± 1.05	20.27 ± 2.13	21.92 ± 2.67	25.56 ± 3.71	14.47 ± 1.65	34.96 ± 4.34
Tvarog H	6.21 ± 0.62	85.64 ± 0.74	20.05 ± 1.23	19.42 ± 3.23	21.81 ± 1.56	13.19 ± 1.22	38.28 ± 3.92
Effect							
Breed	*x*	ns	ns	ns	ns	*x*	ns
Season	*x*	*xx*	*x*	*xx*	ns	*x*	*x*
Culture	*xx*	ns	ns	*x*	*x*	ns	*x*

Tvarog A – milk from Simmental cows, summer season, culture 1; Tvarog B – milk from Simmental cows, summer season, culture 2; Tvarog C – milk from Simmental cows, winter season, culture 1; Tvarog D – milk from Simmental cows, winter season, culture 2; Tvarog E – milk from Holstein-Friesian cows, summer season, culture 1; Tvarog F – milk from Holstein-Friesian cows, summer season, culture 2; Tvarog G – milk from Holstein-Friesian cows, winter season, culture 1; Tvarog H – milk from Holstein-Friesian cows, winter season, culture 2; *x* – significant at $P \leq 0.05$; *xx* – differences significant at $P \leq 0.01$; ns – not significant.

greatest possible degree in the final product. In traditional cheese production methods, however, a substantial portion of these nutrients are lost with whey. During acid coagulation, most minerals in the milk (about 65%) are transferred to whey, while about 35% are bound with protein and remain in cheese (Chmura et al. 2002).

The results of our study indicate variation in the content of individual minerals in both the milk used and the tvarogs produced from it (Tables 1-2). The degree of retention of minerals from the raw material to the product was varied as well (Table 3). The milk obtained from Simmentals had significantly ($p \leq 0.01$) higher content of calcium (1,501.65 mg L^{-1}) than the milk of Holstein-Friesian cows (1,388.54 mg L^{-1}). Irrespective of the breed, milk obtained in the winter season was a richer source of the elements analysed, and the differences were statistically significant ($p \leq 0.05$) in the case of calcium, magnesium and potassium. Król et al. (2016), while evaluating the content of minerals in milk produced on low-input farms, found that the season significantly ($p \leq 0.01$) influenced only Ca content. Higher content of this element, as in the present study, was noted in the milk obtained in the autumn/winter season (1,753.9 mg L^{-1}). The milk from the spring/summer season contained over 250 mg L^{-1} less calcium. The higher calcium content in the autumn/winter season may be due to the use of silage in the animals' diet during this period. In a study by Zielińska et al. (2008), green forage had slightly lower calcium content than the silage prepared from it, which according to the authors may be due to lactic acid bacteria metabolism. Many authors (Król et al. 2012, Sola-Larrañaga, Navarro-Blasco 2009) claim that differences in the concentration of minerals in milk may be primarily due to variation in their content in feed. Analysis of the content of minerals in the tvarogs revealed no effect of a breed or production season (Table 2). It should be noted, however, that the tvarogs proved to be a poorer source of minerals than the milk from which they were produced. This is linked to the very low retention indices for the minerals from the milk to the tvarog. Calcium retention ranged from 18.64% (tvarog D – milk from Simmental cows, winter season, culture 2) to 26.69% (tvarog E – milk from Holstein-Friesians, summer season, culture 1). It should be emphasised that both the production season and the cultures used significantly influenced the degree of retention. Higher retention parameters were obtained in the summer season with the use of culture 1. In a study by Baran et al. (2011), the mean calcium retention index in goat and sheep cheeses was only 15-18%. The authors obtained much higher indices for acid-rennet cheeses (58-85%). As in the case of Ca, the lowest magnesium retention index was obtained for tvarog D (16.28%) and the highest one for tvarog E (33.85%). Comparable patterns were observed for K and Na, with very low retention indices for sodium, ranging from 11.95% to 18.37%. The highest retention indices were obtained for zinc. They were over 30% for all variants of tvarog, and were significantly influenced by the production season and the culture used. Higher retention indices, in both the summer and winter seasons, were obtained using culture 2, with the most zinc retained in tvarogs made from milk acquired in the summer (39.28% and 40.28%). High zinc retention indices were also obtained by Baran et al. (2011), i.e. 25-43% for acid curd cheeses and 100% for acid-rennet cheeses.

CONCLUSIONS

The yield and quality of tvarogs is integrally linked to the quality of the milk used to produce them. Higher tvarog yield was obtained using milk from Simmental cows in the summer, which can be linked to higher protein content in this milk. The dry matter of tvarogs produced from the milk of Holstein-Friesians contained significantly more fat and less protein. It should be noted that no significant differences were found in the degree of protein retention from the raw material to the products, while a higher retention index was obtained during the summer season. Higher content of minerals was noted in the milk obtained from the Simmental cows in the spring/summer and in the tvarogs produced from it. However, very low retention indices were found for these minerals. It should be emphasised that both the production season and the cultures used significantly determined the degree of retention. Higher retention indices were obtained in the spring/summer season using culture 1.

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