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Chemical composition and microbiological quality of market milk supplied to five dairies in the Lublin region, taking into account the season of purchase

Agnieszka Jarosińska, Joanna Barłowska, Anna Wolanciuk, Robert Pastuszka, Katarzyna Barłowska

University of Life Sciences in Lublin,
Department of Commodity Science and Processing of Raw Animal Materials,
ul. Akademicka 13, 20-950 Lublin

The study included 233 market milk samples (115 in the summer season and 108 in the winter season) collected at five dairies (A, B, C, D and E) in the Lublin region, directly from tank lorries, during the course of one year. The following milk parameters were determined: content of fat, protein, lactose and dry matter, total number of bacteria, total number of psychrotrophic bacteria, and coli titre. The raw material purchased in the winter months had a significantly (α=0.01) more favourable chemical composition. The market milk complied with the current requirements of the European Union in terms of microbiological quality, as the total number of bacteria was on average 73,870/ml, including 46,900/ml psychrotrophic bacteria. The raw material supplied to dairies in the winter was of slightly lower microbiological quality in terms of the total number of bacteria (77,890/ml vs. 69,600/ml). More psychrotrophic bacteria (50,320/ml vs. 43,290/ml) were found in the milk samples collected in the summer months. The coli titre in the raw material ranged from 0 to 10^{-6}. A higher percentage of samples with no *Escherichia coli* contamination (40.9%) was obtained in the summer, while at the same time a higher percentage of samples (5.2%) with a higher level of contamination (10^{-6}) was also noted in this period (compared to 32.4% and 2.8%, respectively, in the winter).

KEY WORDS: market milk / production season (season of purchase) / chemical composition / microbiological quality

Milk obtained from a healthy mammary gland, even in sterile conditions, always contains small quantities of microorganisms. According to Ziarno and Czapska [18], in the case of a healthy cow and hygienic milking, the milk is contaminated with only a small number of saprophytic microbes, up to a few thousand cells per ml. Failure to comply with hygiene standards during milking or improper storage of the raw milk can result in an increase in the number of bacteria, from several hundred thousand to even tens of millions per ml.

The microflora in milk is highly diverse, not only in abundance but in terms of the species present as well. The normal microflora of milk consists of lactic acid bacteria (Gram-positive, non-sporing bacteria). They cause acidification of the milk and also influence the organoleptic characteristics of dairy products [13]. EU regulations lay down general microbiological requirements for raw milk, but do not specify the qualitative composition of its microflora [16].

A significant proportion of the total microbial content of raw milk consists of psychrotrophic bacteria that are capable of rapid reproduction at temperatures of 7°C and lower, irrespective of their optimum growth temperature. They are not part of the natural microflora of the mammary gland of cows and their presence in raw milk is solely due to its contamination during or after milking [17]. They enter the milk not only from the surface of the teats, air or water, but mainly from dirty milking equipment and manure [8, 19]. They include both Gram-negative and Gram-positive bacteria [9]. These bacteria, mainly of the genus *Pseudomonas*, produce lipolytic and proteolytic enzymes that contribute to adverse physicochemical and organoleptic changes in the milk. Proteolytic enzymes cause protein degradation, leading to destabilization of casein resulting in changes in milk texture (thickening or gelation), and their further degradation contributes to the occurrence of flavour and odour defects (a putrid, bitter aftertaste and odour). Lipolytic enzymes hydrolyse milk fat to free fatty acids, causing changes manifested as a rancid, soapy and tallowy taste and odour in the milk [6, 8, 9, 15, 19]. They are present in small numbers in milk of good quality (about 10% of the total number of microbes), but constitute over 75% of the microflora in milk produced in unhygienic conditions [10]. Their number increases with the storage time of raw milk in refrigerated conditions [18].

Milk may also contain coliform bacteria, which are an indicator of whether proper hygiene is maintained during milking. The source of contamination is dirty mammary glands, bedding or milking equipment. These bacteria alter the organoleptic characteristics of the milk and cause gas blowing in cheese [19].

The aim of the study was to evaluate the chemical composition and microbiological quality of commercial milk delivered to five dairies in the Lublin region, taking into account the season of purchase.

Material and methods

The material for the study consisted of 233 samples of commercial milk collected in the same year directly from the cisterns at five dairy plants (A, B, C, D and E) located in the Lublin region. The milk samples for analysis were taken, when possible, from the same suppliers in two seasons: summer (115 samples) and winter (108 samples). The milk was collected into sterile 40 ml plastic containers and then transported in thermal bags with freezer packs to the laboratory of the Department of Commodity Science and Processing of Raw Animal Materials, University of Life Sciences in Lublin.

The following were determined in the milk:

– proximate chemical composition, i.e. fat, protein, lactose and dry matter content, with an Infrared Milk Analyzer (Bentley Instruments)

– total microbial count, according to PN-EN ISO 4833:1998 'General principles for determining the number of microorganisms. Plate method at 30°C' (simplified method)

– total number of psychrotrophic organisms by the plate method, according to PN-ISO-17410:2004 'Microbiology of food and feed. Horizontal method for determining the number of psychrotrophic microorganisms'

– coliform bacteria titre by the fermentation method, according to PN ISO 4831:2007 'Microbiology of food and feed. Horizontal method for detection and determination of coliform bacteria. Most probable number method.'

The analysis of the results took into account the season of purchase and the dairy plant.

The statistical analysis of the results was performed using StatSoft Inc. STATISTICA software ver. 6, by on one- and two-way analysis of variance with interaction. The significance of the differences between means for the groups was determined by the LSD test, at $\alpha = 0.05$ and $\alpha = 0.01$.

Results and discussion

The raw milk purchased in the winter had a significantly ($\alpha = 0.01$) more favourable chemical composition (Table 1), as it contained more protein, by 0.16 percentage points (pp), lactose, by 0.29 pp, and dry matter, by 0.98 pp. Fat content was at similar levels. Separate analysis of the content of these constituents for each dairy revealed substantial differences in the raw milk purchased, while no clear trend was observed for the influence of the season on these parameters (Table 1, Figures 1 and 2). Most authors [2, 3, 4, 14] confirm that milk obtained in the autumn and winter has a more favourable chemical composition. Gardzina-Mytar et al. [7] state that diet is a significant factor influencing milk yield in cows and the chemical composition of milk, especially fat, and to a lesser extent protein. In eastern Poland, despite progressive changes in the structure of dairy farming (an increase in the number of farms with intensive milk production involving modern feeding systems – TMR and PMR), there are still many small dairy farmers using traditional feeding systems, which contributes significantly to seasonal changes in the proximate composition of milk.

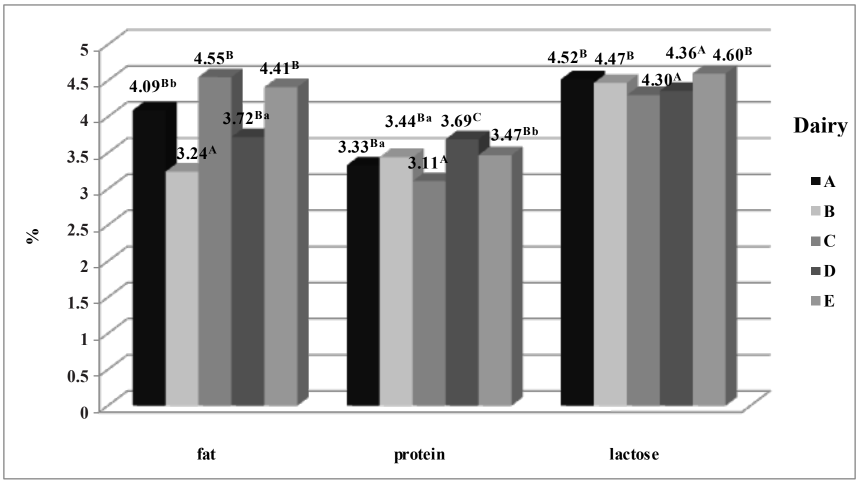
The commercial milk was found to meet current EU requirements in terms of microbiological quality, as the total bacterial count (TBC) was on average 73,870/ml, including 46,900/ml psychrotrophic bacteria (Tab. 1). The raw milk delivered to the plants in the winter, however, was of slightly worse microbiological quality in terms of TBC (77,890/ml vs. 69,600/ml). On the other hand, milk samples taken during the summer months had a higher content of psychrotrophic bacteria (50,320/ml vs 43,290/ml). However, these differences were not statistically confirmed. Similar results were obtained by Kukuła [12], who reported a higher number of psychrotrophic bacteria at warmer times of the year. This is explained by the favourable conditions for the proliferation of microorganisms at higher temperatures. The authors showed that where the total bacterial count in the milk was 10^6 CFU/ml, the share of psychrotrophic bacteria remained lower by 1 to 2 orders of magnitude. Kobus and Kmiecik [11] found no significant diffe-

Table 1

Chemical composition and hygienic quality of market milk with respect to the recipient (dairy) and season of purchase

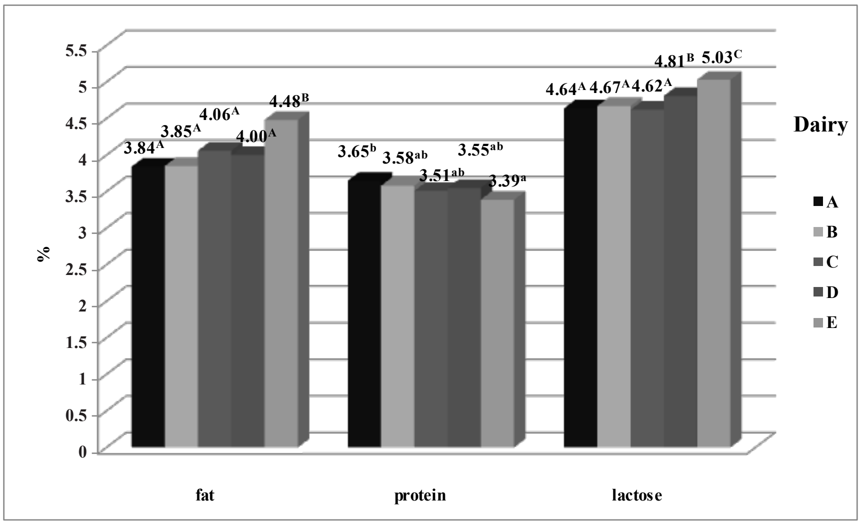
Specification		Recipient (dairy) A		B		C		D		E		Total in the season		Average	Influence of the factor	
Season of production (purchase)		summer	winter	summer	winter	summer	winter	summer	winter	summer	winter	summer	winter		dairy	interaction of dairy x season of production
Number of samples		19	16	20	21	37	37	20	18	19	16	115	108	223		
Fat (%)	$\bar{x}$	4.09	3.84	3.24^{A}	3.85^{B}	4.55^{B}	4.06^{A}	3.72^{A}	4.00^{B}	4.41	4.48	4.08	4.04	4.06	xx	xx
	SD	0.69	0.46	0.46	0.14	0.34	0.77	0.20	0.33	0.54	0.15	0.67	0.54	0.61		
Protein (%)	$\bar{x}$	3.33^{A}	3.65^{B}	3.44^{A}	3.58^{B}	3.11^{A}	3.51^{B}	3.69^{B}	3.55^{A}	3.47	3.39	3.37^{A}	3.53^{B}	3.45	xx	xx
	SD	0.29	0.29	0.19	0.10	0.14	0.50	0.13	0.19	0.33	0.13	0.30	0.33	0.33		
Lactose (%)	$\bar{x}$	4.52	4.64	4.47^{A}	4.67^{B}	4.30^{A}	4.62^{B}	4.36^{A}	4.81^{B}	4.60^{A}	5.03^{B}	4.43^{A}	4.72^{B}	4.57	xx	xx
	SD	0.19	0.39	0.10	0.08	0.34	0.29	0.14	0.09	0.21	0.09	0.26	0.27	0.30		
Dry matter (%)	$\bar{x}$	12.20^{A}	12.91^{B}	11.44^{A}	12.87^{B}	11.85^{A}	12.97^{B}	12.51^{A}	13.23^{B}	12.82^{A}	13.69^{B}	12.11^{A}	13.09^{B}	12.59	xx	ns
	SD	0.38	0.55	0.74	0.32	0.56	1.14	0.20	0.59	0.14	0.39	0.66	0.81	0.88		
Total number of bacteria (thousand/ml)	$\bar{x}$	85.32	97.81	67.70	84.57	95.50	92.57	50.35	56.44	27.11	32.88	69.60	77,89	73,87	xx	ns
	SD	60.73	116.39	46.65	76.34	54,62	72.29	56.55	43.39	18.17	33.61	55.61	109,14	89,20		
Total number of psychrotrophic bacteria (thousand/ml)	$\bar{x}$	74.79	70.63	50.95	51.05	75.92	50.87	21.20	12.72	7.37^{a}	22.63^{b}	50.32	43,29	46,90	xx	ns
	SD	63.54	51.93	46.14	66.28	62.17	59.91	31.61	27.44	5.41	29.76	56.04	54,91	55,48		

A, B – differences between seasons within a dairy significant at α=0.01; a, b – differences significant at α=0.05; influence of the factor: xx – at α=0.01, x – at α=0.05, ns – not significant



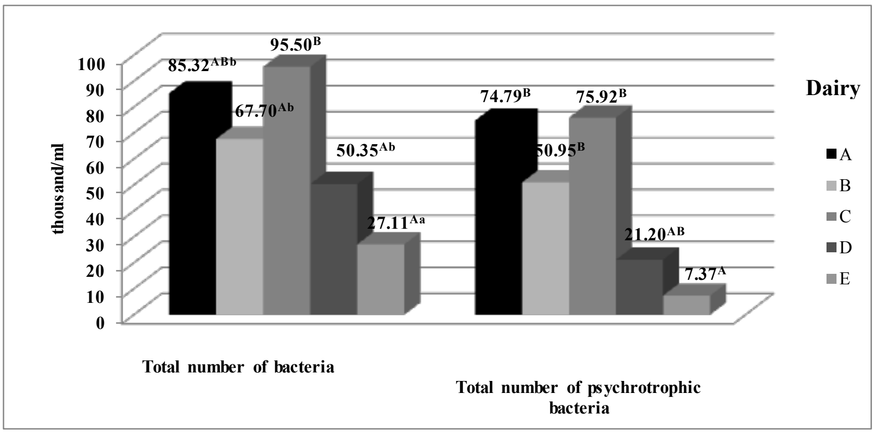
A, B, C – differences between daires significant at α=0.01; a, b – differences significant at α=0.05

Fig. 1. Content of fat, protein and lactose in market milk supplied to five dairies in the Lublin region during the summer season



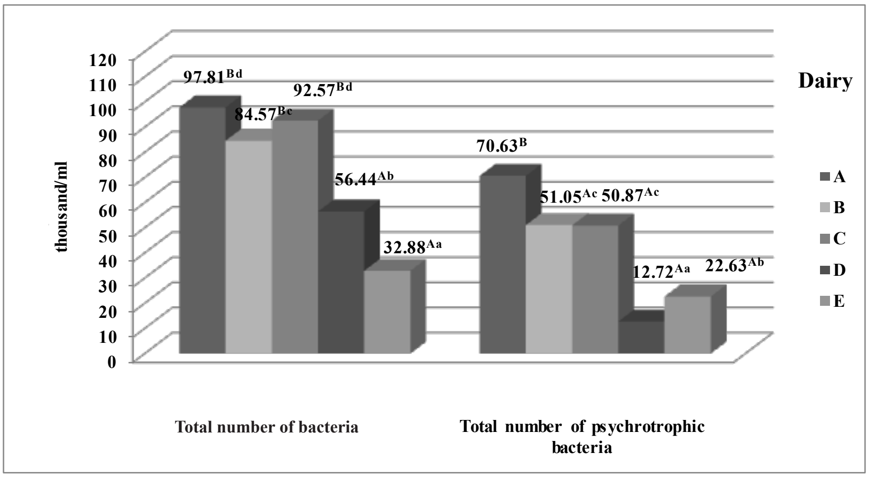
A, B, C – differences between daires significant at α=0.01; a, b – differences significant at α=0.05

Fig. 2. Content of fat, protein and lactose in market milk supplied to five dairies in the Lublin region during the winter season



A, B, C – differences between daires significant at α=0.01; a, b – differences significant at α=0.05

Fig. 3. Microbiological quality of milk supplied to five dairies in the Lublin region during the summer season



A, B – differences between daires significant at α= 0.01; a, b, c, d – differences significant at α=0.05

Fig. 4. Microbiological quality of milk supplied to five dairies in the Lublin region during the winter season

rences in the microbiological quality of commercial milk assessed in different months of the year. Cempirkova [5] reports a high correlation between the total microbial count in raw milk and the number of psychrotrophic bacteria ($r = 0.69$; $p \leq 0.01$).

Considerable differences were noted in the hygienic quality of the milk delivered to different dairies (Table 1, Figures 3 and 4). The lowest total bacterial count, including psychrotrophic bacteria, was found in the milk samples collected from cisterns supplying milk to dairy E in the summer – 27,110/ml and 7,370/ml, respectively. The worst in this respect was the raw milk purchased by dairy A (TBC of 97,810/ml in the samples collected in the winter and 85,320/ml in the summer) and dairy C (95,500/ml in the summer and 92,570 in the winter).

A measure of the purity of raw material (in this case milk) is the degree of its contamination with *Escherichia coli*, a faecal-borne bacterium. According to Ali and Abdelgadir [1], *E. coli* most often enters the milk from infected udders (clinical and subclinical mastitis). Other sources of contamination may also be the animal's skin, the milker, or contaminated water used to wash the milking equipment [13]. The data in Table 2 show that the range of this indicator in the raw milk tested was 0 to 10^{-6}. The milk delivered to dairies D and E was shown to be of relatively high quality in this regard (57.9% and 48.5% of samples free of *E. coli*). In the samples of milk delivered to these dairies, there was no *E. coli* titre as high as 10^{-6}, and in the case of plant E, none was as high as 10^{-5}. The highest degree of contamination with *Escherichia coli* was noted in the raw milk purchased by dairy A, as only 22.9% of the samples were free of this bacterium, while a titre of 10^{-4} was noted in as many as 40.0%, 10^{-5} in 25.7% and 10^{-6} in 5.7%. Analysis of the effect of the season (Table 2) revealed that the percentage of samples that were not contaminated with *E. coli* (40.9%) was higher in the summer, but the proportion of samples with the highest level of contamination, i.e. 10^{-6} (5.2%), was higher as well (in winter, 32.4% and 2.8%, respectively).

The two-way analysis of variance showed that the place of delivery, i.e. the dairy, had a significant ($\alpha = 0.01$) effect on the proximate chemical composition of the milk and the total number of aerobic mesophilic and psychrophilic bacteria. The season of purchase significantly influenced ($\alpha = 0.01$) the content of protein, lactose and dry matter. Furthermore, significant ($\alpha = 0.01$) interactions were noted for the dairies and season of purchase in the case of fat, protein and lactose content.

In conclusion, the production season is no longer as important a determinant of the microbiological quality of milk as it was a decade or so ago. This is because since that time dairy farms have been subject to strict sanitary and veterinary controls, including the obligation to refrigerate the milk. Nevertheless, the production season still has a significant impact on the proximate chemical composition of the raw milk supplied to the dairy, which is largely explained by the varied diets of cows in traditional farming systems. This is particularly true in the case of smaller milk producers. Although the purchased milk met the requirements of the Council Regulation [16] in terms of the total number of microorganisms, the results for the total number of psychrotrophic bacteria and the *E. coli* titre are not yet fully satisfactory.

Table 2
Level of *Escherichia coli* contamination in market milk with respect to the dairy and the season of purchase

Specification	Number of samples	Coli titre													
		0		10^{-1}		10^{-2}		10^{-3}		10^{-4}		10^{-5}		10^{-6}	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Dairy															
A	35	8	22.9	–	–	–	–	2	5.7	14	40.0	9	25.7	2	5.7
B	41	11	26.8	–	–	–	–	9	22.0	13	31.7	7	17.1	1	2.4
C	73	24	32.4	–	–	–	–	9	12.2	19	25.7	16	21.6	6	8.2
D	38	22	57.9	–	–	–	–	10	26.3	4	10.5	2	5.3	–	–
E	36	17	48.5	–	–	2	5.7	15	42.9	1	2.9	–	–	–	–
Season of production (purchase)															
summer	115	47	40.9	–	–	–	–	18	15.7	29	25.2	15	13.0	6	5.2
winter	108	35	32.4	–	–	2	1.9	27	25.0	22	20.4	19	17.6	3	2.8
total	223	82	36.8	–	–	2	0.9	45	20.2	51	22.9	34	15.2	9	4.0

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