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Analysis of metabolic activity of lactic acid bacteria and yeast in model kefirs made from goat's milk and mixtures of goat's milk with mare's milk based on changes in electrical conductivity and impedance

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Abstract

The aim of this study was to analyse the metabolic activity of lactic acid bacteria and yeast based of changes in electrical conductivity and electrical impedance during fermentation of goat milk and mixtures of goat's milk and mare's milk (1:1, 1:2). As a result of fermentation, conductivity increased 1.4-fold. The conductivity of kefir prepared from goat and mare milk mixed at a ratio of 1:2 (6.210 $\Omega^{-3}\cdot cm^{-1}$) was lower than that of the 1:1 mixture or of goat milk alone (7.242 $\Omega^{-3}\cdot cm^{-1}$). A significant dependence of electrical conductivity and pH ($0.970 \le r \le 0.993$) was recorded during fermentation. The addition of mare milk to goat milk significantly slowed down the growth of LAB (Δ_{λ} = 0.8 h) and yeasts during kefir production.

Key words: conductivity, electrical impedance, goat's milk, mare's milk

Introduction

There is a high correlation between the counts of metabolically active lactic acid bacteria (LAB) and the increased conductivity (Lanzanova et al., 1993). Electrical conductivity (EC) and electrical impedance (EI) reflect the dynamics of milk acidification, since they depend on its chemical composition and electrolyte dissociation (St-Gelais and Champagne, 1995; Zhuang et al., 1997; Mabrook and Petty, 2003). It has even been shown that EC may be used as an alternative to pH measurements in monitoring the activity of cheese starters (Paquet et al., 2000).

However, almost all studies describing EC and EI concern cow's milk. There is a lack of information concerning the electrical properties of goat's and mare's milk, and particularly on mixtures of these milks, subjected to fermentation processes. Such information might be useful for commercial practice in dairies and it is thus crucial to investigate this problem, since many innovative dairy products may be produced from goat's and mare's milk (Cais-Sokolińska et al., 2015; 2016a). Due to the rich composition and unique properties of goat's milk, goat dairy products are being increasingly developed. Target group of consumers address

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contemporary educated adults, children, the elderly, but also the well informed and convalescent individuals (Silanikove et al., 2010). The nutritional value of goat milk dairy products may be further enhanced with the addition of mare's milk, characterized by beneficial properties such as high levels of unsaturated fatty acids and vitamin C, as well as the optimal Ca/P ratio (Cieślak et al., 2015; Markiewicz-Kęszycka et al., 2015). Mare's milk contains numerous bioactive components, like enzymes, the long-chain polyunsaturated fatty acids, oligosaccharides, hormones, growth factor/protein and carnitine (Uniacke-Lowe et al., 2010; Markiewicz-Kęszycka et al., 2013; 2014).

The aim of this study was to characterize the metabolic activity of bacteria and yeast cultures based on changes in the EC and EI of model kefir produced from goat's milk and mixture of goat's milk with mare's milk. The electrical impedance dynamics during fermentation was established using the Gompertz mathematical model parameters.

Materials and methods

Goat's milk, mare's milk and mixtures thereof

Milk was collected from Polish White Improved goats and Polish Cold-blooded multiparous mares kept on an equine dairy farm in Western Poland. The microbiological quality and cytological parameters of this milk have previously been described by Cais-Sokolińska et al. (2016a; b). The composition of goat's milk was as follows - 28.6 $g \cdot kg^{-1}$ (N×6.38) protein, 40.1 $g \cdot kg^{-1}$ lactose, 8.8 $g \cdot kg^{-1}$ ash and pH value of 6.66. Mare's milk consisted of 24.0 $g \cdot kg^{-1}$ (N×6.38) protein, 53.0 $g \cdot kg^{-1}$ lactose, 5.7 $g \cdot kg^{-1}$ ash and pH value 6.58. The whole mare's milk contained 1.5 % fat, so the goat's milk was corrected for fat content to the same level. Mixtures (*v/v*) of goat's and mare's milk were prepared at ratios of 1:1 and 1:2. The milk and milk mixtures were pasteurized at 85 °C for 5 min.

Production of kefir samples

The goat's milk, mare's milk, and the mixtures thereof were subjected to combined lactic-acid–alcoholic fermentation. A starter culture under the joint trading code of 75106 (Abiasa Inc., Quebec, Canada) was used. Starter cultures were introduced in lyophilized form according to producers' instructions (30 u.a. / 100 L milk). Fermentation was performed at 22 °C for 24 h. The dose of introduced cultures was selected to ensure that the end point of fermentation would be a product with a pH value of 4.4. The products were analysed 48 hours after the end of the fermentation process.

Conductivity

A CC 401 conductometer coupled with an EC-60 sensor and a CT2B-121 temperature sensor was used (Elmetron, Zabrze, Poland). The conductivity sensor was equipped with black-coated platinum electrodes with K = 1 cm^{-1} (St-Gelais and Champagne, 1995).

Acidity

The pH value was measured using a CP-502 pH-meter (Elmetron, Zabrze, Poland) equipped with a ESAgP-301W type combined electrode probe (Eurosensor, Gliwice, Poland). The measurement was performed in accordance with the guidelines specified by Martinez-Villaluenga et al. (2006).

Electrical impedance for estimating the metabolic activity of bacteria and yeast culture

Electrical impedance was measured using a BacTrac^{Tm} 4100 analyser (SyLab, Purkersdorf-Wienna, Austria) (Čurda and Plockova, 1995; Walker et al., 2005). The *M* value was recorded, determining changes in impedance in the space between the electrodes, and the *E* value was measured in the layers adjacent to the electrodes. The threshold of impedance changes established was TV = 3 % (Pless et al., 1994). The intensity of CO_2 release as an indicator of yeast growth was a measure of binding by 0.2 % KOH solution. Special tubes of 10 mL and 20 mL, respectively, were used to assess the growth of LAB and yeast (Yang and Bashir, 2008). The tubes were sterilized before use at 121 °C for 15 min. Each tube was charged with a substrate (9 mL) and 1 mL of inoculum from the test starter culture. Within 15 min of inoculation, the tubes were placed in a thermostat and incubated for 24 h at 30 °C. Under these conditions, the impedance detection time (IDT) was recorded at a measurement frequency of 1 kHz (Flint and Brooks, 2001).

Statistical analysis

A level of significance at $\alpha = 0.05$ was adopted to verify the statistical hypotheses. The statistical calculations were performed using the Statistica data analysis software system (version 10, StatSoft, Inc., 2011).

Results

Conductivity changes

Prior to fermentation, goat's milk was characterized by a greater conductivity (5.211 $\Omega^{-3}\cdot cm^{-1}$) than the mare's milk (3.950 $\Omega^{-3}\cdot cm^{-1}$) ($P<0.05$). The conductivity of the unfermented mixture of mare's milk and goat's milk (1:1) (4.885 $\Omega^{-3}\cdot cm^{-1}$) did not differ significantly from that of goat's milk ($P>0.05$). During fermentation, the conductivity of the samples increased (on average Δ_{EC} = 2.121 $\Omega^{-3}\cdot cm^{-1}$; 46.4%). The conductivity of kefir made from the goat's milk was 7.244 $\Omega^{-3}\cdot cm^{-1}$, while that of the mixtures was 7.219 $\Omega^{-3}\cdot cm^{-1}$ (1:1) ($P>0.05$) and 6.210 $\Omega^{-3}\cdot cm^{-1}$ (1:2) ($P<0.05$). High values of normal distribution (SW–W ≥ 0.731) were obtained (Fig. 1). The conductivity of the goat's milk and its mixtures reflects the changes in pH during acidification ($0.970 \le r \le 0.993$).

Metabolic activity of LAB and yeast on the basis of changes in the impedance of their growth medium

The strongest proliferation of LAB was observed in goat's milk (IDT = 1.1 h) while the addition of mare's milk slowed LAB growth. In the mare's milk, IDT = 2.3 h (Table 1). Analysis of the Gompertz model parameters confirmed that LAB started to proliferate faster in the goat's milk than in the mare's milk (Table 2). However, no differences in bacterial growth initiation were shown between goat's milk (λ = 6.32) and the 1:1 mixture with mare's milk (λ = 6.33). The LAB growth rate was not consistent with the growth dynamics of the yeast (Table 1). The impedance DT values for yeast growth in the goat's milk and the mixtures did not differ ($P > 0.05$) and amounted on average 2.5 h. Yeast growth began the latest (3.0 h) in the mare's milk.

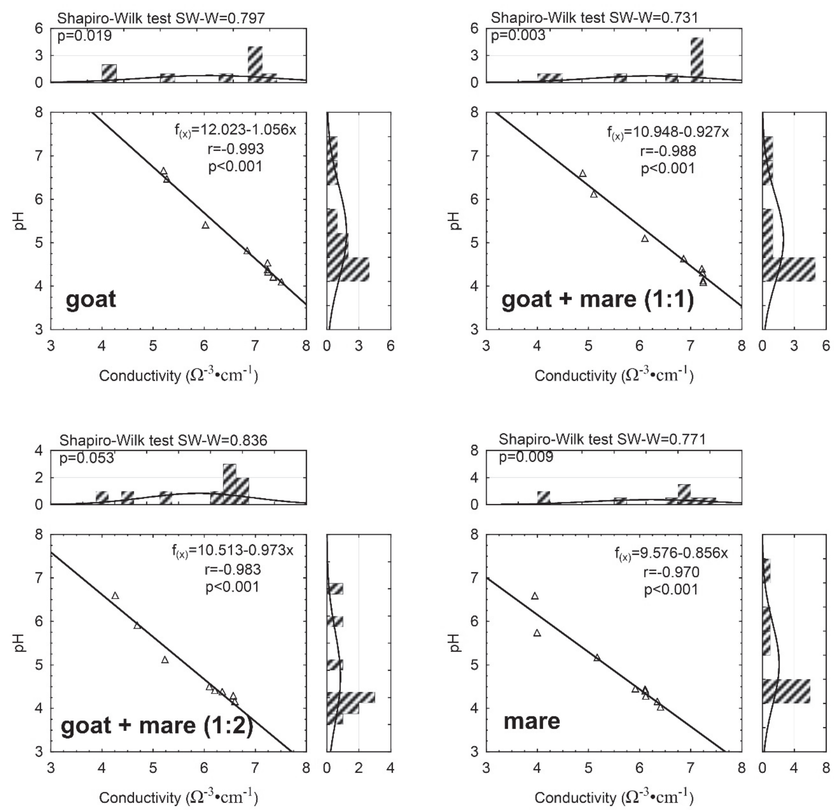

Figure 1. Analysis of the interdependence between conductivity and pH in kefir from goat milk and its mixtures, n=9

Discussion

Similar conductivity values for the goat's milk (from 4.3 to 13.9 $\Omega^{-3}\cdot cm^{-1}$) were reported by Park et al. (2007). Highly increased milk conductivity was observed within the first 6-7 h of fermentation as a result of changes in the colloid system, the formation of the casein micelle network and changes in the colloid solubility of calcium and phosphorus (Bornaz et al., 2010). Goat's milk contains larger amounts of minerals and casein than does mare's milk, which may also be responsible for the differences recorded in conductivity. A linear dependence between EC and pH in reconstituted acidified skim milk with a pH ranging from 6.4 to 4.6 was shown by St-Gelais and Champagne (1995). The fermentation dynamics reflect the metabolic activity of the LAB and yeast, which is the reason such diverse DT values were recorded. In investigating LAB growth Walker et al. (2005), showed a broad range of impedance DT (ranging from 6.0 h to 10.5 h) was required for LAB counts to reach 10^8 CFU mL^{-1}.

Conclusions

The electrical conductivity and impedance of the goat's milk and of the mixtures with mare's milk reflect the dynamics of fermentation and the metabolic activity of the characteristic microflora. The values of conductivity during lactic-acid–alcohol fermentation are strongly correlated with pH. The measurement of conductivity may be used to control the production process of goat's milk kefir with the addition of mare's milk. However, the addition of mare's milk to goat's milk slows the growth of LAB and yeast, which is indicated by the impedance kinetics and parameters of the Gompertz mathematical model. This may result in the application of other time and temperature parameters, or the modulation of the amounts of starter cultures in the production of such products from goat's milk.

Table 1. Changes in impedance parameters for growth of LAB and yeasts in goat milk and its mixtures (mean ± SD)

Impedance detection time for growth (h)	Milk sample			
	goat	goat + mare 1:1	goat + mare 1:2	mare
LAB	1.1±0.1^a	1.3±0.3^a	2.0±0.3^b	2.3±0.4^c
yeast	2.5±0.3^a	2.5±0.2^a	2.6±0.2^a	3.0±0.3^b

a-c, different small letters with mean values in rows indicate statistically significant differences at α=0.05; SD- standard deviation

Table 2. Assessment of LAB activity in goat milk and its mixtures based on changes in impedance kinetics and parameters of the Gompertz mathematical model

Milk sample	Model parameters $y=a\cdot e^{(-e^{(b-cx)})}$				Parameters of dynamics of impedance changes		
	a (-)	b (-)	c (-)	r	I_{max} (%h)	T_1 (h)	λ (h)
goat	21.53	1.93	0.31	0.978	2.45^c	1.51^a	6.32^c
goat + mare 1:1	20.37	1.83	0.30	0.977	2.33^c	1.67^b	6.33^c
goat + mare 1:2	17.15	1.25	0.25	0.964	1.54^b	2.24^c	5.26^b
mare	14.35	0.96	0.24	0.951	1.27^a	2.31^c	4.24^a

e - constant mathematical model, e=2.7183 (-); a-c, different small letters with mean values in column with dynamics parameters indicate statistically significant differences at α=0.05

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Analiza metaboličke aktivnosti bakterija mliječne kiseline i kvasaca u modelnim uzorcima kefira pripravljenim od kozjeg mlijeka i mješavina kozjeg i kobiljeg mlijeka na temelju promjena električne vodljivosti i otpora

Sažetak

Cilj ovog istraživanja bio je ispitati metaboličku aktivnost bakterija mliječne kiseline i kvasaca na temelju promjena u električnoj vodljivosti i otporu izmjerenim tijekom fermentacije kozjeg mlijeka i mješavina kozjeg i kobiljeg mlijeka (1:1, 1:2). Uslijed fermentacije električna vodljivost povećala se 1,4 puta. Vodljivost kefira napravljenog od mješavine kozjeg i kobiljeg mlijeka u omjeru 1:2 (6,210 $\Omega^{-3}\cdot cm^{-1}$) bila je manja u usporedbi s električnom vodljivosti kefira pripravljenog samo od kozjeg mlijeka (7,242 $\Omega^{-3}\cdot cm^{-1}$), odnosno od mješavine kozjeg i kobiljeg mlijeka u omjeru 1:1. Tijekom fermentacije utvrđena je statistički značajna ovisnost između električne vodljivosti i pH vrijednosti ($0,970 \leq r \leq 0,993$). Dodavanje kobiljeg mlijeka kozjem mlijeku značajno usporava rast bakterija mliječne kiseline (Δ_{λ} = 0,8 h) i kvasaca tijekom proizvodnje kefira.

Ključne riječi: električna vodljivost, električni otpor, kozje mlijeko, kobilje mlijeko

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