THE EFFECT OF RAW MILK QUALITY AND STARTER CULTURES ON THE YIELD AND NUTRITIONAL VALUE OF POLISH ACID CURD CHEESE (TVAROG)*

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Abstract

The aim of the study was to characterize variants of traditional acid curd cheese obtained in Poland in artisanal conditions in terms of their yield and nutritional value. The curd cheeses were made from raw milk obtained in various conditions: with and without the use of pasture forage (i.e. in traditional and intensive systems), in two seasons of production (spring/summer and autumn/winter), from two breeds raised in Poland (Polish Holstein-Friesian and Simmental), and on the basis of two types of starter culture (i.e. freeze-dried and traditional cultures). The basic chemical composition and content of selected fat-soluble vitamins, i.e. A, D₃ and E, were determined in samples of bulk milk and cheese. The cheese yield was calculated as the number of litres of milk used to produce 1 kg of curd cheese. The nutritional value of the experimental curd cheeses was determined by calculating the calorific value and the Index of Nutritional Quality (INQ). All analysed factors except for the bacterial cultures significantly affected the chemical composition of the cheese. Curd cheese made from the milk of Simmental cows had higher content of protein and fat-soluble vitamins (A, D₃ and E) than cheese from the milk of Holstein-Friesian cows. However, curd cheeses produced in a traditional system and/or in the spring and summer were a richer source of vitamins, mostly vitamin E. The high INQ values obtained indicate that curd cheese is a rich source of protein and vitamin A.

Key words: cow milk, cheese, cheese yield, lipophilic vitamins, INQ

Acid curd cheese, known in Poland as “white cheese” or “tvarog”, is highly popular among Polish consumers due to centuries-old tradition, dietary habits, and low cost (Gołabek, 2011; Król et al., 2018). Curd cheeses comprise a large and varied group of dairy products with an established position in the domestic market. About 20% of the milk supplied to purchasing centres is used for their production (Żylińska

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et al., 2014). Tvarog owes its high nutritional value primarily to protein content. This protein is characterized by very high digestibility of approx. 96–97%. Moreover, curd cheese has relatively low calorific value and enriches the human diet with easily digestible milk fat, vitamins, and minerals (Górska-Warsewicz et al., 2019; Siemianowski and Szpendowski, 2014). It is also a potential source of bioactive peptides, which exert a positive effect on human health (Barać et al., 2017). Owing to these features, curd cheeses can be used in the diet of healthy people and in various non-pathological states (e.g. pregnancy or sport), as well as in pathological states (e.g. trauma, infections, and the post-operative period) (Siemianowski and Szpendowski, 2014).

Curd cheeses are included among fresh cheeses, manufactured for immediate consumption or for further processing. They are a characteristic and attractive product not only in Poland but in other countries of Central and Eastern Europe as well. Different countries have specific traditional methods for producing these cheeses, as well as names that are often associated with a given region (Schultz-Collins and Senge, 2004). Depending on the method used to coagulate the milk proteins, we can distinguish acid cheese and cheese produced using both acid and enzymes. There are many cheese varieties where milk is coagulated by acid instead of the more common practice of using rennet. These include cottage cheese, quark, and cream cheese (Lucey, 2011). For the traditional curd cheese that is highly valued in Poland, acid coagulation is used. The production technology of acid curd cheese involves specific treatment of curd obtained as a result of acidification by lactic acid bacteria to the isoelectric point of casein ($\text{pH} = 4.6$), without the use of coagulating enzymes or other additives. The curd is cut, mixed, and heated, and then separated from the whey. The separated mass is moulded, pressed, cooled, and packaged.

In Poland, as in other EU countries, recent years have seen an increase in consumption of cheese, including tvarog. In 2019 consumption of this group of products in EU countries amounted to 19.9 kg per capita, and in Poland 10.68 kg (Institute of Agricultural and Food Economics, 2020). The average annual consumption of tvarog in Polish households is over 5 kg, amounting to 5.40 kg per capita in 2019. It is estimated that curd cheese accounts for about one fifth of an average household's expenditures for dairy products.

Cheese-making efficiency (cheese yield) is an important parameter indicating the degree to which the solids constituents of milk are recovered, which in turn affects the economics of production (Abd El-Gawad and Ahmed, 2011). Low protein content in milk significantly reduces cheese yield per unit of volume, which in consequence raises the production costs of dairy products. By reducing the amount of milk used to produce 1 kg of cheese by just 0.1 litre, 10 kg more cheese can be made from 100,000 litres of milk, which amounts to 3,000 kg of cheese a year (Chojnowski and Nowak, 2013).

The yield and nutritional value of curd cheese are mainly determined by the quality of the raw milk used and the production process, including the choice of starter cultures (Manuelian et al., 2017; Revilla et al., 2014). Therefore, the aim of the study was to characterize variants of traditional acid curd cheese (tvarog) obtained in artisanal conditions in terms of their yield and nutritional value. The curd cheeses were made from raw milk obtained in various conditions: with and without the use of

pasture forage (i.e. in traditional and intensive systems), in two seasons of production (i.e. in the spring/summer and autumn/winter seasons), from two breeds raised in Poland (Polish Holstein-Friesian and Simmental), and on the basis of two types of starter culture (freeze-dried and traditional cultures).

Material and methods

Research material

Characteristics of farms

Milk was obtained from two farms located in south-eastern Poland (Lublin region), on which both Holstein-Friesian and Simmental cows were raised. On one farm the cows were fed in a traditional system, while on the other they were fed intensively from a feed wagon (TMR – total mixed ration). The cows raised in the intensive system were kept in a free-stall cowshed, while the cows raised in the traditional, low-input system were housed in tie-stall barns. Feeding in the spring/summer season was based mainly on pasture grazing supplemented with hay and cereal meal, while in the autumn/winter the cows were fed grass silage supplemented with hay and cereal meal (Table 1).

Table 1. Characteristics of farming systems included in the research

Specification	Traditional farming	Intensive (TMR) farming
Number of farms	1	1
Breed of cows	Holstein-Friesian and Simmental	Holstein-Friesian and Simmental
Number of cows	15	100
Feeding of cows	<u>Spring/summer season:</u> pasture – <i>ad libitum</i> hay – 3 kg grain meal – 2 kg <u>Autumn/winter season:</u> haylage – 20 kg hay – 3 kg grain meal – 2–3 kg	<u>Spring/summer and autumn/winter seasons:</u> maize silage – 29 kg haylage – 6 kg grain meal – 2–3 kg soybean meal – 1 kg rapeseed meal – 1 kg salt licks – <i>ad libitum</i>
Milking system	bucket milking system	milking parlour
Animal housing	tie-stall	free-stall barns

All farms were subject to use value assessment for dairy cattle and met the requirements for milk production specified in Commission Regulation (EC) No. 1662/2006 of 6 November 2006 amending Regulation (EC) No. 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin.

Raw milk collection

A total of 16 bulk milk samples (250 mL) were collected for assessment of physicochemical properties and hygiene quality. At the same time, 16 bulk milk samples of 10 L each were collected for cheese production. Eight bulk milk samples were

collected on each farm (16 batches of milk in total), i.e. four in the spring/summer season and four in autumn/winter – two from each breed. Raw milk from each breed was collected and processed separately.

Acid curd cheese (tvarog) production

The acid curd cheeses (tvarog) were made in artisanal conditions by a traditional method according to the procedure presented in Table 2. Two variants of starter cultures were used: starter variant 1 – freeze-dried DVS starters, used to directly inoculate milk with lactic acid bacteria (Flora Danica by Chr. Hansen, Denmark), and starter variant 2 – a traditional working starter. The acid curd cheeses were made twice in each season, i.e. in the spring/summer and autumn/winter seasons. In total, 32 cheeses were produced using two starter cultures, i.e. 8 cheeses per breed, 8 cheeses per system and 8 cheeses per season.

Table 2. Technological parameters for manufacture of acid curd cheese (tvarog)

Procedure	
Pasteurization	80°C for 10 min
Cooling the milk	35°C
Adding the starter	Starter culture variant 1 or 2
Milk coagulation	
Temperature time	30–32°C, 12 h to pH of about 4.6
Curd cutting	4x4 cm cubes
Heating the mass	60°C, 10 min
Draining the curd	60 min
Moulding the curd	Filling the moulds, pressing for 2–2.5 h
Pressing	Force of 1–3 kG/kg; 24 h

Milk and tvarog analyses

Milk analysis

The proximate chemical composition (protein, fat, lactose, and solids) of the raw milk was determined with an Infrared Milk Analyser (Bentley Instruments, USA). Casein percentage was analysed according to AOAC (2000). To assess the hygienic quality of the raw milk, the total bacterial count at 30°C (PN-EN ISO 4833-2:2013) and somatic cell count (SCC) were determined with the Somacount 150 (Bentley Instruments, USA).

Acid curd cheese (tvarog) analysis

The cheese yield was calculated as the number of litres of milk used to produce 1 kg of curd cheese (Bohdziewicz, 2010). The protein content of cheese samples was analysed according to the Kjeldahl method, multiplying the nitrogen content obtained by a factor of 6.38 (PN-EN ISO 8968-1:2014). The fat content was assayed by the Weibull-Stoldt method, involving acid hydrolysis (37% HCl, w/w) of lipids bound to proteins and carbohydrates followed by hexane extraction. In addition, the active acidity (pH) was determined with a pH meter (Elmetron CP-401, Poland), and

the titratable acidity by the titration method (0.25 N NaOH with phenolphthalein as an indicator; the result was given in Soxhlet-Henkel degrees – °SH). For this purpose 5 g of cheese was weighed out and ground in a mortar with 50 mL distilled water with a temperature of 40°C. The moisture content of the cheese was determined using a drying method at 102°C according to the Polish standard (PN-A-86232:1973). The analyses were carried out in three replications.

The concentrations of selected fat-soluble vitamins, i.e. A, D₃, and E, in the milk and cheese were determined by reversed-phase high performance liquid chromatography (RP-HPLC) using a ProStar Varian liquid chromatograph equipped with a fluorescence detector (Brodziak et al., 2018). Samples were prepared by extracting fat according to the Röse-Gottlieb method as modified by Hewavitharana et al. (1996). Compounds were separated on a Pursuit XRs 3-C18 column (Varian, USA) with a length of 150 mm and a diameter of 4.6 mm. The mobile phase was a mixture of acetonitrile, methanol, water and dichloromethane (Sigma, Germany), and the flow rate was set at 1 ml/min. Reference substances were analysed under identical conditions. Standard solutions of vitamins were used for this purpose: α -tocopherol (vitamin E) with $\geq 97\%$ purity (HPLC), cholecalciferol (vitamin D₃) with $\geq 98\%$ purity (HPLC), and retinol (vitamin A) $\geq 99\%$ (Sigma, Germany). Qualitative identification of each substance was based on analysis of retention times read from individual chromatograms using Star 6.2 Chromatography Workstation software (Varian, USA). Quantitative analysis was performed by the external standard method. The analyses were carried out in three replications.

The percentage of the RDA (recommended dietary allowance) for vitamins contained in 100 g of curd cheese was determined for the Polish population according to nutrition standards developed by the Food and Nutrition Institute in Warsaw. Men and women were distinguished as population groups (NFNI, 2017).

The nutritional value of the experimental curd cheeses was determined by calculating the calorific value and the Index of Nutritional Quality (INQ) from the mean values for the chemical composition. The calorific value was calculated using net Atwater equivalents, according to which 1 g protein = 4.0 kcal; 1 g carbohydrates = 4.0 kcal; and 1 g fat = 9.0 kcal (Kruskall et al., 2003). INQ values were calculated for protein, fat, and vitamins in relation to the average recommended intake (Regulation (EC) No. 1169/2011 of the European Parliament and of the Council), separately for women and men according to the following equation:

$$INQ = \frac{\text{content of nutrient in 100 g of product} \times \text{energy requirement}}{\text{energy value of 100 g of product} \times \text{requirement for nutrients}}$$

Statistical analysis

The hypothesis was tested using non-parametric tests, i.e. the Mann-Whitney test and the Kruskal-Wallis test. The Shapiro-Wilks test was used to check for normality of distribution. The significance of differences between means for groups was determined at a level of p (α) = 0.05. The results are presented as means $\pm$ standard error (SE). StatSoft Inc. Statistica ver. 13.1 (Dell, 2016) was used for the calculations.

Results

Raw milk

The bulk milk used for curd cheese production was of high hygienic quality, meeting the requirements of Commission Regulation (EC) No. 1662/2006 of 6 November 2006. An important component of milk that determines its suitability for processing is its content of crude protein, including casein. Raw milk from the intensive system contained more protein ($P \leq 0.01$), including casein ($P \leq 0.05$), than milk from the traditional system (Table 3). Of the two production seasons, higher content of these components was obtained in the autumn/winter season, with significant ($P \leq 0.01$) differences obtained for total protein. The milk of Simmental cows was a richer source of total protein, including casein, than that of Holstein-Friesian cows ($P \leq 0.05$). Breed also had a significant influence ($P \leq 0.05$) on the protein-to-fat ratio. A higher ratio was obtained for the milk of Simmental cows (0.88) than that of Holstein-Friesians (0.80). All factors (breed, system, and season) statistically significantly ($P \leq 0.01$) influenced the content of solids, which was mainly determined by the content of fat and protein.

Significantly higher content of fat-soluble vitamins (A and D₃) was noted in the milk obtained from cows raised in the traditional system in the spring/summer season (Table 4). In the case of vitamin E, the breed of cow also influenced the concentration of this vitamin in the milk, with higher ($P \leq 0.01$) content noted in the milk of Simmental cows (1.725 mg/L) compared to Holstein-Friesians (1.349 mg/L).

Acid curd cheese (tvarog)

Yield

Analysis of the tvarog yield showed that it was significantly influenced by the experimental factors (breed, housing system, season, and starter cultures) – Table 5. In the intensive system, 22% less milk ($P \leq 0.01$) was used to produce 1 kg of cheese than in the traditional system (4.65 L vs 5.96 L per kg of cheese). Lower ($P \leq 0.01$) yield (by 17% on average) was noted in the autumn/winter than in the spring/summer. The cheese yield from milk obtained from Holstein-Friesian cows was 5.55 L/kg. Using the milk of Simmental cows reduced the amount of milk needed to produce 1 kg of cheese by about 10% on average ($P \leq 0.05$). Higher ($P \leq 0.01$) cheese yield was obtained when the traditional starter was used (variant 2). In this case, about 15% less milk was needed to produce 1 kg of cheese.

Acidity and chemical composition

A factor that significantly ($P \leq 0.01$) influenced the active acidity of the tvarog was the type of starter cultures used, with a higher pH value noted in the cheese made using the freeze-dried starter (culture variant 1). The active acidity of the cheese made using the second starter variant (traditional starter) was significantly lower, at 4.47. The starter cultures also influenced the potential acidity of the cheese ($P \leq 0.05$). Higher potential acidity was obtained in the cheese produced using the traditional starter (variant 2). The acidity of the cheeses was not significantly influenced by the production system, nor was it affected by the season or the breed of cow (Table 5).

Table 3. Proximate chemical composition of bulk milk in relation to the experimental factors

Parameter		Breed		System		Season	
		Holstein-Friesian	Simmental	traditional	intensive	autumn/ winter	spring/ summer
Number of samples	N	8	8	8	8	8	8
Fat (%)	$\bar{x}$	4.40 B	4.16 A	4.04 a	4.39 b	4.41 b	4.12 a
	SE	0.09	0.12	0.12	0.10	0.11	0.13
Protein (%)	$\bar{x}$	3.46 a	3.59 b	3.43 A	3.63 B	3.63 B	3.42 A
	SE	0.09	0.07	0.07	0.04	0.06	0.10
Protein-to-fat ratio	$\bar{x}$	0.80 a	0.88 b	0.86	0.82	0.83	0.84
	SE	0.02	0.02	0.02	0.03	0.02	0.03
Casein (%)	$\bar{x}$	2.67 a	2.82 b	2.74 a	2.87 b	2.82	2.73
	SE	0.06	0.07	0.05	0.04	0.05	0.04
Lactose (%)	$\bar{x}$	4.77	4.71	4.75	4.70	4.73	4.76
	SE	0.09	0.07	0.14	0.10	0.08	0.12
Solids (%)	$\bar{x}$	13.23 B	12.78 A	12.66 A	13.44 B	13.49 B	12.60 A
	SE	0.23	0.26	0.21	0.28	0.29	0.29

a, b, A, B – statistically significant differences within factor: a, b – at $P \leq 0.05$; A, B – at $P \leq 0.01$.

Table 4. Content of selected fat-soluble vitamins in the raw milk in relation to the experimental factors

Parameter		Breed		System		Season	
		Holstein-Friesian	Simmental	traditional	intensive	autumn/ winter	spring/ summer
Number of samples	N	8	8	8	8	8	8
Vitamin A	mg/L	0.361	0.397	0.417 B	0.314 A	0.324 A	0.462 B
	SE	0.039	0.036	0.033	0.017	0.021	0.032
µg/g fat	$\bar{x}$	8.93	9.57	10.54 B	7.60 A	7.40 A	11.00 B
	SE	0.60	0.59	0.80	0.64	0.68	0.96
Vitamin D ₃	µg/L	0.562	0.591	0.605 B	0.496 A	0.479 A	0.707 B
	SE	0.056	0.057	0.051	0.030	0.049	0.057
µg/g fat	$\bar{x}$	0.015	0.016	0.017 B	0.011 A	0.010 A	0.019 B
	SE	0.001	0.002	0.002	0.001	0.001	0.002
Vitamin E	mg/L	1.349 A	1.725 B	1.803 B	1.213 A	1.319 A	1.870 B
	SE	0.124	0.166	0.155	0.102	0.120	0.194
µg/g fat	$\bar{x}$	31.55 A	41.00 B	48.30 B	25.85 A	27.62 A	46.65 B
	SE	2.14	2.86	3.34	1.80	2.26	3.48

A, B – statistically significant differences within factor at $P \leq 0.01$.

Table 5. Yield, acidity and proximate chemical composition of curd cheese in relation to the experimental factors

Parameter	Breed		System		Season		Cultures*	
	Holstein-Friesian	Simmental	traditional	intensive	autumn/winter	spring/summer	1	2
Number of samples	N	16	16	16	16	16	16	16
Yield L/kg	$\bar{x}$ SE	5.55 b 0.21	5.04 a 0.13	5.96 B 0.11	4.65 A 0.18	4.82 A 0.11	5.80 B 0.20	5.65 B 0.22
Acidity	$\bar{x}$ SE	4.50 0.02	4.60 0.02	4.53 0.02	4.58 0.03	4.64 0.02	4.56 0.02	4.60 B 0.02
$^{\circ}\text{SH}$	$\bar{x}$ SE	74.29 1.57	72.32 1.20	74.23 0.86	73.50 1.95	72.82 0.95	74.75 1.76	72.48 a 1.68
Fat in dry matter (%)	$\bar{x}$ SE	43.29 B 0.60	38.59 A 0.96	39.20 A 0.64	42.84 B 1.19	41.91 b 0.86	39.53 a 0.88	40.58 0.92
Protein (%)	$\bar{x}$ SE	12.81 A 0.37	15.04 B 0.39	11.84 A 0.24	16.03 B 0.35	15.25 B 0.36	12.73 A 0.39	13.75 0.29
Dry matter (%)	$\bar{x}$ SE	29.93 b 0.61	26.82 a 0.36	26.32 A 0.42	30.35 B 0.74	29.86 b 0.36	26.91 a 0.68	27.40 0.59

*starter culture variant: 1 – freeze-dried starter; 2 – traditional starter
a, b, A, B – statistically significant differences within factor: a, b – at $P \leq 0.05$; A, B – at $P \leq 0.01$.

Table 6. Content of selected fat-soluble vitamins in the curd cheese in relation to the experimental factors

Parameter		Breed		System		Season		Cultures*	
		Holstein-Friesian	Simmental	traditional	intensive	autumn/winter	spring/summer	1	2
Number of samples	N	16	16	16	16	16	16	16	16
Vitamin A	mg/kg	1.247	1.345	1.380 b	1.219 a	1.141 a	1.450 b	1.280	1.306
	SE	0.027	0.032	0.013	0.029	0.023	0.023	0.024	0.020
µg/g fat	µg/g fat	9.65	12.25	12.94 a	9.12 a	8.80 a	13.15 b	11.50	10.86
	SE	0.34	0.80	0.85	0.61	0.70	1.02	0.94	1.04
Vitamin D ₃	µg/kg	1.585 a	1.746 b	1.882 b	1.451 a	1.369 a	1.966 b	1.659	1.673
	SE	0.026	0.040	0.026	0.010	0.026	0.043	0.038	0.032
µg/g fat	µg/g fat	0.010	0.015	0.017 B	0.010 A	0.010 A	0.016 B	0.015	0.013
	SE	0.001	0.002	0.003	0.001	0.001	0.002	0.001	0.002
Vitamin E	mg/kg	3.272 a	3.520 b	3.540 b	3.255 a	3.059 A	3.745 B	3.406	3.390
	SE	0.049	0.049	0.032	0.026	0.037	0.058	0.054	0.050
µg/g fat	µg/g fat	23.13 a	31.21 b	29.67 b	24.86 a	22.00 A	32.37 B	28.15	26.23
	SE	0.28	0.24	0.32	0.18	0.25	0.40	0.35	0.28

*starter culture variant 1 – freeze-dried starter; 2 – traditional starter.

a, b, A, B – statistically significant differences within factor: a, b – at $P \leq 0.05$; A, B – at $P \leq 0.01$.

Table 7. Degree to which a portion of experimental curd cheese covers the RDA (recommended dietary allowance) for fat-soluble vitamins according to standards for the Polish population

Recommended daily allowance for an adult		Degree to which 100 g of cheese covers the RDA (%)									
		breed		system		season		starter cultures*			
		Holstein-Friesian	Simmental	traditional	intensive	autumn/winter	spring/summer	1	2		
Women											
Vitamin A	0.5 mg	$\bar{x}$	25.00 a	26.85 b	28.97 B	22.88 A	25.49	26.56	26.83	24.75	
		SE	0.45	0.35	0.49	0.29	0.42	0.34	0.33	0.28	
Vitamin D ₃	15 µg	$\bar{x}$	1.06	1.18	1.24 b	0.97 a	1.05	1.17	1.10	1.12	
		SE	0.03	0.04	0.06	0.02	0.03	0.03	0.04	0.03	
Vitamin E	8 mg	$\bar{x}$	4.09	4.41	4.70 B	3.81 A	4.07 a	4.42 b	4.25	4.24	
		SE	0.56	0.72	0.62	0.49	0.54	0.83	0.62	0.70	
Men											
Vitamin A	0.63 mg	$\bar{x}$	19.84 a	21.31 b	23.03 B	18.15 A	20.07	21.07	20.92	19.89	
		SE	0.21	0.28	0.24	0.14	0.31	0.36	0.38	0.30	
Vitamin D ₃	15 µg	$\bar{x}$	1.06	1.18	1.24 b	0.97 a	1.05	1.17	1.10	1.12	
		SE	0.03	0.04	0.06	0.02	0.03	0.03	0.04	0.03	
Vitamin E	10 mg	$\bar{x}$	3.24	3.55	3.76 b	3.01 a	3.25 a	3.54 b	3.40	3.38	
		SE	0.17	0.22	0.19	0.07	0.12	0.20	0.10	0.14	

*starter culture variant 1 – freeze-dried starter; 2 – traditional starter.

a, b, A, B – statistically significant differences within factor: a, b – at $P \leq 0.05$; A, B – at $P \leq 0.01$.

Table 8. Calorific value and index of nutritional quality of curd cheese in relation to the experimental factors

Parameter	Breed		System		Season		Cultures*	
	Holstein-Friesian	Simmental	traditional	intensive	autumn/winter	spring/summer	1	2
Calorific value of curd cheese (kcal/100 g)	$\bar{x}$ 163.4	149.5	137.2 a	176.1 b	175.2 b	138.5 a	147.4	165.5
	SE 7.89	6.21	3.18	6.24	8.43	6.17	7.42	7.70
INQ for protein	$\bar{x}$ 2.89 a	3.61 b	3.15	3.37	3.25	3.27	3.24	3.28
	SE 0.09	0.10	0.08	0.09	0.07	0.07	0.11	0.12
INQ for fat	$\bar{x}$ 2.12 a	2.75 b	2.38	2.56	2.43	2.45	2.45	2.46
	SE 0.03	0.02	0.01	0.04	0.02	0.02	0.03	0.02
	$\bar{x}$ 1.96	1.71	1.94	1.79	1.83	1.91	1.89	1.84
	SE 0.05	0.06	0.07	0.05	0.04	0.07	0.06	0.07
	$\bar{x}$ 1.31	1.18	1.26	1.23	1.23	1.27	1.25	1.24
	SE 0.07	0.08	0.05	0.06	0.06	0.07	0.07	0.08
INQ for vitamin A	$\bar{x}$ 3.16	3.56	4.18 B	2.61 A	2.93 A	3.78 B	3.58	3.05
	SE 0.08	0.11	0.06	0.06	0.09	0.09	0.10	0.11
	$\bar{x}$ 2.44	2.84	3.29 b	2.02 a	2.32 a	2.99 b	2.74	2.48
	SE 0.09	0.12	0.05	0.04	0.06	0.07	0.05	0.07
INQ for vitamin D3	$\bar{x}$ 0.13	0.15	0.17	0.11	0.13	0.16	0.15	0.13
	SE 0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
INQ for vitamin E	$\bar{x}$ 0.49	0.60	0.66 b	0.44 a	0.48	0.63	0.57	0.54
	SE 0.06	0.07	0.05	0.03	0.05	0.07	0.06	0.07
	$\bar{x}$ 0.41	0.47	0.55 b	0.35 a	0.39	0.50	0.46	0.43
	SE 0.03	0.05	0.06	0.02	0.04	0.07	0.03	0.04

INQ – Index of Nutritional Quality.

*starter culture variant 1 – freeze-dried starter; 2 – traditional starter.

a, b, A, B – statistically significant differences within factor: a, b – at $P \leq 0.05$; A, B – at $P \leq 0.01$.

The content of dry matter constituents of the tvarog was directly linked to the quality of the milk used. The cheese made from the milk of Holstein-Friesian cows, irrespective of the production system and season, had significantly higher dry matter content (and thus lower water content) as well as higher fat content in the dry matter ($P \leq 0.05$). Comparison of farming systems showed differences at $P \leq 0.01$ in favour of the intensive system, while the differences between seasons were significant at $P \leq 0.05$, with higher dry matter content in cheese produced from milk obtained in the autumn/winter (Table 5). The milk of Simmental cows and the cheese made from it were richer sources of protein ($P \leq 0.01$).

Content of and requirements for fat-soluble vitamins

The content of fat-soluble vitamins in the curd cheese depended on the quality of the raw milk. The system and season of production and the breed of cow from which the milk was obtained significantly affected the content of vitamins in the tvarog (Table 6). Higher content of these vitamins (by over 30%) was noted in the cheese produced from the milk of cows kept in the traditional system. Comparison of production seasons showed a higher concentration of vitamins in the cheese produced from spring/summer milk. This can be linked to their higher content in the raw milk, which is associated with pasture grazing. Tvarog made from milk obtained in the pasture season contained about 40% more vitamin D₃, 27% more vitamin A, and 22% more vitamin E, as compared to the autumn/winter. Curd cheese made from the milk of Simmental cows had significantly higher content of vitamins D₃ and E than cheese from the milk of Holstein-Friesians.

The starter cultures used were not found to significantly affect the concentrations of vitamins in the experimental curd cheese. It should be noted that the content of fat-soluble vitamins in the cheese was several times as high as in the raw milk. The greatest differences were noted for vitamin A, whose concentration in the cheese was five times that found in the milk. In addition, the content of vitamins D₃ and E was three times as high in the cheese as in the milk (Table 6).

Table 7 presents the degree to which consumption of 100 g of experimental curd cheese covers the adult requirements for vitamins A, D₃ and E (separately for men and women). The Simmental breed, the traditional system, and the spring/summer were clearly the most favourable in this respect. Among the vitamins tested, consumption of 100 g of cheese was found to provide the highest percentage of the recommended intake of vitamin A. Curd cheese made from the milk of cows kept in a traditional system was shown to satisfy 28.97% of the vitamin A requirement for women and 23.03% of the requirement for men. Consumption of the same portion of cheese produced from the milk of cows kept in an intensive system provides 5–6% less of the required intake. The amount of curd cheese needed to meet the requirements for vitamins D₃ and E is also smaller in the case of cheese produced from the milk of cows kept in a traditional system ($P \leq 0.05$). It should be noted, however, that curd cheese is not a rich source of vitamins D₃ and E. A 100 g portion of curd cheese made from the milk of cows raised in a traditional manner covered 1.24% of the requirement for vitamin D₃, and in the case of vitamin E, 4.70% for women and 3.76% for men. Comparison of cheese produced from the milk of cows of different

breeds showed that consumption of cheese made from the milk of Simmental cows covered the recommended intake of fat-soluble vitamins to a greater extent. The difference was significant in the case of vitamin A. No significant differences were found for the remaining vitamins. The requirement for fat-soluble vitamins was also found to be covered to a greater degree by consumption of curd cheese made from milk obtained in the spring/summer. Statistically significant differences in favour of curd cheese from that season were found for vitamins D₃ and E ($P \leq 0.05$). The starter cultures did not affect the degree to which the experimental curd cheeses covered the recommended intake of fat-soluble vitamins (Table 7).

Calorific value and indices of nutritional quality

Table 8 presents the calorific value of the experimental curd cheese and the index of nutritional quality determined for protein, fat, and fat-soluble vitamins by gender. The calorific value of the curd cheese ranged from 137.2 to 176.1 kcal/100 g of product. Higher ($P \leq 0.05$) calorific value (by 27%) was found for the tvarog made from the milk of cows kept in the intensive system as compared to the traditional system. Evaluation of cheese produced from milk obtained in different seasons revealed that the cheeses from the autumn/winter season had higher (by 20%; $P \leq 0.05$) energy value than the cheese from the spring/summer (138.5 kcal/100 g). In the case of the breed of cow, a higher calorific value was noted in the cheese made from the milk of Holstein-Friesian cows, but the differences were not statistically significant. The starter cultures were also not found to significantly affect the calorific value of the curd cheese.

The INQ for protein in the cheeses ranged from 2.89 to 3.61 for women and from 2.12 to 2.75 for men. An especially high INQ value was shown for the cheese produced from the milk of Simmental cows, and the difference between breeds was confirmed statistically. Cheese made from the milk of Holstein-Friesian cows had a 27% lower index of nutritional quality for protein. The other factors (housing system, season, and starter cultures) were not found to significantly affect the INQ for protein in the experimental curd cheeses. There were also no statistically significant differences in the fat INQ of the cheeses, which averaged 1.71–1.96 for women and 1.18–1.31 for men, indicating a good balance of this nutrient in relation to energy. The INQs calculated for vitamin A indicate that the curd cheeses are a very good source of this vitamin. They were 2.61–4.18 for women and 2.02–3.29 for men. Tvarog produced from the milk of Simmental cows had a significantly higher INQ. It was 3.56 for women and 2.84 for men, and was 17% and 16% higher than the INQ for cheese made from the milk of Holstein-Friesians. Curd cheese made from the milk of cows kept in a traditional system was a much better source of vitamin A, with an INQ of 4.18 for women and 3.29 for men, compared to cheese produced from cows raised in the intensive system, for which the INQ was 60% lower. A significantly higher INQ was also found for the tvarog produced from milk acquired in the spring/summer – 3.78 for women and 2.99 for men, which was 27% higher than for cheese from the autumn/winter. In the case of vitamin D₃, the INQ was far below 1, which indicates inadequate content of this vitamin in the curd cheese. All factors analysed were not found to affect the INQ for vitamin D₃. The INQ for vitamin E was

also below 1. Significantly higher INQ values were found only for curd cheese made from the milk of cows kept in a traditional system – 0.66 and 0.55 for women and men, respectively, compared to the intensive system: 0.44 and 0.35. There were no statistically significant differences in the INQ for vitamins depending on the starter cultures.

Discussion

Raw milk

One of the major determinants of the value of dairy products is the quality of the raw milk used. Only raw milk of adequate hygienic quality and an appropriate chemical composition will result in a palatable product with optimal nutritional value and a long shelf-life, thus fully meeting consumer expectations. The results of the analyses indicated that the raw milk was of high hygienic quality, in compliance with legal requirements (Regulation (EC) No. 1662/2006 of 6 November 2006) regarding total bacterial count (100,000 cfu/mL) and SCC (400,000 cells/mL). The milk of Simmental cows, due to its high content of protein, including casein, and its higher protein-to-fat ratio, is more suitable for processing, which has also been reported by other authors (Brodziak et al., 2018; Król et al., 2008; Teter et al., 2019).

Curd cheese yield

According to many authors (Barłowska et al., 2014; Król et al., 2018; Żywica et al., 2008; Verdier-Metz et al., 2001), cheese yield is primarily determined by the content of protein, especially the casein fraction. All of these authors reported a relationship between the content of these milk constituents and cheese yield. In the present study, higher cheese yield was obtained from milk with higher protein content, higher casein content, and a higher protein-to-fat ratio. Król et al. (2018) found that the use of homofermentative cultures instead of heterofermentative cultures reduced the amount of milk needed to produce 1 kg of curd cheese by 6%. In the present study as well, less raw milk (by 12%) was needed when the traditional starter (homofermentative culture) was used. As in the study by Król et al. (2018), less milk was used to produce 1 kg of cheese in the case of Simmental cows compared to the Holstein-Friesian breed. As in the present study, Żywica et al. (2008) showed that the reduction in protein content in the spring/summer relative to autumn/winter resulted in an increase in the amount of raw milk needed to produce 1 kg of curd cheese.

Acidity and chemical composition of cheese

One of the most important quality parameters of curd cheese is its acidity. Active acidity of curd cheese ranging from 4.4 to 4.6 indicates that the milk acidification process proceeded correctly, resulting in proper coagulation and a suitably firm curd (Godula et al., 2018; Mulawka et al., 2019). In the present study, the acidity was within this range in all of the curd cheeses tested. Dmytrów (2015), in a study on curd cheese from a retail chain, reported a pH of 4.5–4.6. However, the potential acidity was fairly low, at 31.0–52.5 °SH. The author explained the low level of titrat-

able acidity by important factors leading to changes in the acidity of the curd cheeses – temperature and storage time. Godula et al. (2018) showed lower pH (3.98–4.17), which they explain by the higher content of protein and mineral salts in the raw milk, which increases its buffer capacity and thus slows down the increase in the active acidity of the curd cheeses. Mulawka et al. (2019), using various types of starter cultures in curd cheeses, showed that the starter cultures significantly affected the pH of acid cheese. The active acidity of the curd cheeses ranged from 4.11 to 4.45 and the potential acidity from 54 to 63 °SH (higher values for the MSO-11 starter). According to the authors, the differences in the acidity of the curd cheese following the use of different starter cultures can be explained by differences in the metabolic activity of the bacteria contained in them (Mulawka et al., 2019). This theory is also reflected in the results of the present study. Jasińska et al. (2010) investigated the effect of the cow feeding system (traditional vs TMR) on the properties of curd cheese. No direct relationship was found between the feeding system and the acidity of the curd cheese. This is consistent with the results of the present study. As in a study by Siemianowski et al. (2013), the titratable acidity of the curd cheeses in the present study was found to increase with the content of dry matter in the cheese. Jasińska et al. (2010) did not confirm these tendencies in their study. The authors found more water and less fat in curd cheese made from milk obtained in the autumn/winter. The results of the present study are in agreement with those reported by Król et al. (2018). The authors showed significantly higher ($P \leq 0.01$) content of dry matter, fat, and protein in curd cheese from the winter compared to the summer. The starter cultures used were not found to affect the basic chemical composition of the curd cheese. Other authors have also found that the starter used did not affect the proportions of fat and water in curd cheese (Mulawka et al., 2019; Dmytrów et al., 2007).

Content of fat-soluble vitamins in the raw milk and experimental curd cheeses

According to many authors (Brodziak et al., 2018; Butler et al., 2008; Gabryszuk et al., 2013; Kuczyńska et al., 2011), the content of fat-soluble vitamins in milk is significantly influenced by the system in which the cows are kept. Milk obtained from low-input, traditional farms has higher levels of these vitamins. Many authors (Nozière et al., 2006; Shingfield et al., 2005) report that fresh pasture sward has higher levels of vitamin E and provitamin A than preserved feedstuffs, and thus pasture feeding has a beneficial effect on their content in milk. Due to their exposure to UV radiation, grazing cows also produce milk with increased content of vitamin D₃ (Butler et al., 2008; Gabryszuk et al., 2013; Kuczyńska et al., 2011). The present study also showed significant seasonal differences in favour of the spring and summer. Radkowska (2013) reported similar content of vitamins A and E in the milk of Holstein-Friesian cows raised in two groups, i.e. exclusively in a cowshed and with access to a cattle run – 0.329 µg/mL and 0.334 µg/mL, respectively, for vitamin A and 0.970 µg/mL and 0.974 µg/mL for vitamin E. However, the author observed that cows that grazed in the pasture produced milk with significantly higher content of these vitamins: A – 0.458 µg/mL and E – 1185 µg/mL. The increase in the content of these fat-soluble vitamins in the milk of cows whose diet included pasture forage is

supported by other studies as well (Brodziak et al., 2018; Kalač, 2011; Kuczyńska et al., 2011; Strusińska et al., 2010).

The literature lacks studies on the content of fat-soluble vitamins in curd cheese, and the few available studies mainly concern ripened cheese. Lucas et al. (2006), in an analysis of five French varieties of ripened farmhouse cheese, showed that the milk composition affected ($P < 0.001$) the composition of the cheese, with a significant linear correlation ($P < 0.001$) between the content of fat-soluble vitamins in the cheese and in the raw milk. It is worth noting that Rocamadour cheese, produced by acid coagulation, had higher content of vitamins A and E than the other cheeses. As in the present study, Revilla et al. (2014) reported that the season of milk production significantly influenced the concentration of vitamin A in both the raw milk and the ripened cheese produced from it. The vitamin A content of the cheese was $212.79 \mu\text{g}/100 \text{ g fat}$ in the summer and $200.70 \mu\text{g}/100 \text{ g fat}$ in the winter. The season was not shown to affect the content of vitamin E. The authors observed a decrease in the concentrations of both vitamins in cheese during ripening. They suggest that seasonal differences in retinol content may be explained by different concentrations of the retinol precursor in the animals' diet and/or their bioavailability.

Calorific value and indices of nutritional quality of the experimental cheeses

Determination of the nutrient density of products is a good use of information on nutritional value in assessing the relationship between the diet and the human body. It takes into account not only the absolute nutritional value of the product, but energy requirements as well (Laskowski and Górska-Warsewicz, 2014). A good measure of the nutrient density of food is INQ. An INQ close to 1 describes food products that are well balanced in terms of the content of a given nutrient relative to its energy value. An $\text{INQ} < 1$ indicates that the product does not supply an adequate amount of the nutrient in relation to the energy supplied, while an $\text{INQ} \geq 1$ means that the product is a rich source of a given nutrient and can be used to supplement the composition of products lacking in this nutrient (Laskowski and Górska-Warsewicz, 2014; Siemiąnowski et al., 2015). The calorific value of the experimental curd cheese was similar to that of high-fat curd cheese, which according to Kunachowicz et al. (2019) is $177 \text{ kcal}/100 \text{ g}$. The INQ values indicate that curd cheese is a relatively low-calorie, rich source of protein and vitamin A. Similar conclusions have been reported by Siemiąnowski et al. (2013).

Conclusions

In conclusion, the factors analysed, i.e. breed, system and season of milk production, and starter cultures, significantly affected the yield of the experimental curd cheeses. Higher cheese yield was obtained from milk with higher protein content, including casein content, and a higher protein-to-fat ratio. When the traditional starter was used, about 15–25% less milk was needed to produce 1 kg of cheese. The content of dry matter components of the tvarog, including fat-soluble vitamins, was directly linked to the quality of the raw milk used. The starter cultures, on the other hand, were not found to affect the level of fat-soluble vitamins in the cheese. The curd cheeses (irrespective of the cow breed) were found to be a good source of

protein and vitamin A in the diet. However, curd cheese made from the milk of Simmental cows had higher content of protein and vitamin A (based on the INQ value) than cheese from the milk of Holstein-Friesian cows. Generally, curd cheeses produced in a traditional system and/or in the spring and summer were a richer source of vitamins, mostly vitamin A.

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