Acta Sci. Pol., Technol. Aliment. 10(2) 2011, 155-163
ISSN 1644-0730 (print) ISSN 1889-9594 (online)

THE FERMENTATION DYNAMICS OF SHEEP MILK WITH INCREASED PROPORTION OF WHEY PROTEINS

Agata Lasik, Jan Pikul, Romualda Danków, Dorota Cais-Sokolińska
Poznań University of Life Sciences

Introduction. A new trend in food production is to use a whey proteins as a food additives. The aim of this research project was to evaluate the impact of increased proportions of whey proteins in sheep milk on the course of its fermentation process.
Material and methods. The materials used in the experiments was sheep and cow milk of natural ratio of whey to casein proteins (1:4) and sheep milk in which the ratio of whey to casein proteins amounted to 1:1 and 4:1. The dynamics of the fermentation process and the stability of final products was described on the basis of pH value, titratable acidity, lactose, galactose and lactic acid content.
Results. Higher, in relation to natural, ratios of whey to casein proteins (1:1 and 4:1) in sheep milk increased the acidity of the final product after incubation and decreased the degree of fermented lactose. The cooling storage of fermented milks failed to affect changes in pH value, titratable acidity, galactose and lactic acid content of the final products.
Conclusion. Changes in ratios of whey to casein proteins result in differences in dynamics of sheep milk fermentation but not influenced on final product's stability.

Key words: sheep milk, whey proteins, fermentation, dynamics

INTRODUCTION

Global annual production of sheep milk fluctuates at the level of approximately 8.2 million tons with such countries as: China, Italy, Turkey, Greece, Iran, Syria, the Sudan, Romania and Spain as leaders in this field. Majority of sheep milk is used to manufacture cheeses and yoghurts. These products, most commonly considered regional, are protected by legal regulations which guarantee their taste and aroma typical for a given area and which they usually owe to traditional production technology. From the nutritional point of view, sheep milk is more valuable than cow and goat milk. In developing

Corresponding author – Adres do korespondencji: Mgr inż. Agata Lasik, Department of Dairy Technology of Poznań University of Life Sciences, Wojska Polskiego 28, 60-624 Poznań, Poland, e-mail: alasik@up.poznan.pl

countries, sheep milk – as a rich source of protein, calcium and energy – is utilised to overcome malnutrition [Haenlein and Wendorff 2006].

Protein content in sheep milk amounts to about 6% comprising the following six groups of proteins: α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, α-lactoalbumin and β--lactoglobulin. Sheep milk fat contains, primarily, triacylglycerols with much less monoacylglycerols, diacylglycerols, phospholipids and sterols all of which together constitute from 5.5 to 6% of total quantity of lipids. Lactose content in sheep milk amounts to about 4.5%. The content of vitamins and minerals is considerably higher than in the milk of other mammals and amounts to approximately 1% [Park 2006, Pandya and Ghodke 2007].

Whey proteins, which are by-products developed during the process of cheese production, are utilised, mainly, as feed for animals due to their high nutritive value. In recent years, successful attempts have been undertaken to use whey proteins as a food additive. Whey proteins include: α-lactoalbumin and β-lactoglobulin, immumoglobulins and albumin serum. When compared with milks of other mammals, sheep milk is characterised by the highest β-lactoglobulin content, which is responsible for the transport of retinol and fatty acids, is characterised by antioxidative activity and anticancerogenic and antiviral properties [Król et al. 2008].

A growing interest has been observed in recent years, accompanied by increasing consumption, in fermented milks. This dynamic sales growth of these milk products is preconditioned by their sensoric traits and pro-salubrious properties. According to the definition of the Food and Agriculture Organization of the World Health Organisation (FAO/WHO) and International Milk Federation (FIL/IDF), fermented milk is a product obtained from whole milk, partially or completely defatted, thickened or regenerated in powder and subjected to fermentation by specific organisms which are capable of fermenting lactose, the pH value of milk and cause protein coagulation [Kudełka 2005, Pikul et al. 2006]. Nutritional value of fermented milk is affected significantly by the type of milk from which these drinks have been manufactured [Zmarlicki 2006]. At the present time, products manufactured from sheep milk have been gaining in market popularity. This milk is characterised by enhanced content of individual nutritional constituents. In addition, this type of milk contains such bioactive components as: immumoglobulins, lysozyme and lactoferrin. All the above-mentioned constituents impart products manufactured from sheep milk pro-salubrious properties and make them a perfect supplementation of our daily diets [Manzi et al. 2007, Blasi et al. 2008].

Investigation on fermented milk products manufactured from sheep milk carried out so far have focused, primarily, on the quality assessment of the finished product. Very little attention has been paid, in the available literature on the subject, to the characterisation of the process itself of the sheep milk fermentation as well as to sheep milk with enhanced proportion of whey proteins. Therefore, the objective of this research project was to evaluate the impact of increased proportions of whey proteins in sheep milk on the course of its fermentation process.

MATERIAL AND METHODS

The raw material used in the experiments was sheep and cow milk. The applied sheep milk was obtained from the Experimental Station in Złotniki which is a part of Poznań University of Life Sciences, while cow milk – from Obra Dairy Cooperative in Kościan. In order to increase the proportion of whey proteins in sheep milk, it was first subjected to a process of centrifugation and then the defatted milk was subjected to the process of microfiltration. Microfiltration was conducted using Isolux membranes with modified filtrating layers. The above process was carried out at the temperature of 20°C. The initial pressure amounted to 6 atm. and was reduced to 3 atm. during the process. Following the microfiltration process, a threefold concentrated retentat of increased content of casein proteins was obtained as well as a filtrate of increased content of whey proteins. In the course of the experimental part of investigations, the following samples of milk of 1.5% fat content were prepared: sheep milk of natural ratio of whey to casein proteins (1:4), sheep milk in which the ratio of whey to casein proteins amounted to 1:1, sheep milk in which the ratio of whey to casein proteins amounted to 4:1 and cow milk of natural ratio of whey to casein proteins (1:4).

The prepared samples of processed milk were inoculated with a freeze-dried starter culture Kefir DC, Danisco Biolacta Ltd. After inoculation, it was incubated at the temperature of 30°C for the period of 18 h. The applied time and temperature incubation parameters were selected in relation to essential conditions necessary to decrease pH values to 4.6 in sheep and cow milk of natural ratio of whey to casein proteins. On incubation termination, the obtained product was cooled down to the temperature of 4°C. The manufactured fermented milk were stored in cold-store conditions (4-6°C).

The dynamics of the course of the fermentation process of the prepared milk samples was described on the basis of active acidity measurements using, for this purpose, an electronic pH-meter type CP 411 Elmetron with temperature compensation [Hermanowicz et al. 1999], determination of titratable acidity [PN-75/A-86130] as well as determination of lactose, galactose and lactic acid content using high performance liquid chromatography (HPLC) [Mullin and Emmons 1997]. The employment of the HPLC technique required initial protein precipitation from the analysed samples. For this purpose, 4.5 cm^3 0.013N sulphuric acid (VI) (samples before inoculation) or 0.010N sulphuric acid (VI) (samples after inoculation) were added. Samples were thoroughly mixed (vortex, 15 s) and placed in a boiling water bath for about 10 min. Next, the samples were left at room temperature for approximately 20-30 min to allow them to cool and then they were centrifuged (3000 g, 10 min). The obtained supernatant was filtered using Millex-LCR filters (Millipore) Low Protein Binding Hydrophilic LRCPTFE 0.45 nm [Chick et al. 2001]. Samples prepared in this way in the amount of 20 µl were transferred onto a column (HPX 87H, BioRad) connected with an RI detector. The mobile phase was 0.005M solution of sulphuric acid (VI). The flow velocity through the column amounted to 0.6 cm^3/min and the time of analysis at the temperature of 30°C was 30 min.

The obtained results were subjected to statistical analysis using for this purpose the STATISTICA 8 (StatSoft) package.

RESEARCH RESULTS AND DISCUSSION

The conditions adopted in the experimental part for the course of the souring process of sheep and cow milks characterised by natural ratio of whey to casein proteins caused a drop in the pH value to about 4.6 after 18 hours of incubation. When the ratio of whey to casein proteins increased in sheep milk (ratio 1:1 and 1:4), a significant increase in the acidity of these fermented milks took place after the same incubation time (Table 1).

Table 1. Influence of incubation time on milk acidity

Milk	Ratio of whey to casein proteins	Incubation time h	Active acidity pH	Titratable acidity °SH
Sheep	1:4	0	6.38[e]	7.67[a]
		18	4.37[b]	13.67[b]
Sheep	1:1	0	6.13[d]	7.67[a]
		18	4.20[a]	15.67[bc]
Sheep	4:1	0	6.29[de]	8.33[a]
		18	4.17[a]	16.67[c]
Cow	1:4	0	6.93[f]	8.79[a]
		18	4.58[c]	14.24[bc]

a-f – means with different superscripts within same column are significantly different ($P < 0.05$).

Similar observations appear to result from earlier experiments [Martin-Diana et al. 2003, Herrero and Requena 2006]. The above-mentioned researchers found that a significant increase occurred in fermented milks manufactured from goat milk containing increased proportions of whey proteins, although it was not correlated with the highest lactic acid content in those samples.

The titratable acidity of all manufactured fermented milks increased as a result of the performed fermentation process. A twofold increase of this parameter was observed during sheep milk incubation of increased ratio of whey to casein proteins.

Literature data confirm that high performance liquid chromatography was applied to determine the content of organic acids as well as mono- and disaccharides in fermented milks [Mullin and Emmons 1997, Tormo and Izco 2004, Álvarez-Martin et al. 2008, Pescuma et al. 2008]. Investigations on metabolites developing during the process of milk fermentation conducted by Mullin and Emmons [1997], as well as by Álvarez-Martin et al. [2008] confirmed possibilities of the occurrence, apart from lactic acid, also of acetic acid (0.02%), propionic acid (0.03%) and citric acid (0.2%). However, the amounts in which the above acids were determined were negligible. In the presented research results, no other organic acids, except lactic acid, were found to occur.

The fermented milk manufactured from sheep milk containing different proportions of whey proteins, as well as from cow milk were characterised by variations in the content of lactose, galactose and lactic acid (Table 2). No statistically significant differences were observed in lactose concentrations between sheep and cow milks containing natural ratios of whey to casein proteins. The content of milk sugar in the analysed samples decreased significantly as a result of changes caused by the fermentation process. In sheep milk samples containing enhanced ratios of whey to casein proteins, the content of lactose before and after the incubation process did not differ statistically significantly. Fermented drinks manufactured from sheep milk characterised by increased proportions of whey proteins distinguished themselves by significantly lower concentrations of galactose and lactic acid in comparison with drinks manufactured from milk of natural ratio of whey to casein proteins.

Table 2. Influence of incubation time on lactose, galactose and lactic acid content

Milk	Ratio of whey to casein proteins	Incubation time h	Lactose $g\cdot dm^{-3}$	Galactose $g\cdot dm^{-3}$	Lactic acid $g\cdot dm^{-3}$
Sheep	1:4	0	38.26^{d}	nd	nd
		18	21.92^{a}	2.18^{c}	3.36^{c}
Sheep	1:1	0	30.90^{ab}	nd	nd
		18	29.41^{b}	1.49^{a}	2.28^{a}
Sheep	4:1	0	30.38^{b}	nd	nd
		18	29.42^{b}	1.59^{a}	2.25^{a}
Cow	1:4	0	36.13^{cd}	nd	nd
		18	33.94^{c}	1.86^{b}	2.86^{b}

a-d – means with different superscripts within same column are significantly different ($P < 0.05$).
nd – not detectable at 0.002 $mg\cdot dm^{-3}$ level.

In order to assess the stability of the obtained milk products, they were stored for the period of 14 days in cold storage conditions. No statistically significant impact was observed of the storage time on the active acidity of all analysed fermented milks (Table 3). A statistically significant increase was in titratable acidity of the fermented milks manufactured from sheep milk containing enhanced ratio of whey to casein proteins (4:1) as well as the fermented milks from cow milk during cold storage. The fact was probably cause by product ripening. The content of lactose during cold storage did not undergo statistically significant changes in fermented milks manufactured from sheep milk with increased ratio of whey to casein proteins (1:1) as well as in fermented milks

Table 3. Influence of storage time on fermented milks acidity changes

Milk	Ratio of whey to casein proteins	Storage time day	Active acidity pH	Titratable acidity °SH
Sheep	1:4	3	4.40^{a}	15.67^{a}
		14	4.39^{a}	17.23^{a}
Sheep	1:1	3	4.19^{a}	25.33^{b}
		14	4.18^{a}	26.22^{bc}
Sheep	4:1	3	4.19^{a}	24.33^{b}
		14	4.17^{a}	30.44^{c}
Cow	1:4	3	4.50^{a}	24.77^{b}
		14	4.49^{a}	28.89^{c}

a-c – means with different superscripts within same column are significantly different ($P < 0.05$).

from cow milk. Amounts of galactose and lactic acid did not undergo statistically significant changes during the 14-day period of cold storage in fermented milks from cow and sheep milks of natural protein proportions. The changes in lactose, galactose and lactic acid concentrations recorded in the examined products manufactured from sheep and cow milks could have been caused by the presence in them of live microorganisms which, despite unfavourable temperature conditions, carried on essential, basic living processes using distinctly fewer substrates and producing smaller quantities of lactic acid (Table 4).

Table 4. Influence of storage time of fermented milks lactose, galactose and lactic acid content changes

Milk	Ratio of whey to casein proteins	Storage time day	Lactose $g \cdot dm^{-3}$	Galactose $g \cdot dm^{-3}$	Lactic acid $g \cdot dm^{-3}$
Sheep	1:4	3	23.46^{b}	2.56^{d}	3.50^{d}
		14	19.72^{a}	2.41^{d}	3.63^{d}
Sheep	1:1	3	29.77^{c}	1.56^{a}	2.35^{a}
		14	29.39^{c}	1.93^{b}	2.80^{b}
Sheep	4:1	3	29.71^{c}	1.56^{a}	2.40^{a}
		14	23.61^{b}	1.97^{b}	2.83^{b}
Cow	1:4	3	34.68^{d}	2.03^{bc}	3.04^{c}
		14	33.51^{d}	2.17^{cd}	3.10^{c}

a-d – means with different superscripts within same column are significantly different ($P < 0.05$).

Similar observations were reported by Herrero and Requena [2006] in the course of their investigations on the stability of fermented products from goat milk during their storage. The pH value of samples analysed by these researchers declined from 4.48 to 4.30, and the content of lactic acid increased from 0.70% to 0.77% during a 28-day cold storage. However, the above changes were not statistically significant.

The application of the second degree multinomial regression made it possible to evaluate the relationship between the amount of fermented lactose and the amount of the developed lactic acid in the analysed fermented milks (Fig. 1). High R values calculated by STATISTICA 8 package testify to a significant correlation between the declining lactose content and the increasing concentration of lactic acid. The directional coefficient of milk containing natural ratios of proteins fractions was negative and in samples with increased ratios of whey proteins the coefficient was positive. The plotted trend lines of the above-mentioned correlation made it possible to divide the analysed samples from the point of view of the shape of the plotted curves. The first group comprises the fermentation process carried out on cow and sheep milks containing natural ratios of whey to casein proteins, whereas the second group includes sheep milk samples of increased ratios of whey to casein proteins (1:1 and 4:1) whose trend line adopted a shape of a hyperbola.

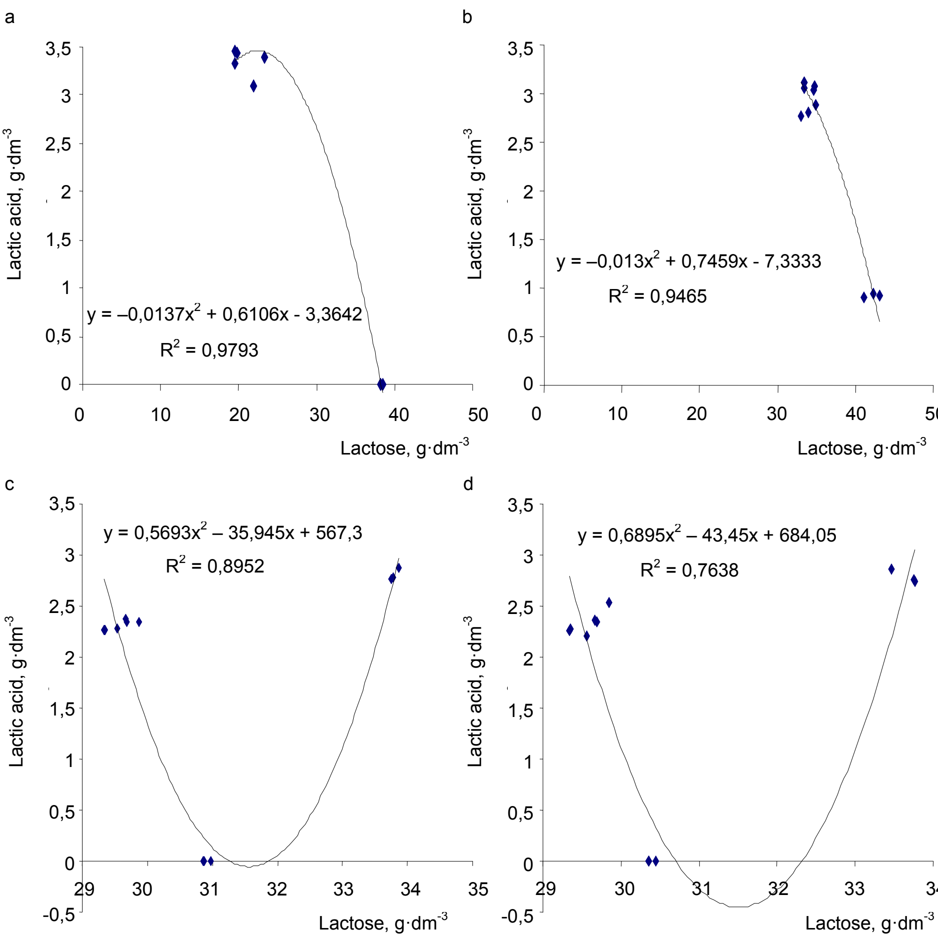

Fig. 1. The decline of lactose and the rise of lactic acid dependency in fermented milk produced from: a – sheep milk of natural ratio of whey to casein proteins (1:4), b – cow milk of natural ratio of whey to casein proteins (1:4), c – sheep milk in which the ratio of whey to casein proteins amounted to 1:1, d – sheep milk in which the ratio of whey to casein proteins amounted to 4:1

CONCLUSIONS

1. Higher, in relation to natural, ratios of whey to casein proteins (1:1 and 4:1) in sheep milk increased the acidity of the final product after 18-hour incubation.

2. The applied modification of the two major proteins in the processed sheep milk involving enhanced whey to casein proteins ratio decreased the degree of fermented lactose and, consequently, decreased the content of lactic acid in the examined fermented milk products.

3. The storage of fermented milk products in cold storage conditions for the period of 14 days failed to affect changes in pH value of the final products.

4. During the 14-day cold storage of fermented milk products manufactured from sheep and cow milks a statistically significant increase in titratable acidity, galactose and lactic acid content were recorded at a simultaneous decline in lactose content.

5. Increased ratio of whey to casein proteins in sheep milk resulted in a change from negative to positive curve trend coefficient reflecting the dependence of the fermented lactose on the quantity of the manufactured lactic acid.

REFERENCES

Álvarez-Martin P., Flórez A.B., Hernández-Barranco A., Mayo B., 2008. Interaction between dairy yeasts and lactic acid bacteria strains during milk fermentation. Food Control 19, 62-70.

Blasi F., Montesano D., De Angelis M., Maurizi A., Ventura F., Cossignani L., Simonetti M.S., Damiani P., 2008. Results of stereospecific analysis of triacylglycerol fraction from donkey, cow, ewe, goat and buffalo milk. J. Food Comp. Anal. 21, 1-7.

Chick H., Shin H.S., Ustunol Z., 2001. Growth and acid production by lactic acid bacteria and Bifidobacteria growth in skim milk containing honey. J. Food Sci. 66, 478-481.

Haenlein G.F.W., Wendorff W.L., 2006. Sheep milk. In: Handbook of milk of non bovine mammals. Eds Y.W. Park, G.F.W. Haenlein. Blachwell Publ. Oxford, 137-194.

Hermanowicz W., Dojlido J., Dożańska W., Kosiorowski B., Zerze J., 1999. Fizyko-chemiczne badania wody i ścieków [Phisyco-chemical investigations of water and waste]. Arkady, Warszawa [in Polish].

Herrero A.M., Requena T., 2006. The effect of supplementing goats milk with whey protein concentrate on textural properties of set-type yogurt. Int. J. Food Sci. Technol. 41, 87-92.

Król J., Litwińczuk A., Zarajczyk A., Litwińczuk Z., 2008. Alfa-laktoalbumin and beta-laktoglobulin as a bioactive compounds of milk protein fraction. Med. Wet. 64, 1375-1379.

Kudełka W., 2005. The characteristic of fermented milk in European Union and in Poland. Zesz. Nauk. AE Krak. 678, 149-160.

Martin-Diana A.B., Janer C., Peláez C., Requena T., 2003. Development of a fermented goat's milk containing probiotic bacteria. Int. Dairy J. 13, 827-833.

Manzi P., Marconi S., Pizzoferrato L., 2007. New functional milk-based products in the Italian market. Food Chem. 104, 808-813.

Mullin W.J., Emmons D.B., 1997. Determination of organic acid and sugars in cheese, milk and whey by high performance liquid chromatography. Food Res. Int. 30, 147-151.

Pandya A.J., Ghodke K.M., 2007. Goat and sheep milk products other than cheese and yogurt. Small Rumin. Res. 68, 193-206.

Park Y.W., 2006. Goat milk – chemistry and nutrition. In: Handbook of milk of non bovine mammals. Eds Y.W. Park, G.F.W. Haenlein. Blachwell Publ. Oxford, 34-58.

Pescuma M., Hebert E.M., Mozzi F., Font de Valdez G., 2008. Whey fermentation by termophilic lactic acid bacteria: Evolution of carbohydrates and protein content. Food Microb. 25, 442--451.

Pikul J., Cais-Sokolińska D., Górecka D., Flaczyk E., 2006. Mleko i koncentraty mleczne [Milk and milk concentrates]. In: Towaroznawstwo produktów spożywczych. Eds E. Flaczyk, D. Górecka, J. Korczak. Wyd. AR Poznań, 59-84 [in Polish].

PN-75/A-86130. 1975. Mleko i przetwory mleczarskie. Napoje mleczne. Metody badań [Milk and milk products. Milk beverages. Analytical methods] [in Polish].

Tormo M., Izco J.M., 2004. Alternative reverse-phase high-performance liquid chromatography method to analyze organic acids in diary products. J. Chromat. A, 1033, 305-310.

Zmarlicki S., 2006. The heath aspects of milk and dairy products. Zdrow. Publ. 116, 142-146.

DYNAMIKA UKWASZANIA MLEKA OWCZEGO O ZWIĘKSZONYM UDZIALE BIAŁEK SERWATKOWYCH

Wstęp. Przeprowadzone dotychczas badania dotyczące mlecznych napojów fermentowanych wyprodukowanych z mleka owczego skupiały się głównie na ocenie jakości gotowego produktu. Celem pracy była ocena wpływu zwiększonego udziału białek serwatkowych w mleku owczym na przebieg procesu jego fermentacji.
Materiał i metody. Surowcem do badań był mleko owcze i krowie. Dynamikę procesu fermentacji oraz stabilność wytworzonych napojów opisano za pomocą wartości pH, kwasowości miareczkowej, zawartości laktozy, galaktozy oraz kwasu mlekowego.
Wyniki. Zwiększony w stosunku do naturalnego udział białek serwatkowych do kazeinowych w mleku owczym (1:1 oraz 4:1) wpłynął na zwiększenie kwasowości gotowego produktu po 18 h inkubacji oraz obniżenie stopnia odfermentowania laktozy. Chłodnicze przechowywanie napojów wpłynęło na zmianę wartości pH, kwasowości miareczkowej, zawartości galaktozy i kwasu mlekowego w produktach.
Wnioski. Zwiększenie udziału białek serwatkowych w mleku owczym jest przyczyną zmiany dynamiki procesu fermentacji, lecz nie wpływa na stabilność gotowego produktu.

Słowa kluczowe: mleko owcze, białka serwatkowe, fermentacja, dynamika

Received – Przyjęto: 16.08.2010

Accepted for print – Zaakceptowano do druku: 6.12.2010

For citation – Do cytowania: Lasik A., Pikul J., Danków R., Cais-Sokolińska D., 2011. The fermentation dynamics of sheep milk with increased proportion of whey proteins. Acta Sci. Pol., Technol. Aliment. 10(2), 155-163.