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ORIGINAL ARTICLE

Content of free fatty acids, lipolytic bacteria and somatic cells in relation to milking technology

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Abstract
The contents of free fatty acids (FFA) and counts of total bacteria, psychrotrophic lipolytic bacteria (PLiBC) and somatic cells were determined in 150 samples of cow's bulk raw milk on 20 farms with three different milking technologies in South Bohemia during 2008–10. FFA were determined using an extraction-titration method. Within the compared technologies, the highest mean values of FFA (3.88 mmol 100 g^{-1}; $P<0.001$) and PLiBC (696 CFU ml^{-1}) were observed on farms with pipeline milking in stalls. The lowest mean FFA level (1.54 mmol 100 g^{-1}) was determined on farms with an automatic milking system. Medium values were determined on farms with parlour milking.

Key words: free fatty acids; psychrotrophic lipolytic bacteria; somatic cells; lipolysis; milking technology; mechanical stress of milk

Abbreviations:

TBC	total bacterial count
LPL	lipoprotein lipase
SCC	somatic cell count
PLiBC	psychrotrophic lipolytic bacteria
FFA	free fatty acids

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INTRODUCTION

Imperfect cleaning and disinfection of milking machines remains the main reason for the occurrence of psychrotrophic bacteria in raw milk. On average, the psychrotrophic microflora of bulk raw milk accounts for about 10 to 50% of the total bacterial count (Chambers 2005).

Lipolysis takes place through the enzymatic hydrolysis of milk fat, which leads to the accumulation of free fatty acids (FFA) in milk (Walstra et al. 1999). Lipases are enzymes catalysing the hydrolysis of triacylglycerols, the main lipid component of milk. The reaction products are free (non-esterified) fatty acids, partial glycerol esters (monoacylglycerols, diacylglycerols) and even glycerol in some cases

(Deeth 2006). Research results indicate that the lipolytic enzyme lipoprotein lipase (LPL) is responsible for almost all lipolytic activity in cow's raw milk (Olivecrona et al. 2003). A large number of LPL are present in milk but most of them are inactive. Lipase activators naturally occur in blood and enter the milk in higher concentrations for example during an udder infection, during the mammary gland involution at the end of lactation and when dairy cows are exposed to stress or malnutrition (Thomson et al. 2005).

The content of FFA also increases during the formation of incomplete fat globules, usually due to metabolic disorders in the dairy cows (Hanuš et al. 2008). These disorders may result from stress and from a lower ability of the dairy cows to adapt themselves to high requirements for milk production (Frelich et al. 2010).

Milk pasteurisation reduces the initial bacteria count and the activity of original milk lipase. This is the reason why thermostable lipolytic and proteolytic enzymes of psychrotrophic bacteria become a limiting factor for maintenance of the taste quality of liquid milk and dairy products (Hantis-Zacharov and Halpern 2007). The activity of lipases is facilitated by physical processes acting during milk processing such as homogenisation, sudden changes in temperature, intensive stirring or milk turbulence in the pipeline that may damage the lipoprotein membrane of fat globules and make the fat accessible to the action of lipases (Janštová et al. 2004).

Thus, an increased content of FFA may indicate i) the poor health status of the dairy cows or ii) fat breakdown due to milk contamination by psychrotrophic bacteria when the appropriate conditions of milk storage are not observed or iii) the exposure of the milk to excessive mechanical stress. The content of FFA has therefore been used recently as an indicator of milk quality influencing milk sale and acceptability. A high FFA content causes the deterioration of milk technological properties (e.g. a worse whipping ability of cream) and sensory characteristics of milk, especially of taste and flavour, which is reflected in a rancid off-taste that may negatively influence the quality of dairy products (Antonelli et al. 2002, Hanuš et al. 2008). The maximum permissible FFA content is 3.2 mmol 100 g^{-1} for the extraction-titration method of determination (Czech Standard ČSN 57 0529).

When lipolytic (Azzara and Dimick 1985) and proteolytic (Verdi and Barbano 1988) activities in milk increases the somatic cell count (SCC), which is of crucial importance from the aspect of the required hygienic quality of milk (Ma et al. 2000). In European countries, Australia and New Zealand the upper limit for SCC in bulk milk is 400×10^3 ml^{-1}. The limit of 500×10^3 ml^{-1} is defined in Canada while in the USA it is 750×10^3 ml^{-1} (Fetrow et al. 2000). At a higher milking frequency (for example with automatic milking systems), an increase in milk yield is accompanied by a decrease in somatic cell count (Allen et al. 1986, Berglund et al. 2002). According to Hillerton (1991), the microbial quality of milk is improved. A probable cause is the elution of bacteria from the udder before they can cause an inflammation.

It is hypothesized that differences in herd size, feeding, milking and housing strategies of cows may influence the microbial quality of milk (Dankow et al. 2004, Torkar and Teger 2008). The objective of the present paper was to determine the levels of free fatty acids, total bacterial count, psychrotrophic lipolytic bacteria count and somatic cell count in bulk samples of cow's raw milk on farms with different milking technology.

MATERIAL AND METHODS

The FFA content and counts of somatic cells, psychrotrophic lipolytic bacteria (PLiBC) and total bacteria (TBC) were monitored in 150 non-preserved samples of cow's raw milk during 2008–10. Samples originated from twenty farms in South Bohemia using three milking technologies (14 farms with milking in a milking parlour, 3 farms with pipeline milking in stalls and 3 farms with an automatic milking system). The cows were mostly milked twice a day. Bulk samples of cow's raw milk from morning milking were collected into sterile samplers and transported for 90 min in insulated boxes with a cooling pad. The milk temperature did not exceed 6 °C during the transportation. Samples were processed immediately after the delivery to the laboratory. Characteristics of the farms are shown in Table 1.

The content of FFA was determined by an extraction-titration method in accordance with the standard ČSN 57 0533 (1997). Milk fat was extracted with a mixture of isopropyl alcohol and petroleum ether (4:1) and acidified with sulphuric acid. FFA were titrated with 0.02 M potassium hydroxide.

Lipolytic bacteria counts and total bacterial counts were determined by a culture cultivation method in accordance with International Dairy Federation standards (ISO 6610, 6730; 1992).

Sterile Ringer's solution with peptone was used for the sample dilution. The medium tempered to 45 °C was poured onto one ml of the inoculum of the respective dilution. The samples were inoculated by three successive dilutions in duplicate. Plate Count Skim Milk Agar (Merck) was used for the determination of TBC. The plates were incubated at 30 °C for 72 hours. The plates with a count of 10 to 300 colonies were enumerated. Tributyrin Agar (Merck) was employed for the culture cultivation of PLiBC. The incubation was carried out at 6.5 (± 0.5) °C for ten days. Colonies with a clear lytic zone were enumerated.

The somatic cell count in bulk samples of raw milk was determined pursuant to the standard ČSN EN ISO 13366-3 (1997) Milk – Determination of Somatic Cell Count, Part 3: Fluoro-opto-electronic Method, using a Fossomatic 5000 instrument.

Geometric means, standard deviation, minimum and maximum values were computed from the actual values of FFA, SCC, PLiBC and TBC. Statistical evaluation of data was carried out using the Statistica CZ 7 software. Before the statistical analysis was carried out, the particular values of TBC, FFA, SCC and PLiBC were transformed logarithmically in order to provide for normal distribution. Microbial contamination of milk and content of FFA in relation to milking technology were evaluated by Tukey's test.

Table 1. Characteristics of the farms tested farms

Farm	Altitude above sea level	Milking	Technology of housing	n	Breed %	Average daily milk yield (l)
BR	550	Automatic	loose slatted-floor litterless	226	H	29
BO	502	Automatic	loose cubicle littered	570	C	20.0
SR	540	Milking parlour	loose cubicle littered	40	C90, L10	16.0
KR	570	Milking parlour	loose cubicle littered	200	H	30.0
CHL	510	Automatic	loose cubicle littered	80	C80, H20	16.0
LI	450	Milking parlour	loose cubicle littered	105	H	24.0
VJ	800	Milking parlour	loose cubicle littered	120	C92, H8	20.0
CHO	520	Milking parlour	loose cubicle littered	290	C92, H8	21.0
HD	420	Milking parlour	loose cubicle littered	120	H70, HxC30	17.8
ZU	600	Milking parlour	loose cubicle littered	315	H70, C30	20.8
CD1	410	Milking parlour	loose cubicle littered	320	H100	12.5
CD2	410	Pipeline milking in stalls	stanchion littered	74	H100	12.0
TE	700	Pipeline milking in stalls	stanchion littered	146	C100	14.0
RY	650	Pipeline milking in stalls	stanchion littered	123	C60, H40	16.2

n – number of milking cows, C – Czech Fleckvieh, H – Holstein cattle, L – Czech Red cattle

Table 2. Aggregated characteristics for 150 milk samples

Parameter	FFA (mmol 100 g^{-1})	SCC (10^3 ml^{-1})	PLiBC (CFU ml^{-1})	TBC (CFU ml^{-1})
Mean	2.49	281	628	15 392
S.D.	1.56	159	2 619	50 448
Min	0.69	71	50	500
Max	8.40	1 342	19 500	550 000

FFA – free fatty acids, SCC – somatic cell count, PLiBC – psychrotrophic lipolytic bacteria count, TBC – total bacterial count, S.D. – standard deviation

Table 3. Mean values of the determined parameters in relation to milking technology for 150 milk samples

Parameter	Automatic milking system	Milking parlour	Pipeline milking in stalls
n	10	97	43
FFA (mmol 100 g^{-1})	1.54	2.15	**3.88
SCC (103 ml^{-1})	237	283	289
TBC (CFU ml^{-1})	17 781	17 170	11 631
PLiBC (CFU ml^{-1})	560	607	696

For the abbreviations see Table 2, ** difference at significance level of $P<0.01$

RESULTS AND DISCUSSION

The TBC values were in the range of 500–550,000 CFU ml^{-1} (Table 2) with the mean 15,392 (± 50,448). This value is lower than that reported by Vyletělová et al. (2000), who determined for a set (n = 85) the mean value of milk contamination by mesophilic microorganisms 25,118 CFU ml^{-1}. Foltys and Kirchnerová (2006) reported a very similar range of variation in TBC (500 to 500,000) and the mean 13,679 CFU ml^{-1}.

The counts of PLiBC ranged widely between 50 and 19,500 CFU ml^{-1} with the mean value of 628 (± 2,619) CFU ml^{-1} (Table 2). These values are higher than those of Vyletělová et al. (1999) who determined in a set (n = 82) the mean PLiBC value of 105 CFU ml^{-1} with the range of variation 10 to 7,400 CFU ml^{-1}. The highest values of PLiBC were determined in the system of pipeline milking in stalls where the values were 696 (± 2,731) CFU ml^{-1} (Table 3). As a result of preceding research, Cempírková et al. (2009) reported the same trend of higher PLiBC in the system of pipeline milking in stalls.

Somatic cell counts reached on average 281 (± 159) × 10^3 ml^{-1} with the range between 71 × 103 and 1,342 × 10^3 ml^{-1}. The hygienic limit for SCC (≤ 400 × 10^3 ml^{-1}; Regulation No. 853/2004, 2004) was exceeded in 17% of samples. Almost identical values were determined in the preceding set (Cempírková et al. 2009). Moderately higher SCC values (283 × 10^3 and 289 × 10^3 ml^{-1}) were found in conventional milking technologies (Table 3) and lower values of SCC were determined on farms with automatic milking systems (237 × 10^3 ml^{-1}). A similar comparison was made by Helgren and Reinemann (2006), who also reported lower mean SCC in the automatic milking system (268 × 10^3 ml^{-1}) than in conventional milking (288 × 10^3 ml^{-1}). The lowest values of SCC were determined on the farms using an automatic milking system The influence of milking technology on somatic cell count in milk was not statistically significant, which confirmed the findings of other authors (Svennersten-Sjaunja et al. 2000, Shoshani and Chaffer 2002) who also did not demonstrate any significant influence of milking technology. Nevertheless, further factors can affect somatic cell counts. According to Oleggini et al. (2001), larger herds had lower SCC than smaller herds. The influence of different cow breeds on SCC in raw milk was reported by Bytyqi et al. (2010).

The mean FFA content was 2.49 (± 1.56) mmol 100g^{-1} (Table 2) with the range of 0.69–8.4 mmol 100 g^{-1}. The maximum permissible content of FFA (3.2 mmol 100 g^{-1} fat for the extraction-titration method; ČSN 57 0529) was exceeded in 31% of samples. The results documenting the highest mean values of FFA on farms with pipeline milking in stalls (Table 3), where the mean level of FFA reached 3.88 mmol 100 g^{-1} and the permissible limit was exceeded in 69.7% samples, are of particular interest. The same finding has previously been reported (Cempírková et al. 2009), and can be explained by the fact that on farms with pipeline milking, milk flows through a longer pipeline than milk in the milking parlour, which causes mechanical stress on milk and induces lipolysis. Shorter intervals between milkings could also contribute to an increase in FFA content. The lowest levels of FFA (1.54 mmol 100 g^{-1}) were recorded on farms with an automatic milking system (Table 3). Nevertheless, FFA can be affected by further factors. According to Ferlay et al. (2006), milk lipolysis was lower in diets based on concentrates with maize silage than in diets based on grass silage or pasture.

In addition, FFA were determined in a further set of 150 milk samples from various regions of the Czech Republic during 2008–10. The contents of FFA were determined by infrared spectroscopy, using a Milkoscan FT 6000 instrument (ČSN 57 0536; 1999). The mean value was 0.45 mmol 100 g^{-1}. This level can be compared with the

reported mean FFA content of 0.725 mmol 100 g^{-1} (Hanuš et al. 2008) determined by the same method. This method was calibrated according to the results of a referential (churning) method (the maximum allowed FFA content is 1.3 mmol 100 g^{-1} for a churning method or 3.2 mmol 100 g^{-1} for an extraction method). Thus, this method gives a lower FFA values than the extraction-titration determination.

The evaluation of the influence of milking technology on some qualitative parameters of milk demonstrated significantly higher values of FFA and PliBC in the system of pipeline milking in stalls as compared to milking parlour or automatic milking. Milk flows through a longer pipeline, which increases the mechanical stress on milk, resulting in damage to the membranes of the fat globules. For this reason, pipeline milking was found to be less suitable. The average count of mesophilic bacteria in raw milk (expressed by the parameter TBC) is consistent with the European Union hygiene rules. The permitted hygienic limit for SCC was exceeded in 17% of milk samples. The influence of milking technology on somatic cell count in milk was not proved. The results of this study showed a convenient influence of automatic milking systems on milk quality parameters, especially the content of free fatty acids and PLiBC.

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