Biotechnology in Animal Husbandry 21 (5-6), p 237-240, 2005 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637. 1

THE EFFECT OF HEAT TREATMENT ON THE FREE FATTY ACIDS IN EWE'S MILK[1]

Agata Reguła, Genowefa Boncza, H. Pustkowiak[2]

Abstract: The objective of this study was to evaluate the influence of pasteurization's methods (72˚C/30s; 85˚C/20min.; 95˚C/5min.) on the free fatty acids (FFAs) profile in ewe's milk. Identification and quantification of FFAs were performed by capillary gas chromatography.

The major free fatty acid in raw milk as well as in pasteurized milks, was palmitic followed by myristic. It was found that pasteurization, depending on its type, caused the different changes in FFAs profile. Heat treatment at 72˚C/30s affected statistically significantly the total amount of FFAs. This type of pasteurization caused also the highest increase in almost all the FFAs; especially in myristic by 107%, in lauric by 71% and in palmitic by 40%. It was observed that pasteurization at 95˚C/5min contributed to the decrease in most of FFAs. The highest decrease was found for stearic and butyric acid (by 85% and by 75% respectively).

*Key words***:** free fatty acids, heat treatment, ewe's milk

Introduction and literature review

Free fatty acids (FFAs), although in different amount, are always present in raw milk. These compounds in milk can originate from three sources: from incomplete synthesis of fatty acids, from blood – bound to serum albumin, by hydrolysis of milk glycerides (*Cardak et al., 2003*). The amount of FFAs is affected by many factors, among which the most important are: lactation period, feedings regimes, kind of milking, and factors associated with further milk's treatment: cooling, homogenization (*Walstra and Jennes, 1985; Fox 1995).*

FFAs affect the sensory properties of milk and milk derived products. Some amount of these compounds is even required to assure the organoleptic characteristic and acceptance of e.g. cheese, fermented milks. In case of raw milk, to high concentration of FFAs may limit raw material to the manufacture (*Santos, 2003*). FFAs, especially schort-chain free fatty acids (butyric, caproic, caprylic capric) may cause flavor defect described as "rancid". Some of FFAs may also inhibit growth and activity of starter culture (*Szczepanik and Libudzisz, 2001).*

The basic process during manufacture of many milk products is pasteurization. As the results of heat treatment the vegetative forms of microorganisms and milk enzymes are inactivated. Heating contribute also to the changes in milk compounds (proteins, salts, fat).

The aim of the present study was to estimate the effect of three different forms of pasteurization (72˚C/30s; 85˚C/20min; 95˚C/5min.) on the FFAs profile in ewe's milk.

Material and methods

Ewe's milk was collected three times (mornings) in the September from Polish mountain ewes, bred at a farm in Opatkowice near Kraków. Milk was divided into four equal portions. 1st batch was used for raw milk's analysis, 2nd batch was heated to 72˚C/30s, 3rd batch to 85˚C/20min., 4th batch to 95˚C/5min. After pasteurization all batches were cooled to the 20˚C.

Analytical methods

In raw milk dry matter content, the protein content from the crude nitrogen according to the Kjeldahl method and the total fat and lactose contents using the Gerber and Bertrand methods were evaluated (*AOAC, 1990; Budsławski,1973*). The milk density was measured using a densitometer (*Budsławski, 1973*). The pH of the milks was measured using a digital pH meter. The titratable acidity was determined by Soxhlet-Henkel method (*Budsławski, 1973*). Extraction of the free fatty acids was performed according to the method described by *Huerta-Gonzalez and Wilbey* (*2001*). Identification and quantification of FFA were determined using gas chromatograph equipped with capillary column "Nukol" (length 30m). The carrier gas (helium) flow rate was 20 cm^3/min, and the temperature was raised from: 70˚C to 200˚C at 5˚C/min, then held at 200˚C for 30 min. The concentration of individual free fatty acids was expressed as µg/g fat (in text values: mg/l milk also was used). Statistical analyses were carried out using computer's program Statistica 6.0.

---

1 Original scientific paper – originalni naučni rad

2 Mgr inż. Agata Reguła, dr hab.Genowefa Bonczar, prof AR: Department of Animal Products Technology, Agricultural University, Kraków, Poland; Mgr inż. Henryk Pustkowiak: Department of Cattle Breeding, Agricultural University of Kraków, Kraków, Poland

Results of investigations and discussion

Chemical composition (g/100g) of raw ewe's milk was as follows: dry matter 16.83 (± 0.42, fat) 6.3 (± 0.15), protein (5.9 ± 0.07), lactose 4.42 (± 0.14). Density of milk was 1.032, pH 6.68 (± 0.08), and titratable acidity 8.07 °SH (± 1.34). The values of these parameters were comparable with those reported by other authors (*Haenlein,2001*; *Kondyli and Katsiari, 2002).*

The total FFAs concentration in raw milk: 293,94 µg/g fat (i.e. 19,1 mg/l milk) was lower than the results obtained by *Kondyli and Katsiari (2002*). However, in the total FFAs evaluated by these authors were also acetic as well as oleic, linoleic and liolenic acids. *Diaz et al*.(*2004*) estimated total FFAs, depending on the time of milking (morning, evening) on the level 25.6 mg/l and 60.5 mg/l milk respectively. The amount of these compounds in milk depends on so many factors, that the results of researchers can give only a some view of FFAs concentration in milk. There is known that agitation, cooling, stirring affect increase in FFA, what is due to the destruction of milk fat globule membranes (MFGM), which protect glycerides against lipolysis (*Danthine et al*., *2000; Evers, 2004*). Very important are also: stage of lactation and regime's form: green forage contribute to the decrease in lipolysis, while dry feed may caused an effect inverse. Low level of FFAs concentration in the present study may be attributed to fact that milk was collected morning, was not cooled and not stirred. Ewes were fed on the pasture.

The short-chain free fatty acid (SFFA: butyric, caproic, caprylic capric) in raw milk should be present in amount as low as possible. These compounds are responsible for rancid flavor. Total concentration of SFFA in raw ewe's milk (5,3mg/l milk) was in agreement with the results of *Diaz et al*. (*2004*). Comparable values regarding butyric and capric acid found *Kondyli and Katsiari* (*2002)* but concentrations of caproic and caprylic acids reported by these authors are higher. Pasteurization had an influence on the SFFA. Heating at 72˚C/30s caused an increase in butyric and in capric acid, but temperature 95˚C/5min and 85˚C/20min decreased butyric acid content by 75% and by 54% respectively. It was found statistically significantly differences (with regard to butyric acid amount) between raw milk and milk heated at 95˚C/5min ($p \leq 0.01$) and 85˚C/20 min ($p \leq 0.05$). Differences between forms of pasteurization also were significant.

The amount of medium-chain free fatty acids (MFFA: lauric and myristic) in raw ewe's milk was twofold lower than values observed by *Kondyli and Katsiari* (*2002*) and simultaneously higher than findings reported by *Diaz et al.* (*2004*). The concentration of MFFA increased regardless of the pasteurization's method. The higher increases in lauric and in myristic acid (by 71% and 107% respectively) was found for 72˚C/30s. Pasteurization at 85˚C/20 min also caused an appreciable increase in both acids. Amount of lauric and myristic acids were higher (by 54% and 62% respectively) than in raw material. The effect of this heating was statistically significant. It could be supposed that pasteurization at 72˚C/30s and at 85˚C/20 min was insufficient for inactivation the native lipase. According to *Chavari et al.* (*1998*) inactivation of lipoprotein lipase in ewe's milk after pasteurization can range from 73% to 95%. Activity of the heat stable bacterial lipases that survived pasteurization also can not be omitted. The most diversification between forms of pasteurization was stated in the case of myristic acid.

Table 1. Variation in free fatty acids (µg/g milk fat) in ovine milk (raw and after pasteurization)

Free fatty acids	Raw milk	Pasteurization		
		72˚C/30s	85˚C/20 min	95˚C/5 min
C_4	8,56 ± 0,41Aa	9,89 ± 1,96Bb	3,93 ± 0,80ab	2,13 ± 1,07AB
C_6	14,44 ± 1,82	14,77 ± 0.34	13,39 ± 1,32	13,16 ± 1,84
C_8	15,60 ± 1,78	15,47 ± 0,57	14,40 ± 0,98	15,05 ± 1,90
C_{10}	42,33 ± 5,92	54,41 ± 3,41	43,38 ± 4,22	44,03 ± 7,07
C_{12}	25,28 ± 0,57ab	43,34 ± 2,12a	39,16 ± 2,61b	31,58 ± 6,31
C_{14}	44,97 ± 2,87AB	93,27±3,64ACD	73,28 ± 1,58 BCE	46,98 ± 4,62DE
C_{16}	111,78 ± 12,10a	156,75 ± 3,96ab	118,19 ± 1,91	103,17 ± 22,5b
C_{18}	30,98 ± 4,22A	25,46 ± 1,82B	23,67 ± 3,42C	4,37 ± 1,3ABC
Total	293,94 ± 4,57A	413,36 ± 1,23ABa	329,4 ± 2,45a	260,47 ± 3,21B

Abbreviations used: FFA- free fatty acids, C_4- butyric acid, C_6 –caproic acid, C_8 – caprylic acid, C_{10} –capric acid, C_{12} -lauric acid, C_{14} – myristic acid, C_{16} - palmitic acid, C_{18}- stearic acid

A,B,C,D,E – statistically significant difference between averages in the line ($p \leq 0.01$)

a, b, - statistically significant difference between averages in the line ($p \leq 0.05$)

The concentrations of palmitic and stearic acid in raw material were lower than values reported by other authors (*Kondyli and Katsiari, 2002; Diaz et al., 2004*). These differences can be attributed to the way of milking (mornings, by hand) and further milk handling (it was not cooled and not stirred). Heat treatment at 72˚C as well as at 85˚C affected the increase in palmitic acid and slightly decrease in stearic acid. Pasteurization at 95˚C/5min. caused the decrease in both acids. Drop in stearic acid content by 85% was statistically significant ($p \leq 0.01$).

Conclusion

As a results of our investigation it may be found out that heating, depending on its form, contributed to the different changes in FFAs profile. Heat treatment at 72˚C/30s caused the highest increase in total amount of FFAs (by 40,6%) as well as in most of individual acids. Pasteurization at 95˚C/5min affected the decrease in FFAs especially in butyric and stearic acids (drop by 75% and by 85% respectively). On the basis on our results it can be concluded that heat treatment at 72˚C/30s should be limited in the manufacture. The most suitable seems to be temperature 95˚C/5min.

We must however emphasize that results of the present study concerned only ewe's milk and further researchers regarding milk of other ruminants (cow, goat) are necessary to evaluate the effect of the heat treatment on the FFAs concentration in these milks.

UTICAJ TOPLOTNOG TRETMANA NA SLOBODNE MASNE KISELINE U OVČIJEM MLEKU

Agata Reguła, Genowefa Bonczar, H. Pustkowiak

Rezime

Slobodne masne kiseline (FFAs) su uvek prisuten u svežem mleku, ali njihov nivo zavisi od mnogih faktora: perioda laktacije, režima ishrane, muže, itd. Ove kiseline utiču na senzorne osobine mleka i mlečnih proizvoda. Određena količina ovih jedinjenja osigurava organoleštičke karakteristike sireva i fermentisanih napitaka. Kod sirovog mleka visoka koncentracija FFAs nije poželjna. Posebno masne kiseline kratkog lanca (buturna, kapronska, kaprilna, kaprinska kiselina) mogu izazvati oštećenje ukusa/aroma mleka koji se često opisuje kao "užegao". Neke FFAs mogu inhibirati rast i aktivnost starter kultura (*Szczepanik and Libudzisz, 2001).* U proizvodnji mlečnih proizvoda jedan od prvih koraka je pasterizacija. Tolpotni tretman međutim menja sastav mleka. Cilj ovog rada je bio da oceni uticaj tri oblika pasterizacije (72˚C/30s; 85˚C/20min; 95˚C/5min) na profil slobodnih masnih kiselina FFAs u ovčijem mleku.

Ovčije mleko sakupljano je tri puta (jutro) tokom septembra I to od poljskih planinskih ovaca gajenih na farmi u mestu Opatkowice u blizini Krakova. Fizičko-hemijske osobine ovčijeg mleka su bile u skladu sa rezultatima ostalih istraživača (*Haenlein,2001*; *Kondyli and Katsiari, 2002).* Slobodne masne kiseline u sirovom mleku kao I u pasterizovanom su ocenjivane prema metodi opisanoj kod *Huerta-Gonzalez and Wilbey* (*2001*). Identifikacija i kvantifikacija FFA je urađena pomoću gasne hromatografije. Glavna slobodna masna kiselina kod svih istraženih mleka je bila palmitinska a zatim I miristinska kiselina. Oblici pasterizacije su imali signifikantan uticaj na profil FFAs. Toplotni tretman na 72˚C/30s je statistički signifikantno uticao na ukupnu količinu FFAs (povećanje za 40,6%) u poređenju sa sirovim mlekom. Ovaj oblik pasterizacije je izazvao najveće povećanje u svim slobodnim masnim kiselinama (posebno miristinskoj za 107%, laurinskoj za 71% i palmitinskoj za 40%). Utvrđeno je da pasterizacija na 95˚C/5min doprinosi smanjenju većine FFAs. Najveći pad je utvrđen kod stearinske i buterne kiseline (za 85% i 75% respektivno). Na osnovu naših rezultata može se zaključiti da toplotni tretman na 72˚C/30s treba da bude ograničen samo na proizvodnju ovčijeg mleka. Najpogodnija je pasterizacija na temperature od 95˚C/5min.

References

1. AOAC. (1990): in Official methods of analysis, Arlington: A. Press
2. BUDSŁAWSKI J. (1973): Badanie mleka i jego przetworów. PWRiL, Warszawa
3. CARDAK A.D., YETISMEYEN A. and BRUCKNER H. (2003): Quantitative comparison of free fatty acids in camel, goat and cow milk. Milchwissenschaft, 58, 127-130.
4. CHAVARI F., SANTISTEBAN A., VIRTO M. and de RENOBALES M. (1998): Alkaline phosphatase, acid phosphatase, lactoperoxidase, and lipoprotein lipase activities in industrial ewe's milk and cheese. Journal of. Agricultural Food Chemistry, 46, 2926-2932.
5. DANTHINE S., BLECKER C., PAQUOT M., INNOCENTE N. and DEROANNE C. (2000): Évolution des connaissances sur la membrane du globule gras du lait: synthèse bibliographique. Lait 80, 209-222.
6. DIAZ J.R., PERIS C., RODRIGUEZ M., MOLINA M.P. and FERNANDEZ N. (2004): Effect of milking pipeline height on machine milking efficiency and milk quality in sheep. Journal of Dairy Science, 87, 1675-1683.
7. EVERS J.M. (2004): The milk fat globule membrane-compositional and structural changes post secretion by the mammary secretory cell. Int. Dairy J., 14, 661-674.
8. FOX P. F. (1995): Heat – induced changes in milk. IDF, Brussels.
9. HAENLEIN G.F.W. (2001): Past, present and future perspectives of small ruminant dairy research. Journal of Dairy Science, 84, 2097-2115.
10. HUERTA-GONZALEZ L. and WILBEY R.A. (2001): Determination of free fatty acids produced in filled-milk emulsion as result of the lipolytic activity of lactic acid bacteria. Food Chemistry, 72, 301-307.
11. KONDYLI E. and KATSIARI M.C. (2002): Fatty acid composition of raw ewe's milk of Boutsiko breed during lactation. Milchwissenschaft 57, 74-76.
12. SANTOS M.V., MA Y., CAPLAN Z. and BARBANO D.M. (2003): Sensory threshold of off-flavors caused by proteolysis and lipolysis in milk. Journal of Dairy Science, 86, 1601-1607.
13. SZCZEPANIK A. and LIBUDZISZ Z. 2001. Przydatność technologiczna mleka koziego. Przemysł Spożywczy, 2, 35-36.
14. WALSTRA P. and JENNES R. (1985): Lipids in Dairy Chemistry and Physics. A Wiley-Interscience Publication. John Wiley & Sons. New York 58-70