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Characteristics of fermented ewe's milk product with an increased ratio of natural whey proteins to caseins☆

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ABSTRACT

The aim of the study was to produce fermented ewe's milk with an increased ratio of native whey proteins and to characterize selected properties (metabolic activity, mono- and disaccharides, lactic acid and ethanol content, aroma profile, texture, color and sensory properties) of experimental milk and final product. Increasing the ratio of whey proteins to caseins in experimental milk resulted in higher activity of microorganisms belonging to the starter culture used in fermentation and higher content of volatile ketones in total volatile compounds identified during experiment. Increase in the lactic acid content and brightness parameter in fermented milk was observed in product made from experimental milk with modified ratio of whey proteins to caseins. It was concluded that changing the ratio of the main protein fractions in experimental milk differences in the physical and chemical properties of the final product, but these changes did not have a negative influence on sensory properties and product desirability.

1. Introduction

Developing new nutritional needs and sensory features of food products is associated with the growing interest in functional foods enriched with certain ingredients with documented health-promoting properties. Ewe's milk and the products derived from it are the source of many ingredients with a high biological value, which stimulate physiological processes for maintaining good health, boost the immune system and contribute to reducing the risk of lifestyle diseases (Haenlein and Wendorff, 2006; Park et al., 2007). In the last few years, a significant role in stabilizing vital functions was assigned to milk proteins as a source of biologically active peptides. Whey proteins are particularly rich in valuable and active peptides, which control the level of iron in blood, have antibacterial and antithrombotic properties and counteract the formation of free radicals. Their properties are mainly associated with high levels of branched-chain amino acids such as isoleucine, leucine and valine, which stimulate muscle protein synthesis (Gad and Sayed, 2009; Gustaw and Kozioł, 2011).

Nowadays, whey proteins concentrates (WPC) are widely used in yoghurt production to improve flavor, texture, nutritional value, extend shelf life of product and improve the activity of probiotic bacteria. The other positive aspects of using WPC are costs reduction and possibility to avoid addition of nondairy ingredients such as starch, gelatin or pectin (Glibowski, 2004; Onwulata and Tomasula, 2006). It is known, that as a result of the concentration process during production of WPC, a part of functional properties of whey proteins subject to deterioration and some of these even disappear. The explanation for this fact can be found in utilization for the production of WPC a whey obtained after cheese manufacturing which was already subjected to the action of acid or an enzyme. The use of membrane processes for the separation of whey proteins and caseins from raw milk allows to keep all of the functional properties of both protein fractions (Miocinovic et al., 2016). An additional advantage is the ability to fully utilize caseins in cheese production and relieve cheese production from whey as a by-product. It is very

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important approach especially in the processing of small ruminant milk targeted in particular for the production of cheese.

In recent years, the food market has abounded in functional and convenience food. Increased public awareness of healthy diets has changed eating habits and consumer preferences (Gad and Sayed, 2009; Fluegel et al., 2010; Bhat and Bhat, 2011). Bovine and non-bovine species milk have presented increasing trends of consumer interests for consumption of healthy foods.

The aim of the study is to evaluate the possibility of manufacturing fermented ewe's milk with an increased ratio of native whey proteins to caseins (from 1:4 to 1:1) and to evaluate selected properties of experimental milk and final product.

2. Material and methods

2.1. Preparation of experimental ewe's milk

The experimental milk was consisted of processed ewe's milk derived from animals fed traditionally (Cais-Sokolińska et al., 2015; Dankow et al., 2015). In order to prepare the processed milk, raw ewe's milk had undergone membrane separation preceded by centrifugation, as a result of which cream and skimmed milk were obtained (Fig. 1). The cream was pasteurized at 98 °C for 5 min, and skimmed milk was used for microfiltration process. Double-modeled microfiltration was carried out using a system comprising two membranes, Isoflux membranes (TAMI, Nyons, France) with modified filter layer characterized by a cut-off of 1.4 μm (purpose: reduction of microorganisms number) and 0.2 μm (purpose: protein separation). The temperature at which the process was carried out was 20 °C. The initial pressure was 6.08 bar, and during the process it was reduced to 2.03 bar. The transmembrane pressure was 0.41 bar and the velocity through the membrane 2 m/s. The milk used for further experiments was combined with a suitable proportion of raw milk, cream and double filtrate (characterized by reduced number of microorganisms and contain only whey proteins). The fat content was standardized to 3%. This milk was then subjected to homogenization (152 bar), pasteurization at 80 °C for 15 s and after all these processes milk was cooled to 4–6 °C. As a result of these processes, 2 experimental milk samples were prepared: MO-1 (milk with a natural ratio of whey proteins to casein of 1:4) and MO-2 (milk with an increased ratio of whey proteins to caseins of 1:1). Milk samples prepared in accordance with the procedure described above were inoculated with a 0.1 g/L commercially used starter culture (*Lactobacillus delbruckii* subsp. *bulgaricus*, *Lactococcus lactis*, *Lactococcus lactis* subsp. *cremoris*, *Saccharomyces kefir*, *Candida kefir*). Incubation was carried out at 37 °C, until the active acidity had been reduced to pH 4.6. After incubation, the fermented milk was subjected to cooling at 4–6 °C. The final product was obtained after cooling for 24 h.

2.2. Methods

2.2.1. Rating metabolic activity of bacteria and yeast culture

Analysis of the metabolic activity of bacteria and yeast was performed using the method of measuring changes in the electrical impedance of the medium (Flint and Brooks, 2001; Gomez et al., 2002; Lasik and Nowak, 2010). In the study, a BacTrac 4100's Sy-Lab, Austria Automatic Analyzer of Microorganism Growth was used. Special tubes with a capacity of 10 mL, equipped with four electrodes were used to measure the metabolic activity of the bacteria. 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 of the Automatic Analyzer of Microbial Growth BacTrac 4100 and incubated for 24 h at 30 °C. Changes in the electrical impedance of the cultured medium were registered automatically every 10 min throughout the culture period. A graphical image of the impedance changes during the incubation of microorganisms is in the form of a curve, similar to the waveform of microbial growth.

Evaluation of the metabolic activity of the yeast was made using the indirect method, which is based on measurement of impedance changes not directly in the culture medium, but indirectly in a KOH solution (Noble et al., 1999). For measuring the special two-piece tubes, were used, consisting of one tube called 'external' with two electrodes and a second, 'internal' sterile tube. The internal sterile tube was filled with 4.5 mL of growth medium which had been inoculated with 0.5 mL inoculum of the test starter culture, and then placed in the external sterile tube, previously loaded with 2 mL of 0.2% potassium hydroxide solution. Within 15 min of inoculation, the tubes were placed in a thermostat of the Automatic Analyzer of Microbial Growth BacTrac 4100 and incubated for 24 h at 30 °C. Changes in the electrical impedance of culture medium were registered automatically every 10 min during incubation. A graphical image of changes in impedance recorded using the indirect method is curved in shape, which is a "mirror image" of the curves obtained using the direct method (negative values). The threshold of detection in the direct and indirect method was set at 5% (Flint and Brooks, 2001; Gomez et al., 2002).

2.2.2. Determination of mono- and disaccharide, organic acid and ethanol content

Analysis of experimental milk and fermented milk for the content of mono- and disaccharides, organic acids and ethanol using high performance liquid chromatography requires protein precipitation (Mullin and Emmons, 1997; Chick et al., 2001; Tormo and Izco 2004; Álvarez-Martin et al., 2008; Pescuma et al., 2008). To do this, 4.5 mL of 0.013N H_2SO_4 (experimental milk samples), or 0.01 N H_2SO_4 (for fermented milk samples) was added to the 0.5 mL of the sample, stirred well (15s, vortex) and placed in a boiling water bath for about 10 min. After this, the sample was allowed to cool to room temperature (20–30 min) and centrifuged (10 min, 3000 × *g*). The supernatant obtained was filtered through a Millex filter – LCR (Millipore) Low Protein Binding Hydrophilic LRCPTFE 0.45 nm (Chick et al., 2001). A volume of 20 μL of the prepared sample was loaded onto a column (HPX 87H, BioRad) connected to the RI detector. The mobile phase is a solution of 0.005 M H_2SO_4. The analysis time was 30 min, temperature 30 °C. The flow rate through the column was 0.6 mL/min.

2.2.3. Determination of aroma profile

2.2.3.1. Extraction of volatile compounds. The extraction of volatile compounds was performed by HS-SPME. Extraction was carried out at 50 °C for 40 min using Carboxen Polydimethylsiloxane (PDMS) 85 μm fiber (Cais-Sokolińska et al., 2011).

2.2.3.2. Determination of volatile compounds. Volatile compounds in experimental milk and final product (fermented milk) were determined by 2D GC–MS. Separation was carried out in two columns. The first column (non-polar), ZB-5, had a length of 30 m, a diameter of 250 μm, a film thickness of 0.25 μm; the second column (polar), Supelcowax 10, had a length of 0.9 m, a diameter of 100 μm and a film thickness of 0.1 μm. Modulation time was 3 s. An initial temperature of 45 °C in the first furnace was maintained for 2 min, and then increased by 8 °C/min to 150 °C, followed by an increase of 25 °C/min to 235 °C (5 min). The initial temperature of 60 °C in the second furnace was maintained for 2 min, increased by 8 °C/min to 175 °C followed by 25 °C/min to 260 °C (5 min). The flow of helium was 0.8 mL/min. Injection attempts were made at 240 °C, with a transfer line at 260 °C. The mass scan range was