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The effects of ultrasonication, fermentation with *Lactobacillus* sp., and dehydration on the chemical composition and microbial contamination of bovine colostrum

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ABSTRACT

The aim of this study was to evaluate the influence of ultrasonication, fermentation with *Lactobacillus plantarum* LUHS135 and *Lactobacillus paracasei* LUHS244, and different methods of dehydration on the chemical composition of bovine colostrum (BC), including the fatty acid and free amino acid profile and the content of micro- and macroelements. In addition, we analyzed the changes in lactic acid bacteria count, microbial contamination (aerobic mesophilic spore-forming bacteria, enterobacteria including *Escherichia coli*, and fungi/yeasts), the abundance of biogenic amines, and the concentration of nucleotide monophosphates. Significant effects of different treatments on the free amino acid profile were established, and an increase of lysine concentration by 1.2 to 95.9% was observed in treated BC. All of the treatments reduced the concentration of cadaverine, histamine, and tyramine in BC. The concentrations of macro- and microelements in BC followed the following order Ca > Na > K > Mg and Zn > Fe > Sr > Ba > Mn > Cu > Al > Se > Mo > Cr > Ni > Sn > Co > Pb > Cd. By combining the fermentation with *Lactobacillus plantarum* strain LUHS135 and vacuum drying, it was possible to increase the abundance of nucleotide monophosphates by more than 100%. All of the treatments reduced the microbial contamination of BC. Thus, the combination of ultrasonication, fermentation, and dehydration can be used for improving the properties and safety of BC.

Key words: bovine colostrum, ultrasonication and fermentation, dehydration, chemical composition, microbial contamination

INTRODUCTION

Bovine colostrum (**BC**) as functional food ingredient has been associated with the prevention and treatment of human diseases (Saad et al., 2016; Chae et al., 2017). Bovine colostrum has been demonstrated to possess antimicrobial activity, the ability to neutralize toxins (Støy et al., 2014), and to increase the growth of intestinal epithelial cells (Rathe et al., 2014). Based on these findings, BC is considered a very promising ingredient for functional food and nutraceutical formulations. However, the composition and physical properties of BC are highly variable due to several factors, including the preservation processes used (McGrath et al., 2016). Up to 60% of BC produced in the United States fail to meet the minimum requirements (i.e., >50 g/L of IgG and a total plate count of <100,000 cfu/mL, respectively; Morrill et al., 2012), raising concerns about the quality of BC. The specific composition of BC (high protein content and the presence of other sensitive or antimicrobial compounds) presents many challenges to industrial processing (McGrath et al., 2016), and only limited research has been carried out regarding the influence of various preservation methods on the detailed chemical composition of BC. The most common methods for the preservation of BC are cooling or freezing

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(Ramya et al., 2016). The possible use of potassium sorbate to preserve BC has been reported (Denholm et al., 2017), and anaerobic fermentation can also be employed to reduce the contamination of BC (Saalfeld et al., 2016). Fermentation is a natural way to preserve BC, but fermentation of proteinaceous material could lead to the formation of biogenic amines (**BA**); therefore, these compounds should be controlled. Another promising treatment for the preservation of BC is ultrasonication. Low-frequency ultrasonication can be used to improve the solubility of proteins and to increase the nutritional value of proteinaceous products (Bhandari and Zisu, 2016). Therefore, we hypothesized that the use of ultrasonication and fermentation, as well as different methods of dehydration, could be promising technologies for BC treatment to ensure biosafety parameters. However, changes of BC chemical composition should be controlled in addition to microbial contamination.

The aim of our study was to evaluate the influence of ultrasonication, fermentation with *Lactobacillus plantarum* strain LUHS135 and *Lactobacillus paracasei* strain LUHS244, and different methods of dehydration on the chemical composition of BC, including the fatty acid (**FA**) and free amino acid (**FAA**) profile and the content of micro- and macroelements. In addition, we analyzed the lactic acid bacteria (**LAB**) count, microbial contamination (aerobic mesophilic spore-forming bacteria, enterobacteria including *Escherichia coli*, and fungi/yeasts), as well as the concentrations of biogenic amines (**BA**) and nucleotide monophosphates (**NM**) in BC.

MATERIALS AND METHODS

Materials

Bovine colostrum was obtained within 2 h of calf delivery (from 20 cows) from Linas Agro agricultural company (Luksiai, Lithuania). The BC was selected in the winter at a farm of Lithuanian Black and White–Holstein dairy cows. The colostrum sample was collected at the same time and kept frozen (−20°C) until analyzed in the laboratory. Animals were cared for according to the requirements for keeping, maintenance and use of animals intended for experimental and other scientific purposes (Lithuanian State Food and Veterinary Service, 2003). All cows passed general health examinations monthly throughout. *Lactobacillus plantarum* LUHS135 and *Lactobacillus paracasei* LUHS244 strains, which previously demonstrated good antimicrobial activity against a variety of pathogenic microorganisms, were selected for BC fermentation (Bartkiene et al., 2017). The LAB strains were stored at −80°C in a Microbank system (Pro-Lab Diagnostics, Toronto, Canada) and grown in de Man, Rogosa, and Sharpe (**MRS**) broth (CM 0359, Oxoid, Hampshire, UK) at 30°C for 48 h before use.

Ultrasonication, Fermentation, and Dehydration of BC

Ultrasonication at low frequency (37 kHz, 160 W) was used for the treatment of BC. The equipment employed was Proclean 3.0DSP model (Ulsonix, Zielona Góra, Poland). Each 20-g sample of BC was processed for 20 min at 40°C and each experiment was performed at least twice.

Lactobacillus plantarum strain LUHS135 and *L. paracasei* strain LUHS244 were grown in MRS medium (Biolife, Milan, Italy) at 30°C. Two percent of the MRS solution (vol/vol), in which were the strains multiplied, were inoculated into fresh medium and propagated for 18 h; further multiplied strains (cell concentration 9.2 log10 cfu/mL, on average) were used for BC fermentation. *Lactobacillus plantarum* strain LUHS135 and *L. paracasei* strain LUHS244 were added to the BC (3%, vol/vol), followed by fermentation in CO_2 atmosphere in incubator (Memmert GmbH + Co. KG, Schwabach, Germany) for 24 h at 30°C. The BC samples after fermentation with LUHS135 and LUHS244 strains, as well as nonfermented BC samples, were dried by (1) freeze drying for 72 h at −40°C (Sublimator 3 × 4 × 5, Zirbus Technology, Bad Grund, Germany, condenser temperature −85°C, pressure 2×10^{-6} mPa), or (2) vacuum drying (temperature 45 ± 2.0°C and pressure 6×10^{-3} mPa in a XF020 vacuum dryer, France Etuves, Chelles, France).

In total, 11 samples were analyzed: fresh BC, freeze-dried at −40°C, vacuum-dried at 45°C, BC fermented with *L. plantarum*, BC fermented with *L. paracasei*, BC fermented with *L. plantarum* and freeze-dried at −40°C, BC fermented with *L. paracasei* and freeze-dried at −40°C, BC fermented with *L. plantarum* and vacuum-dried at 45°C, BC fermented with *L. paracasei* and vacuum-dried at 45°C, BC after ultrasonication and freeze drying at −40°C, and BC after ultrasonication and vacuum drying at 45°C.

Analysis of FA Composition in BC

The FA composition of BC was determined using a 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector (**GC-FID**), as described by Bartkiene et al. (2016).