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

Elena Bartkiene,^{*1} Vadims Bartkevics,^{†‡} Laura Elīna Ikkere,[‡] Iveta Pugajeva,[‡] Paulina Zavistanaviciute,^{*} Vita Lele,^{*} Modestas Ruzauskas,[§] Jurga Bernatoniene,[#] Valdas Jakstas,^{||} Dovile Klupsaite,[¶] Daiva Zadeike,[¶] Pranas Viskelis,^{**} and Grazina Juodeikiene[¶]

^{*}Department of Food Safety and Quality, Lithuanian University of Health Sciences, Tilzes St. 18, LT-47181 Kaunas, Lithuania

[†]Department of Chemistry, University of Latvia, Jelgavas iela 1, LV-1004 Riga, Latvia

[‡]Laboratory of Food and Environmental Investigations, Institute of Food Safety, Animal Health and Environment “BIOR,” Lejupes iela 3, LV-1076 Riga, Latvia

[§]Institute of Microbiology and Virology, Lithuanian University of Health Sciences, Tilzes St. 18, LT-47181 Kaunas, Lithuania

[#]Department of Drug Technology and Social Pharmacy, Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Sukilėlių pr. 13, LT-5016 Kaunas, Lithuania

^{||}Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Sukilėlių pr. 13, LT-5016 Kaunas, Lithuania

[¶]Department of Food Sciences and Technology, Kaunas University of Technology, Radvilėnu Rd. 19, LT-50254 Kaunas, Lithuania

^{**}Biochemistry and Technology Laboratory, Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kauno St. 30, LT-54333 Babtai, Lithuania

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, fermenta-

tion, 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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¹Corresponding author: elena.bartkiene@lsmuni.lt

(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 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, Basingstoke, 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, was inoculated into fresh medium and propagated for 18 h; further multiplied strains (cell concentration $9.2 \log_{10}$ 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 $3 \times 4 \times 5$, Zirbus Technology, Bad Grund, Germany, condenser temperature -85°C , pressure 2×10^{-6} mPa), or (2) vacuum drying (temperature $45 \pm 2.0^{\circ}\text{C}$ 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).

Determination of the FAA Profile

The FAA were extracted with 0.1 M HCl solution and the extracts were processed by ion exchange solid phase extraction and chloroformate derivatization using EZ:faast technology (Phenomenex, Torrance, CA), and then analyzed by GC-FID method (Agilent 6890N instrument) as described by Bartkiene et al. (2016). All elution and derivatization agents were provided by the EZ:faast amino acid analysis kit for protein hydrolysates by GC-FID or GC-nitrogen phosphorus detector.

Analysis of the BA Content

The procedures for extraction and determination of BA in BC samples were based on a report by Ben-Gigirey et al. (1999). An Agilent 1200 HPLC instrument with diode array detector and Chemstation LC software (Agilent 1200 Series, Waldbronn, Germany) was used for this analysis.

Analysis of Micro- and Macroelements in BC

The analysis of micro- and macroelements in BC was performed by inductively coupled plasma mass spectrometry (ICP-MS) according to a published method (Bartkiene et al., 2016), using an Agilent 7700x ICP-MS instrument with Mass Hunter Work Station software for ICP-MS, version B.01.01 (Agilent Technologies, Tokyo, Japan).

Analysis of NM in BC

The analysis of NM in BC was performed using liquid samples (2 g each) and freeze-dried samples (0.5 g each). The freeze-dried samples were dissolved in water before the analyses. The samples were mixed with 5 mL of aqueous 5% trichloroacetic acid solution. Each mixture was centrifuged (at $2,328 \times g$ for 15 min, 4°C) and the supernatants were filtered through 0.2- μ m polyethersulfone membrane filters before the instrumental analyses.

The chromatographic analysis was performed using an Acquity UPLC system (Waters, Milford, MA) coupled to a QTrap 5500 mass selective detector (AB Sciex, Framingham, MA). The separation of NM was achieved with a Phenomenex Kinetex 1.7- μ m C18 column (100 $\times$ 3.0 mm). Water acidified with 0.1% formic acid (A) and acetonitrile (B) were used as the mobile phases (flow rate = 0.3 mL/min, ambient column temperature, injection volume = 10 μ L, and gradient according to the following program: 0 min 100% A, 5 min 99% A, 6.5 min 95% A, 7 min 0% A, 7.5 min 0%

A, 12 min 100% A). The conditions for the detection by MS-MS were curtain gas at 206,842.7 Pa, ion spray voltage 4,500 V, temperature 550°C, ion source gas at 275,790.3 Pa, ion source gas at 413,685.4 Pa, and declustering potential 30 V. We used ion transitions adenosine monophosphate (AMP) 348 $\rightarrow$ 136, uridine monophosphate (UMP) 325 $\rightarrow$ 97, cytidine monophosphate (CMP) 324 $\rightarrow$ 112, guanosine monophosphate (GMP) 364 $\rightarrow$ 152, and inosine monophosphate (IMP) 349 $\rightarrow$ 137. The collision energy for all analytes was 18 eV, except that 25 eV was used for AMP.

Microbiological Analysis of BC

Microbiological analyses (LAB, aerobic mesophilic spore-forming bacteria, the total number of enterobacteria, *Escherichia coli*, and fungi/yeast counts) were performed with BC samples that were ultrasonicated, fermented or not fermented, and before or after drying. For the evaluation of LAB count, a BC sample (10 g) was homogenized with 90 mL of NaCl solution (9 g/L). Serial dilutions of 10^{-4} to 10^{-8} with NaCl solution were used for sample preparation. Sterile MRS agar (CM0361, Oxoid) was used for bacterial growth on petri plates. The plates after seeding with the sample suspension were incubated under anaerobic conditions at 30°C for 72 h. The number of aerobic mesophilic spore-forming bacteria was determined on plate count agar (CM0325, Oxoid). MacConkey and Tryptone Bile X-glucuronide agar (both from Oxoid) were used for the determination of the total number of enterobacteria and *Escherichia coli* count (at 35–37°C for 18–24 h). The fungi and yeasts were determined in chloramphenicol agar (CM0549, Oxoid) after incubation at 25°C for 5 d. The number of microorganisms was calculated and expressed as $\log_{10}$ of colony-forming units per gram or milliliters. All of the results were expressed as the mean values of 5 determinations.

Statistical Analysis

For the chemical analysis (FA and FAA profile, BA content, micro- and macroelements, NM content), 2 parallel samples were analyzed 3 times. For the microbiological analysis, 5 repetitions were performed. All of the results were expressed as the mean values. The obtained data were subjected to ANOVA using the statistical package SPSS for Windows (Version 15.0, SPSS, Chicago, IL), and the effects of different treatments on the parameters of BC were evaluated. The calculated mean values were compared using Duncan's multiple range test with significance defined at $P \leq 0.05$.

RESULTS AND DISCUSSION

FA Composition of Treated BC Samples

All of the investigated treatments of BC reduced the content of myristoleic (C14:1) acid (25.5%, on average), α -linolenic (C18:3n-3) acid (20.0%, on average), butyric (C4:0) acid (17.9%, on average), caproic (C6:0) acid (18.0%, on average), caprylic (C8:0) acid (22.2%, on average), capric (C10:0) acid (17.1%, on average), lauric (C12:0) acid (18.0%, on average), myristic (C14:0) acid (14.0%, on average), pentadecanoic (C15:0) acid (11.1%, on average), and stearic (C18:0) acid (10.1%, on average), as well as the total content of saturated fats (5.1%, on average), *trans*-fats (43.3%, on average), and n-3 fatty acids (31.7%, on average; Table 1). Opposite trends were found for the content of *cis*-10 heptadecenoic (C17:1) acid (increased by 33.3%, on average, in the treated samples), oleic (C18:1n-9 *cis*) acid (increased by 12.2%, on average, in the treated samples), linoleic (C18:2n-6 *cis*) acid (increased by 12.9%, on average, in the treated samples), *cis*-8,11,14-eicosatrienoic (C20:3n-6) acid (increased by 33.3%, on average, in the treated samples), palmitic (C16:0) acid (increased by 3.5%, on average, in the treated samples), arachidic (C20:0) acid (increased by 300.0%, on average, in the treated samples), the total content of UFA (increased by 10.5%, on average, in the treated samples), n-6 FA (increased by 12.1%, on average, in the treated samples), and n-9 FA (increased by 13.4%, on average, in the treated samples) in treated BC samples. In most cases, significant effects of different treatments on the FA content ($P < 0.001$) were established. The influence of ultrasonication, fermentation with LAB strains LUHS135 and LUHS244, and different methods of dehydration also significantly affected the total content of SFA, the total content of UFA, as well as the presence of n-3, n-6, n-9, and *trans*-fatty acids in BC ($P < 0.0001$). Unsaturated FA elaidic (C18:1n-9 *trans*) acid, γ -linolenic (C18:3n-6) acid, *cis*-11-eicosenoic (C20:1) acid, *cis*-11,14-eicosadienoic (C20:2) acid, *cis*-11,14,17-eicosatrienoic (C20:3n-3) acid, *cis*-13,16-docosadienoic (C22:2) acid, and eicosapentaenoic (C22:5n-3) acid, as well as SFA undecanoic (C11:0) acid, heneicosanoic (C21:0) acid, tricosanoic (C23:0) acid, and lignoceric (C24:0) acids were not detected in any of the treated or untreated BC samples.

Limited data were available about the FA composition of BC, with most of the published studies reporting that BC contains high levels of C18:0 and C18:1 FA (Laakso et al., 1996; Palmquist, 2006). Bovine colostrum contains a lower amount of *trans*-FA, including *cis*-9,*trans*-11-octadecadienoic (C18:2) acid, compared with milk (Paszczyk et al., 2005). Essential FA

are involved in many metabolic processes, with their deficiency or imbalance leading to illnesses (Willett, 2012). Many factors can be involved in changes of FA composition, including fermentation with LAB. It has been reported that some of the LAB strains can change the *cis*-9,*cis*-12 diene structure of C18 FA such as linoleic acid, α -linolenic acid, and γ -linolenic acid into the conjugated *cis*-9,*trans*-11 and *trans*-9,*trans*-11 diene structures (Kishino et al., 2010). Finally, the treatment of BC significantly affected the concentrations of all FA (except C22:1n-9), and in treated BC samples higher total content of UFA, n-6 FA, and n-9 FA was found; it should also be mentioned that *trans* fatty acids were not increased. According to our findings, the used treatments had no negative influence on FA.

FAA Profile of BC

The content of essential FAA and nonessential FAA in BC are presented in Table 2. The lowest amounts of Val, Leu, Ile, and Met were found in freeze-dried BC samples (0.90 ± 0.02 , 1.80 ± 0.02 , 0.70 ± 0.01 , and $0.50 \pm 0.02\%$, respectively); however, freeze-dried BC samples contained the highest amounts of Lys ($66.60 \pm 1.18\%$). Ultrasonication increased the content of Val, Leu, Ile, Thr, and Met in BC samples, with the highest amounts of Val (5.10 ± 0.05 and $4.30 \pm 0.03\%$) found in BC samples treated by ultrasonication and freeze drying or by ultrasonication and vacuum drying at 45°C , respectively. The highest concentrations of Leu, Ile, and Met were established in BC samples after ultrasonication and vacuum drying at 45°C (12.90 ± 0.04 , 5.00 ± 0.04 , and $2.50 \pm 0.01\%$, respectively), and the highest content of Thr was found in BC samples after ultrasonication and freeze drying ($2.80 \pm 0.01\%$). All of the used treatments increased the content of Lys in BC samples [by 1.2 to 95.9% in ultrasonicated and vacuum-dried (45°C) BC and in freeze-dried BC, respectively], except in the case when ultrasonication was combined with freeze drying.

Bovine colostrum has been proposed as a dietary supplement for humans due to its high protein content, as well as the presence of EAA that are important for balanced diet. Leucine is known to have a key role in muscle protein anabolism (Paddon-Jones et al., 2015), and histidine participates in the elimination of excess metal ions from the body and participates in the synthesis of collagen (Villacrs et al., 2015). Histidine is also recommended for muscle mass loss prevention after gastric bypass surgery (Katsanos et al., 2016). Threonine and isoleucine are involved in enzymatic activity regulation (Crdenas, 2015), methionine has an essential role in protein synthesis (Xue et al., 2015), and tyrosine is converted in human body to melanin

Table 1. Fatty acid composition (% of total fatty acid) of bovine colostrum (BC) samples¹

Fatty acid	BC	BC _{lyoph}	BC _{vachdried}	BC _{L,plant}	BC _{L,par}	BC _{L,plant lyoph}	BC _{L,par lyoph}	BC _{L,plant vachdried}	BC _{L,par vachdried}	BC _{ultr lyoph}	BC _{ultr vachdried}
Unsaturated fats											
Myristoleic C14:1	1.1 ± 0.03 ^c	0.8 ± 0.02 ^a	0.9 ± 0.04 ^b	0.8 ± 0.02 ^a	0.8 ± 0.02 ^a	0.8 ± 0.01 ^a	0.8 ± 0.02 ^a	0.8 ± 0.01 ^a	0.9 ± 0.02 ^b	0.8 ± 0.02 ^a	0.8 ± 0.02 ^a
<i>cis</i> -10-Pentadecenoic C15:1	0.2 ± 0.02 ^a	0.2 ± 0.01 ^a	0.2 ± 0.03 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.03 ^a	0.2 ± 0.03 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a
Palmitoleic C16:1n-9 <i>cis</i>	1.3 ± 0.01 ^a	1.9 ± 0.02 ^c	1.9 ± 0.1 ^c	1.9 ± 0.01 ^c	1.9 ± 0.02 ^c	1.8 ± 0.02 ^b	1.8 ± 0.05 ^b	1.9 ± 0.02 ^c	1.9 ± 0.1 ^c	1.9 ± 0.01 ^c	1.9 ± 0.01 ^c
<i>cis</i> -10-Heptadecenoic C17:1	0.3 ± 0.02 ^a	0.4 ± 0.03 ^b	0.4 ± 0.04 ^b	0.4 ± 0.02 ^b	0.4 ± 0.03 ^b	0.4 ± 0.01 ^b	0.4 ± 0.03 ^b	0.4 ± 0.03 ^b	0.4 ± 0.03 ^b	0.4 ± 0.01 ^b	0.4 ± 0.04 ^b
Oleic C18:1n-9 <i>cis</i>	25.1 ± 0.02 ^a	28.4 ± 0.03 ^c	28.3 ± 0.06 ^c	28.1 ± 0.08 ^c	28.2 ± 0.01 ^c	28.3 ± 0.04 ^c	28.3 ± 0.05 ^c	28.1 ± 0.03 ^c	27.5 ± 0.04 ^b	28.0 ± 0.2 ^c	28.3 ± 0.06 ^c
Linoleic C18:2n-6 <i>cis</i>	2.1 ± 0.01 ^a	2.4 ± 0.04 ^c	2.4 ± 0.1 ^c	2.4 ± 0.02 ^c	2.4 ± 0.04 ^c	2.3 ± 0.03 ^b	2.3 ± 0.02 ^b	2.3 ± 0.02 ^b	2.4 ± 0.02 ^c	2.4 ± 0.05 ^c	2.4 ± 0.02 ^c
Linolelaidic C18:2n-6 <i>trans</i>	0.3 ± 0.03 ^b	—	—	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.3 ± 0.02 ^b	0.2 ± 0.01 ^a	0.3 ± 0.03 ^b	0.3 ± 0.01 ^b	—	0.2 ± 0.01 ^a
α-Linolenic C18:3n-3	0.5 ± 0.02 ^b	0.4 ± 0.02 ^a	0.4 ± 0.03 ^a	0.4 ± 0.01 ^a	0.4 ± 0.03 ^a	0.4 ± 0.06 ^a	0.4 ± 0.02 ^a	0.4 ± 0.01 ^a	0.4 ± 0.01 ^a	0.4 ± 0.02 ^a	0.4 ± 0.04 ^a
<i>cis</i> -8,11,14-Eicosatrienoic C20:3n-6	0.3 ± 0.04 ^a	0.4 ± 0.03 ^b	0.4 ± 0.02 ^b	0.4 ± 0.02 ^b	0.4 ± 0.04 ^b	0.4 ± 0.04 ^b	0.4 ± 0.03 ^b	0.4 ± 0.02 ^b	0.4 ± 0.02 ^b	0.4 ± 0.01 ^b	0.4 ± 0.03 ^b
Arachidonic C20:4n-6	0.5 ± 0.02 ^b	0.5 ± 0.01 ^b	0.5 ± 0.01 ^b	0.5 ± 0.03 ^b	0.5 ± 0.01 ^b	0.4 ± 0.06 ^a	0.5 ± 0.03 ^b	0.4 ± 0.01 ^a	0.5 ± 0.03 ^b	0.5 ± 0.02 ^b	0.5 ± 0.04 ^b
Eruic C22:1n-9	0.2 ± 0.06 ^a	0.2 ± 0.01 ^a	0.2 ± 0.02 ^a	0.2 ± 0.02 ^a	0.2 ± 0.03 ^a	0.2 ± 0.01 ^a	0.2 ± 0.05 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a
Docosahexaenoic C22:6n-3	0.1 ± 0.01 ^a	—	—	—	—	0.1 ± 0.01 ^a	—	—	—	—	—
Nervonic C24:1	0.5 ± 0.01 ^b	0.4 ± 0.01 ^a	0.4 ± 0.01 ^a	0.5 ± 0.01 ^b	0.5 ± 0.01 ^b	0.4 ± 0.01 ^a	0.4 ± 0.02 ^a	0.4 ± 0.03 ^a	0.5 ± 0.02 ^b	0.4 ± 0.01 ^a	0.5 ± 0.01 ^b
Saturated fats											
Butyric C4:0	2.4 ± 0.03 ^d	1.9 ± 0.01 ^a	2.1 ± 0.03 ^c	2.1 ± 0.02 ^c	1.9 ± 0.01 ^a	1.9 ± 0.02 ^a	1.9 ± 0.03 ^a	2.0 ± 0.02 ^b	2.0 ± 0.03 ^b	2.0 ± 0.04 ^b	1.9 ± 0.2 ^a
Caproic C6:0	1.5 ± 0.02 ^c	1.2 ± 0.02 ^a	1.2 ± 0.01 ^a	1.2 ± 0.03 ^a	1.2 ± 0.02 ^a	1.2 ± 0.06 ^a	1.2 ± 0.01 ^a	1.3 ± 0.05 ^b	1.3 ± 0.01 ^b	1.3 ± 0.04 ^b	1.2 ± 0.05 ^a
Caprylic C8:0	0.9 ± 0.01 ^b	0.7 ± 0.01 ^a	0.7 ± 0.04 ^a	0.7 ± 0.04 ^a	0.7 ± 0.03 ^a	0.7 ± 0.03 ^a	0.7 ± 0.01 ^a	0.7 ± 0.01 ^a	0.7 ± 0.01 ^a	0.7 ± 0.04 ^a	0.7 ± 0.04 ^a
Capric C10:0	1.7 ± 0.06 ^c	1.4 ± 0.04 ^a	1.4 ± 0.03 ^a	1.4 ± 0.06 ^a	1.4 ± 0.01 ^a	1.4 ± 0.04 ^a	1.5 ± 0.02 ^b	1.4 ± 0.03 ^a	1.5 ± 0.02 ^b	1.4 ± 0.03 ^a	1.4 ± 0.03 ^a
Lauric C12:0	3.0 ± 0.02 ^c	2.4 ± 0.05 ^a	2.5 ± 0.05 ^b	2.4 ± 0.07 ^a	2.4 ± 0.02 ^a	2.5 ± 0.05 ^b	2.5 ± 0.01 ^b	2.5 ± 0.02 ^b	2.5 ± 0.05 ^b	2.5 ± 0.04 ^b	2.4 ± 0.05 ^a
Tridecanoic C13:0	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a
Myristic C14:0	14.1 ± 0.06 ^d	12.0 ± 0.04 ^a	12.1 ± 0.01 ^{ab}	12.0 ± 0.03 ^a	12.0 ± 0.03 ^a	12.2 ± 0.04	12.3 ± 0.09 ^{bc}	12.1 ± 0.07 ^{ab}	12.3 ± 0.04 ^c	12.20 ± 0.05 ^{bc}	12.0 ± 0.07 ^a
Pentadecanoic C15:0	0.9 ± 0.01 ^b	0.8 ± 0.03 ^a	0.8 ± 0.02 ^a	0.8 ± 0.02 ^a	0.8 ± 0.02 ^a	0.8 ± 0.02 ^a	0.8 ± 0.02 ^a	0.8 ± 0.01 ^a	0.8 ± 0.02 ^a	0.8 ± 0.04 ^a	0.8 ± 0.02 ^a
Palmitic C16:0	32.8 ± 0.05 ^a	34.0 ± 0.02	33.8 ± 0.03 ^b	33.8 ± 0.09 ^b	33.9 ± 0.05 ^b	33.8 ± 0.04 ^b	33.8 ± 0.07 ^b	33.9 ± 0.05 ^b	34.3 ± 0.08 ^b	34.1 ± 0.04 ^{bc}	33.9 ± 0.06 ^b
Heptadecanoic C17:0	0.7 ± 0.01 ^b	0.6 ± 0.02 ^a	0.6 ± 0.01 ^a	0.7 ± 0.02 ^b	0.6 ± 0.02 ^a	0.6 ± 0.02 ^a	0.6 ± 0.02 ^a	0.6 ± 0.03 ^a	0.6 ± 0.03 ^a	0.6 ± 0.02 ^a	0.6 ± 0.01 ^a
Stearic C18:0	9.2 ± 0.09 ^c	8.4 ± 0.02 ^d	8.2 ± 0.02 ^b	8.3 ± 0.04 ^c	8.4 ± 0.07 ^d	8.3 ± 0.01 ^c	8.2 ± 0.03 ^b	8.3 ± 0.06 ^c	8.1 ± 0.01 ^a	8.2 ± 0.01 ^b	8.3 ± 0.02 ^c
Arachidic C20:0	0.1 ± 0.01 ^a	0.4 ± 0.01 ^b	0.4 ± 0.04 ^b	0.4 ± 0.02 ^b	0.4 ± 0.03 ^b	0.4 ± 0.02 ^b	0.4 ± 0.01 ^b	0.4 ± 0.02 ^b	0.1 ± 0.01 ^a	0.4 ± 0.01 ^b	0.4 ± 0.01 ^b
Behenic C22:0	0.1 ± 0.02 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.04 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.02 ^a	0.1 ± 0.01 ^a
Total saturated	67.5 ^c	64.0 ^a	64.0 ^a	64.0 ^a	63.9 ^a	64.0 ^a	64.1 ^{ab}	64.2 ^{ab}	64.4 ^{ab}	64.4 ^{ab}	63.8 ^a
Total unsaturated	32.5 ^a	36.0 ^b	36.0 ^b	36.0 ^b	36.1 ^{bc}	36.0 ^b	35.9 ^b	35.8 ^b	35.6 ^b	35.6 ^b	36.2 ^{bc}
<i>trans</i> fatty acids	0.3 ^b	—	—	0.2 ^a	0.2 ^a	0.3 ^b	0.2 ^a	0.3 ^b	0.3 ^b	—	0.2 ^a
n-3	0.6 ^c	0.4 ^a	0.4 ^a	0.4 ^a	0.4 ^a	0.5 ^b	0.4 ^a	0.4 ^a	0.4 ^a	0.4 ^a	0.4 ^a
n-6	2.9 ^a	3.3 ^d	3.3 ^d	3.3 ^d	3.3 ^d	3.1 ^b	3.2 ^c	3.1 ^b	3.3 ^d	3.3 ^d	3.3 ^d
n-9	26.6 ^a	30.5 ^{de}	30.4 ^d	30.2 ^c	30.3 ^c	30.3 ^c	30.3 ^c	30.2 ^c	29.6 ^b	30.1 ^{bc}	30.4 ^d

^{a-e}Mean values with different superscripts are significantly different ($P \leq 0.05$).¹BC_{lyoph} = freeze-dried (−40°C) BC; BC_{vachdried} = vacuum dried (+45°C) BC; BC_{L,plant} = BC fermented with *Lactobacillus plantarum*; BC_{L,par} = BC fermented with *Lactobacillus paracasei*; BC_{L,plant lyoph} = BC fermented with *L. plantarum* and freeze dried (−40°C); BC_{L,par lyoph} = BC fermented with *L. paracasei* and freeze dried (−40°C); BC_{L,plant vachdried} = BC fermented with *L. plantarum* and vacuum dried (+45°C); BC_{L,par vachdried} = BC fermented with *L. paracasei* and vacuum dried (+45°C); BC_{ultr lyoph} = BC after ultrasonication and freeze drying (−40°C); BC_{ultr vachdried} = BC after ultrasonication and vacuum drying (+45°C). Data expressed as mean values (n = 3) ± SD.

Table 2. The content of essential (top) and nonessential (bottom) free amino acids (FAA; %) among the extracted AA from bovine colostrum samples¹

Content (%) among the extracted AA											
Sample	Val	Leu	Ile	Thr	Met	Phe	Lys	His			
BC	3.70 ± 0.12 ^e	12.30 ± 0.17 ^f	4.90 ± 0.21 ^e	1.00 ± 0.07 ^d	2.40 ± 0.14 ^f	14.10 ± 0.43 ^f	34.00 ± 1.46 ^b	12.60 ± 1.26 ^b			
BC _{lyoph}	0.90 ± 0.05 ^a	1.80 ± 0.04 ^a	0.70 ± 0.09 ^a	0.60 ± 0.02 ^b	0.50 ± 0.06 ^a	10.00 ± 0.52 ^b	66.60 ± 1.61 ^g	8.80 ± 0.95 ^a			
BC _{vacdried}	3.40 ± 0.11 ^d	11.90 ± 0.15 ^e	4.00 ± 0.14 ^d	0.80 ± 0.04	2.30 ± 0.12 ^f	12.30 ± 0.27 ^d	41.80 ± 1.72 ^b	10.10 ± 0.81 ^b			
BC _{L-plant}	2.70 ± 0.06 ^c	4.80 ± 0.14 ^b	1.90 ± 0.07 ^b	1.80 ± 0.10 ^e	1.10 ± 0.09 ^b	7.50 ± 0.36 ^a	44.90 ± 2.34 ^d	14.10 ± 1.72 ^c			
BC _{L-par.}	1.10 ± 0.10 ^a	6.40 ± 0.20 ^c	1.90 ± 0.11 ^b	0.70 ± 0.02	1.40 ± 0.12 ^c	14.30 ± 0.71 ^f	44.40 ± 2.76 ^d	18.30 ± 1.39 ^d			
BC _{L-plant lyoph}	3.50 ± 0.15 ^d	11.80 ± 0.18 ^e	4.10 ± 0.08 ^d	0.90 ± 0.03	2.30 ± 0.16 ^f	13.00 ± 1.02 ^e	40.50 ± 2.11 ^c	12.30 ± 1.46 ^b			
BC _{L-par lyoph}	2.40 ± 0.10 ^b	10.50 ± 0.13 ^d	3.40 ± 0.15 ^c	0.50 ± 0.01 ^a	1.80 ± 0.11 ^d	13.20 ± 1.34 ^e	49.00 ± 2.35 ^f	13.10 ± 1.24 ^{bc}			
BC _{L-plant vacdried}	2.80 ± 0.12 ^c	9.60 ± 0.10 ^d	3.50 ± 0.10 ^c	0.70 ± 0.01 ^c	1.50 ± 0.14 ^c	11.10 ± 1.23 ^c	46.90 ± 1.95 ^e	13.10 ± 1.61 ^{bc}			
BC _{L-par vacdried}	2.30 ± 0.14 ^b	9.70 ± 0.09 ^d	3.30 ± 0.13 ^c	0.50 ± 0.05 ^a	1.80 ± 0.09 ^d	12.70 ± 1.08 ^d	50.30 ± 2.14 ^f	11.70 ± 1.33 ^b			
BC _{ultr lyoph}	5.10 ± 0.11 ^g	9.90 ± 0.12 ^d	3.70 ± 0.18 ^c	2.80 ± 0.01 ^f	2.10 ± 0.13 ^e	9.10 ± 1.14 ^b	28.60 ± 0.93 ^a	7.20 ± 0.84 ^a			
BC _{ultr vacdried}	4.30 ± 0.13 ^f	12.90 ± 0.14 ^f	5.00 ± 0.4 ^f	1.10 ± 0.07 ^d	2.50 ± 0.17 ^f	12.50 ± 1.19 ^d	34.40 ± 1.41 ^b	10.30 ± 0.93 ^b			

Sample	Ala	Gly	Ser	Pro	Asp	HYP	Glu	HLV
BC	0.80 ± 0.02 ^d	0.80 ± 0.06 ^f	1.90 ± 0.12 ^f	2.00 ± 0.14 ^e	1.00 ± 0.07 ^d	1.30 ± 0.12 ^g	5.60 ± 0.21 ^c	1.70 ± 0.11 ^f
BC _{lyoph}	0.20 ± 0.01 ^a	0.30 ± 0.03 ^a	0.80 ± 0.03 ^b	1.10 ± 0.11 ^b	0.70 ± 0.05 ^b	0.40 ± 0.06 ^c	5.70 ± 0.18 ^c	0.80 ± 0.07 ^d
BC _{vacdried}	0.80 ± 0.01 ^d	0.60 ± 0.02 ^d	0.90 ± 0.06 ^c	1.80 ± 0.17 ^d	0.90 ± 0.04 ^d	0.30 ± 0.05 ^b	7.80 ± 0.28 ^d	0.40 ± 0.05 ^a
BC _{L-plant}	0.90 ± 0.02 ^e	0.90 ± 0.04 ^g	2.30 ± 0.18 ^g	2.80 ± 0.15 ^g	2.60 ± 0.12 ^f	1.10 ± 0.07 ^f	5.90 ± 0.32 ^c	4.60 ± 0.17 ^g
BC _{L-par.}	1.10 ± 0.06 ^f	0.40 ± 0.02 ^b	2.20 ± 0.17 ^g	0.80 ± 0.09 ^a	1.10 ± 0.14 ^e	2.10 ± 0.11 ^h	2.00 ± 0.14 ^a	1.90 ± 0.21 ^f
BC _{L-plant lyoph}	0.80 ± 0.04 ^d	0.70 ± 0.05 ^e	1.10 ± 0.16 ^d	2.10 ± 0.12 ^e	0.90 ± 0.06 ^d	0.30 ± 0.04 ^b	5.60 ± 0.20 ^c	0.50 ± 0.07 ^b
BC _{L-par lyoph}	0.50 ± 0.07 ^b	0.50 ± 0.06 ^c	0.70 ± 0.08 ^a	1.10 ± 0.12 ^b	0.40 ± 0.04 ^a	0.20 ± 0.03 ^a	2.40 ± 0.19 ^a	0.40 ± 0.03 ^a
BC _{L-plant vacdried}	0.60 ± 0.05 ^c	0.50 ± 0.04 ^c	1.10 ± 0.07 ^d	1.60 ± 0.17 ^c	1.00 ± 0.07 ^d	0.80 ± 0.07 ^e	4.60 ± 0.16 ^b	0.50 ± 0.04 ^b
BC _{L-par vacdried}	0.50 ± 0.03 ^b	0.40 ± 0.07 ^b	1.20 ± 0.09 ^d	1.10 ± 0.09 ^b	0.80 ± 0.04 ^c	0.60 ± 0.05 ^d	2.30 ± 0.12 ^a	0.60 ± 0.07 ^e
BC _{ultr lyoph}	1.70 ± 0.09 ^g	1.50 ± 0.15 ^h	3.30 ± 0.14 ^h	4.40 ± 0.11 ^h	3.80 ± 0.21 ^g	0.30 ± 0.04 ^b	15.30 ± 1.05 ^f	1.10 ± 0.11 ^e
BC _{ultr vacdried}	0.90 ± 0.09 ^d	0.80 ± 0.08 ^f	1.40 ± 0.09 ^e	2.60 ± 0.13 ^f	1.20 ± 0.17 ^e	0.20 ± 0.03 ^a	9.40 ± 0.45 ^e	0.50 ± 0.04 ^b

^{a-h}Mean values with different superscripts are significantly different ($P \leq 0.05$).
¹HYP = 4-hydroxyproline; HLV = hydroxylysine; BC = bovine colostrum; BC_{lyoph} = freeze-dried (−40°C) BC; BC_{vacdried} = vacuum dried (+45°C) BC; BC_{L-plant} = BC fermented with *Lactobacillus plantarum*; BC_{L-par.} = BC fermented with *L. paracasei* and freeze dried (−40°C); BC_{L-plant lyoph} = BC fermented with *L. plantarum* and vacuum dried (−40°C); BC_{L-plant vacdried} = BC fermented with *L. plantarum* and vacuum dried (+45°C); BC_{L-par lyoph} = BC fermented with *L. paracasei* and vacuum dried (+45°C); BC_{L-par vacdried} = BC after ultrasonication and vacuum drying (+45°C). Data expressed as mean values (n = 3) ± SD.

(Wu et al., 2016). The rest of the essential FAA are also involved in the biological tissue maintenance and repair. Thus, the obtained results showing an increase of FAA content in BC as a result of different treatments (with ultrasonication being the most effective) are very promising. Besides the enhancement of EAA content, the availability of NEAA is also important (Brestenský et al., 2015). The highest content of NEAA (Ala, Gly, Ser, Pro, Asp, and Glu) was found in BC samples after ultrasonication and freeze drying, compared with untreated samples (increased by 112.5, 87.5, 73.7, 120.0, 280.0, and 173.2%, respectively). The results of ANOVA indicated a significant effect ($P < 0.0001$) associated with the treatment method on the concentrations of EAA and NEAA in the BC samples. According to results obtained, an increase of FAA (essential and nonessential) content in BC as a result of different treatments (with ultrasonication being the most effective) can be very promising because it leads to modification of BC physiological properties as well as an increase in BC as a functional food component.

Formation of BA During Treatment of BC

The highest concentrations of β -phenylethylamine, putrescine, and spermine were found in BC samples after ultrasonication and freeze drying (235.87 ± 12.5 , 830.07 ± 15.4 , and 7.85 ± 0.11 mg/kg, respectively; Table 3). The highest concentrations of cadaverine, histamine, and tyramine were found in untreated BC samples (28.62 ± 3.1 , 15.75 ± 2.1 , and 662.68 ± 12.4 mg/kg, respectively). Statistical analysis showed a very weak positive correlation between Thr and tyramine ($r = 0.1593$, $P = 0.002$) and a weak negative correlation between the amino acid Lys and cadaverine ($r = -0.3757$, $P = 0.001$) in BC samples. Furthermore, no correlations were found between His and histamine or between Phe and β -phenylethylamine. According to Montet and Ray (2016), arginine can be converted to agmatine and further to ornithine, which can be decarboxylated to putrescine. Likewise, lysine can be metabolized to cadaverine, and cadaverine by methylation to carnitine. Histidine can be decarboxylated to histamine, whereas tyramine, tryptamine, and β -phenylethylamine can be produced from tyrosine, tryptophan, and phenylalanine, respectively. Some BA are more toxic than others, but all BA are very important because they can potentiate the toxicity of each other, and the main precursors of BA are AA. Biogenic amines have specific physiological functions, but the consumption of products with high concentrations of BA can lead to health problems. For example, β -phenylethylamine, tryptamine, histamine, and tyramine can have negative effects on nervous and vascular systems (Papavergou et al., 2012); spermidine

and spermine can lead to allergy (Sugita et al., 2007); and tyramine, spermidine, spermine, putrescine, and cadaverine are precursors of nitrosamines (Önal, 2007). According to our results, different processing regimens should be tested to ensure the safety of ultrasonicated BC with regard to the formation of β -phenylethylamine, putrescine, and spermine. Results of the ANOVA indicated that the treatment method had a significant effect ($P < 0.0001$) on the concentrations of BA in BC samples.

Macro- and Microelement Content in BC

Relatively little is known about the concentrations of micro- and macroelements in BC (McGrath et al., 2016). Our findings about the content of macro- and microelements in BC are presented in Table 4. The content of macroelements in BC samples ranged in the order of $\text{Ca} > \text{Na} > \text{K} > \text{Mg}$. Compared with untreated BC samples, a higher content of macroelements was found in all of the treated BC samples, with the highest content of macroelements observed in all the BC samples that were fermented with *L. plantarum* and freeze dried. Even though Na is an essential macro element, dietary intake of Na typically far exceeds the nutritional requirements, with about 70 to 75% of the total intake provided by processed foods. The presence of high concentrations of Ca in BC has been reported (McGrath et al., 2016). Casein micelles found in BC during the colostrum period are less mineralized than later, during the lactation period. According to Jeong et al. (2009), the concentration of K in BC is 2,845 mg/kg, on average, and can range from 983 to 5,511 mg/kg. The reasons for such a high variation of K content in BC are unknown, but may be related to the nutritional status of the animal as well as environmental and genetic factors (Cashman, 2002).

The contents of microelements in BC samples ranged from $\text{Zn} > \text{Fe} > \text{Sr} > \text{Ba} > \text{Mn} > \text{Cu} > \text{Al} > \text{Se} > \text{Mo} > \text{Cr} > \text{Ni} > \text{Sn} > \text{Co} > \text{Pb} > \text{Cd}$ (Table 5). The presence of Al was observed in 3 out of 11 BC samples, such as those that were fermented with *L. plantarum* and vacuum dried, ultrasonicated and freeze-dried, or ultrasonicated and vacuum dried, at the levels of 0.014, 0.381, and 1.302 mg/kg of dry weight, respectively. According to EFSA (2008), a tolerable weekly intake of Al is 1 mg/kg of BW. The highest concentration of Cr was observed in vacuum-dried BC samples (0.249 mg/kg of dry weight), whereas the lowest concentration was found in BC that was fermented with *L. paracasei* (0.020 mg/kg of dry weight). Chromium in the form of Cr(III) plays a significant role in the metabolism of glucose, fats, and proteins in humans and animals, but data about tolerable daily intake of Cr is limited. The

Table 3. The content of biogenic amines (mg/kg) in bovine colostrum samples¹

BC sample	β-Phenylethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
BC	217.02 ± 10.04 ^f	—	28.62 ± 3.1 ^d	15.75 ± 2.1 ^c	662.68 ± 12.4 ^h	—	—
BC _{lyoph}	81.30 ± 5.2 ^e	186.80 ± 8.4 ^f	14.50 ± 2.4 ^b	—	62.17 ± 5.4 ^f	1.36 ± 0.02 ^c	7.10 ± 0.9 ^c
BC _{vacdried}	22.97 ± 2.3 ^c	48.38 ± 2.6 ^a	17.41 ± 2.6 ^{bc}	—	25.55 ± 2.9 ^d	0.21 ± 0.01 ^a	—
BC _{L-plant}	44.25 ± 3.4 ^d	136.34 ± 5.4 ^e	10.75 ± 2.1 ^a	—	19.66 ± 2.4 ^c	2.60 ± 0.04 ^f	—
BC _{L-par.}	20.25 ± 2.1 ^{bc}	50.30 ± 2.9 ^a	21.11 ± 2.9 ^c	—	18.59 ± 2.1 ^c	2.27 ± 0.06 ^e	—
BC _{L-plant lyoph}	16.57 ± 1.6 ^{ab}	68.22 ± 3.2 ^c	19.84 ± 2.4 ^c	0.47 ± 0.06 ^a	12.89 ± 1.2 ^a	—	—
BC _{L-par lyoph}	47.13 ± 3.7 ^d	113.91 ± 4.9 ^d	16.05 ± 2.4 ^b	2.13 ± 0.9 ^b	46.73 ± 3.7 ^e	3.88 ± 0.09 ^g	1.37 ± 0.04 ^b
BC _{L-plant vacdried}	13.78 ± 1.4 ^a	58.43 ± 3.2 ^{ab}	12.39 ± 2.1 ^a	—	11.10 ± 0.9 ^a	—	0.56 ± 0.01 ^a
BC _{L-par vacdried}	15.35 ± 2.5 ^a	42.91 ± 2.1 ^a	11.28 ± 1.9 ^a	0.51 ± 0.04 ^a	15.58 ± 1.4 ^b	1.82 ± 0.04 ^d	0.50 ± 0.02 ^a
BC _{ultr lyoph}	235.87 ± 12.5 ^g	830.07 ± 15.4 ^b	15.65 ± 2.5 ^b	—	163.94 ± 8.4 ^g	1.19 ± 0.03 ^b	7.85 ± 0.11 ^d
BC _{ultr vacdried}	210.02 ± 11.5 ^f	786.54 ± 14.3 ^g	16.20 ± 2.6 ^b	—	163.94 ± 8.3 ^g	1.19 ± 0.04 ^b	7.85 ± 0.08 ^d
Test of between-subjects effects							
Type III sum of square	244,697.348	2,696,366.527	785.602	662.232	1,105,315.923	47.537	354.681
F	587.622	4,898.922	12.802	139.412	2,019.604	2,821.419	16,963.012
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

^{a-h}Mean values with different superscripts are significantly different ($P \leq 0.05$).

¹BC = bovine colostrum; BC_{lyoph} = freeze-dried (−40°C) BC; BC_{vacdried} = vacuum dried (+45°C) BC; BC_{L-plant} = BC fermented with *Lactobacillus plantarum*; BC_{L-par.} = BC fermented with *Lactobacillus paracasei*; BC_{L-plant lyoph} = BC fermented with *L. plantarum* and freeze dried (−40°C); BC_{L-par lyoph} = BC fermented with *L. paracasei* and freeze dried (−40°C); BC_{L-plant vacdried} = BC fermented with *L. plantarum* and vacuum dried (+45°C); BC_{L-par vacdried} = BC fermented with *L. paracasei* and vacuum dried (+45°C); BC_{ultr lyoph} = BC after ultrasonication and freeze drying (−40°C); BC_{ultr vacdried} = BC after ultrasonication and vacuum drying (+45°C). Data expressed as mean values (n = 3) ± SD.

content of Mn in BC varied from 0.124 to 2.100 mg/kg of dry weight in untreated BC and BC samples that were fermented with *L. paracasei* and vacuum dried, respectively. The highest concentration of Fe (9.838 mg/kg of dry weight) was found in vacuum-dried samples; however, the lowest concentrations of Ni, Cu, Zn, and Sr (0, 0.127, 28.6, and 1.167 mg/kg of dry weight, respectively) were established in the same samples. The microelement Ni is involved in metabolism of Fe, and Ni deficiency in diet can lead to anemia (Shah et al., 2011). The microelements Co, I, Fe, and Zn are known to be often deficient in human nutrition (WHO, 1995). The concentrations of Co ranged from 0.003 to 0.037 mg/kg of dry weight in BC samples that were untreated or fermented with *L. plantarum* and vacuum dried, respectively. The highest content of Se was found in BC samples that were fermented with *L. paracasei* and freeze dried (0.707 mg/kg of dry weight). Selenium is involved in the functions of antioxidant enzymes in humans and animals, and the deficiency of Se can lead to many disorders and illnesses (Kumar and Priyadar-sini, 2014). Cadmium was found in 4 of the 11 analyzed BC samples (0.001 mg/kg of dry weight in samples that were only freeze dried or samples that were fermented with *L. paracasei* and then freeze dried; 0.004 mg/kg of dry weight in vacuum-dried BC samples; and 0.002 mg/kg of dry weight in BC samples that were fermented with *L. paracasei* and vacuum dried). Cadmium is toxic and can lead to destabilization of the reproductive and nervous systems (ATSDR, 2008); tolerable monthly intake of Cd is 25 µg/kg of BW (FAO/WHO 2010). Arsenic and Sb were not detected BC samples, whereas Pb and Sn were detected in 1 and 2 samples, respectively, out of the 11 samples analyzed.

Most of the micro- and macroelements are essential for human nutrition, but can negatively affect health in excessive amounts (Zelalem and Chandravanshi, 2014). It has been reported that the average concentrations of Cu, Fe, Zn, and Mn in BC were 1.7-, 10.7-, 10.9-, and 3.3-fold higher than the values for normal milk (Kehoe et al., 2007).

Influence of Different Treatment Procedures on Concentration of NM in BC

The concentrations of NM in BC samples are presented in Table 5. The results of the ANOVA indicated a significant effect ($P < 0.0001$) due to the treatment method on the concentrations of AMP, CMP, GMP, UMP, and IMP in BC samples. In the liquid samples group (untreated BC and BC fermented with the LAB strains LUHS135 and LUHS244), 4 of the 5 analyzed NM had lower concentrations; however, the concentration of CMP was higher in the BC samples fermented

Table 4. Macro- and microelements in bovine colostrum samples¹

Item (mg/ kg of dry weight)	BC	BC _{lyoph}	BC _{ultr lyoph}	BC _{ultr vacdried}	BC _{vacdried}	BC _{L,plant vacdried}	BC _{L,plant lyoph}	BC _{L,par vacdried}	BC _{L,par lyoph}	BC _{L,plant}	BC _{L,par}
Macroelement											
Na	740 ± 8.5 ^a	4,296 ± 20.4 ^f	4,927 ± 15.6 ^g	4,912 ± 15.4 ^g	5,119 ± 16.4 ^g	4,231 ± 12.3 ^e	5,441 ± 14.1 ^h	4,167 ± 13.8 ^d	5,671 ± 13.4 ⁱ	1,053 ± 10.2 ^b	1,024 ± 10.1 ^c
Mg	461 ± 4.1 ^a	2,023 ± 10.1 ^g	1,974 ± 10.4 ^e	2,003 ± 10.6 ⁱ	2,067 ± 12.4 ^h	1,872 ± 10.4 ^e	1,998 ± 10.9 ^f	1,810 ± 10.8 ^d	2,088 ± 12.7 ^h	487 ± 3.9 ^b	472 ± 3.4 ^b
K	987 ± 9.9 ^b	3,904 ± 13.8 ^f	3,864 ± 13.5 ^e	3,913 ± 14.2 ^f	4,000 ± 14.6 ^g	3,818 ± 13.7 ^d	4,028 ± 13.9 ^g	3,799 ± 12.9 ^c	4,184 ± 12.0 ^h	814 ± 8.6 ^a	812 ± 8.6 ^a
Ca	2,865 ± 13.5 ^b	12,840 ± 9.5 ^k	12,178 ± 20.4 ⁱ	12,570 ± 22.1 ^h	12,767 ± 22.7 ⁱ	11,605 ± 20.9 ^e	12,441 ± 21.9 ^g	11,294 ± 21.5 ^d	12,929 ± 23.8 ⁱ	2,996 ± 12.3 ^c	2,716 ± 12.1 ^a
Microelement											
Al	—	—	—	—	—	—	—	0.014 ± 0.01 ^a	—	0.381 ± 0.02 ^b	1.302 ± 0.04 ^c
Cr	0.057 ± 0.01 ^d	0.02 ± 0.01 ^a	0.249 ± 0.02 ^g	0.121 ± 0.01 ^f	—	0.026 ± 0.01 ^a	0.025 ± 0.01 ^a	0.043 ± 0.02 ^c	0.097 ± 0.03 ^c	0.035 ± 0.01 ^b	0.157 ± 0.04 ^g
Mn	0.124 ± 0.04 ^a	0.448 ± 0.01 ^c	0.491 ± 0.03 ^d	0.871 ± 0.04 ^f	1.122 ± 0.03 ^g	0.568 ± 0.01 ^d	0.313 ± 0.01 ^b	1.562 ± 0.02 ^h	2.100 ± 0.04 ⁱ	0.445 ± 0.01 ^c	0.535 ± 0.03 ^e
Fe	1.410 ± 0.4 ^b	9.004 ± 0.08 ⁱ	9.838 ± 0.09 ^k	3.578 ± 0.07 ^e	0.811 ± 0.01 ^a	5.933 ± 0.1 ^d	6.811 ± 0.2 ^e	7.207 ± 0.4 ^f	7.538 ± 0.1 ^g	8.095 ± 0.2 ^h	10.328 ± 0.4 ^e
Co	0.003 ± 0.01 ^a	0.015 ± 0.01 ^c	0.025 ± 0.01 ^e	0.006 ± 0.02 ^b	0.004 ± 0.01 ^a	0.021 ± 0.01 ^d	0.020 ± 0.01 ^d	0.037 ± 0.02 ^f	0.022 ± 0.02 ^d	0.018 ± 0.03 ^c	0.025 ± 0.02 ^e
Ni	0.044 ± 0.02 ^c	0.043 ± 0.01 ^c	0.221 ± 0.07 ^e	0.085 ± 0.02 ^d	—	0.028 ± 0.01 ^b	0.008 ± 0.01 ^a	0.048 ± 0.01 ^c	0.042 ± 0.01 ^c	0.045 ± 0.01 ^c	0.260 ± 0.04 ^f
Cu	0.219 ± 0.08 ^b	0.664 ± 0.06 ^d	0.767 ± 0.04 ^f	0.299 ± 0.08 ^e	0.127 ± 0.04 ^a	0.657 ± 0.06 ^d	0.727 ± 0.02 ^e	1.050 ± 0.09 ^g	0.657 ± 0.05 ^d	0.663 ± 0.06 ^d	1.424 ± 0.08 ^h
Zn	30.3 ± 2.5 ^b	126 ± 4.6 ^f	131 ± 5.2 ^g	33.6 ± 1.5 ^c	28.6 ± 1.9 ^a	123 ± 5.2 ^e	137 ± 5.4 ^h	121 ± 4.9 ^e	114 ± 4.1 ^d	128 ± 5.2 ^f	126 ± 4.9 ^f
As	—	—	—	—	—	—	—	—	—	—	—
Se	—	0.575 ± 0.01 ^f	0.675 ± 0.02 ^g	—	—	0.562 ± 0.04 ^e	0.707 ± 0.02 ^h	0.432 ± 0.04 ^a	0.553 ± 0.02 ^d	0.506 ± 0.03 ^b	0.518 ± 0.01 ^c
Sr	1.253 ± 0.04 ^b	5.288 ± 0.05 ^g	5.487 ± 0.06 ⁱ	1.245 ± 0.02 ^b	1.167 ± 0.01 ^a	5.142 ± 0.07 ^e	5.569 ± 0.07 ^j	4.971 ± 0.06 ^d	4.758 ± 0.04 ^c	5.328 ± 0.05 ^h	5.182 ± 0.04 ^f
Mo	—	0.177 ± 0.01 ^g	0.090 ± 0.01 ^b	—	—	0.107 ± 0.02 ^c	0.055 ± 0.01 ^a	0.130 ± 0.01 ^d	0.154 ± 0.04 ^f	0.107 ± 0.01 ^c	0.142 ± 0.02 ^e
Cd	—	0.001 ± 0.01 ^a	0.004 ± 0.01 ^b	—	—	—	0.001 ± 0.01 ^a	—	0.002 ± 0.01 ^a	—	—
Sn	—	0.044 ± 0.01 ^a	—	—	—	0.694 ± 0.04 ^b	—	—	—	—	—
Sb	—	—	—	—	—	—	—	—	—	—	—
Ba	0.436 ± 0.01 ^a	2.085 ± 0.04 ^h	2.130 ± 0.05 ^k	0.483 ± 0.01 ^b	0.440 ± 0.01 ^a	1.903 ± 0.06 ^d	2.113 ± 0.05 ⁱ	1.916 ± 0.07 ^e	1.764 ± 0.04 ^c	2.049 ± 0.09 ^f	2.075 ± 0.08 ^g
Pb	—	—	—	—	—	0.167 ± 0.01	—	—	—	—	—

^{a–i}Mean values with different superscripts are significantly different ($P \leq 0.05$).¹BC = bovine colostrum; BC_{lyoph} = freeze-dried (−40°C) BC; BC_{vacdried} = vacuum dried (+45°C) BC; BC_{L,plant} = BC fermented with *Lactobacillus plantarum*; BC_{L,par} = BC fermented with *Lactobacillus paracasei*; BC_{L,plant lyoph} = BC fermented with *L. plantarum* and freeze dried (−40°C); BC_{L,par lyoph} = BC fermented with *L. paracasei* and freeze dried (−40°C); BC_{L,plant vacdried} = BC fermented with *L. plantarum* and vacuum dried (+45°C); BC_{L,par vacdried} = BC fermented with *L. paracasei* and vacuum dried (+45°C); BC_{ultr lyoph} = BC after ultrasonication and freeze drying (−40°C); BC_{ultr vacdried} = BC after ultrasonication and vacuum drying (+45°C). Data expressed as mean values (n = 3) ± SD.

Table 5. The influence of different treatment methods on the concentrations of nucleotide monophosphates in BC¹

BC sample	Nucleotide monophosphates concentration (μg/g)				
	AMP	CMP	GMP	IMP	UMP
BC	0.203 ± 0.017	0.027 ± 0.004	0.066 ± 0.007	0.011 ± 0.002	0.085 ± 0.007
BC _{lyoph}	0.630 ± 0.014	0.150 ± 0.018	0.110 ± 0.020	0.770 ± 0.023	0.580 ± 0.031
BC _{vacdried}	2.04 ± 0.12	1.96 ± 0.13	3.96 ± 0.09	2.59 ± 0.11	2.99 ± 0.12
BC _{L.plant}	0.054 ± 0.009	0.383 ± 0.025	0.018 ± 0.002	0.003 ± 0.001	0.039 ± 0.005
BC _{L.par.}	0.056 ± 0.007	0.318 ± 0.014	0.015 ± 0.002	0.006 ± 0.001	0.049 ± 0.004
BC _{L.plant lyoph}	3.34 ± 0.12	2.32 ± 0.10	2.04 ± 0.07	3.18 ± 0.12	2.94 ± 0.11
BC _{L.par lyoph}	1.29 ± 0.10	0.680 ± 0.017	0.720 ± 0.011	1.26 ± 0.09	1.09 ± 0.09
BC _{L.plant vacdried}	3.53 ± 0.18	4.62 ± 0.16	4.37 ± 0.10	3.46 ± 0.10	6.25 ± 0.13
BC _{L.par vacdried}	5.98 ± 0.23	5.49 ± 0.23	6.43 ± 0.15	5.94 ± 0.14	12.94 ± 0.14
BC _{ultr lyoph}	0.720 ± 0.015	0.120 ± 0.010	0.130 ± 0.024	0.810 ± 0.028	0.550 ± 0.021
BC _{ultr vacdried}	1.62 ± 0.13	2.17 ± 0.14	3.90 ± 0.10	2.21 ± 0.11	3.14 ± 0.10

¹CMP = cytidine monophosphate; GMP = guanosine monophosphate; IMP = inosine monophosphate; UMP = uridine monophosphate; BC = bovine colostrum; BC_{lyoph} = freeze-dried (−40°C) BC; BC_{vacdried} = vacuum dried (+45°C) BC; BC_{L.plant} = BC fermented with *Lactobacillus plantarum*; BC_{L.par.} = BC fermented with *Lactobacillus paracasei*; BC_{L.plant lyoph} = BC fermented with *L. plantarum* and freeze dried (−40°C); BC_{L.par lyoph} = BC fermented with *L. paracasei* and freeze dried (−40°C); BC_{L.plant vacdried} = BC fermented with *L. plantarum* and vacuum dried (+45°C); BC_{L.par vacdried} = BC fermented with *L. paracasei* and vacuum dried (+45°C); BC_{ultr lyoph} = BC after ultrasonication and freeze drying (−40°C); BC_{ultr vacdried} = BC after ultrasonication and vacuum drying (+45°C). Data expressed as mean values (n = 5) ± SD.

with *L. plantarum* and *L. paracasei* (0.383 and 0.318 μg/kg, respectively). Among the BC samples in powder form, the highest content of the all analyzed NM was found in the BC samples after fermentation with *L. paracasei* and vacuum drying; compared with the average concentrations of NM, the BC samples after fermentation with *L. paracasei* and vacuum drying had AMP content that was higher by 149.8%, CMP content higher by 150.8%, GMP content higher by 137.5%, IMP content higher by 135.0%, and UMP content higher by 239.6%. Taking into account that NM are involved in many essential physiological processes (synthesis of proteins, lipids, and carbohydrates), dietary supplements of nucleotides can be beneficial for humans under certain conditions (Dominguez-Álvarez et al., 2017). The EFSA NDA Panel (2014) recommends the supplementation of adapted milk products with up to 5 mg/100 kcal of AMP, CMP, GMP, UMP, and IMP.

Effects of Treatment Procedures on Bacteria, *E. coli*, and Fungi/Yeast Counts in BC

Every year in the United States, approximately 48 million food-borne infections are reported (CDC, 2011). Interest is increasing for the development of food preservation techniques that can guarantee the safety and nutritional value of foods but at the same time are less time- and energy-consuming (Knorr et al., 2011). Pasteurization is the main proposed process for ensuring the safety of BC products; however, it can cause protein denaturation and lower the functional activity of BC (Lima et al., 2009). Our results showed that ultrasonication, fermentation, and dehydration could be used to reduce microbial contamination of BC, because aerobic mesophilic spore-forming bacteria, enterobacte-

ria, *E. coli*, and fungi/yeasts were not found in any of the treated BC samples (Table 6) and negative changes of the BC composition were not established. However, the LAB count ranged from 8.36 to 6.00 log₁₀ cfu/mL in BC samples fermented with *L. plantarum* and in vacuum-dried BC samples, respectively. Antagonistic effects produced by LAB toward other organisms may play an important role in preserving of foods, and several authors have documented the ability of various LAB to inhibit growth of pathogenic microorganisms (Mokoena, 2017). Lactic acid bacteria are recognized as generally recognized as safe microorganisms and are commonly used as starter culture in many fermented foods of many countries. They produce various compounds, such as organic acids, diacetyl, hydrogen peroxide, bacteriocin, or bactericidal proteins, during lactic acid fermentations, and their ability to rapidly produce copious amounts of acidic end-products with a concomitant pH reduction is the major factor in these fermentations (Lindgren and Dobrogosz, 1990). Our results showed that fermentation could be used to reduce microbial contamination of BC. Ultrasound vibrations can cause liquids to expand and contract continuously; when liquids expand beyond their tensile strength, bubbles are formed, which eventually implode in a cavitation process and generate diverse physical and chemical processes at a microscopic level, such as extreme levels of temperature, shock waves, free radicals, reactive oxygen species formation, extreme levels of pressure, and high shear, which all ultimately provoke microbial inactivation (Bastarrachea et al., 2017). The effects of ultrasound on microorganisms are believed to consist of cell membrane disruption, damage of genetic information, denaturation of proteins, and cell lysis (Piyasena et al., 2003). Our results showed that ultrasonication,

Table 6. The influence of different treatment methods on some microbiological parameters of bovine colostrum¹

BC sample	log ₁₀ cfu/g				
	Enterobacteria	<i>Escherichia coli</i>	LAB	TBC	Mold/yeast
BC	6.39 ± 0.12	6.04 ± 0.09	8.32 ± 0.10 ⁱ	8.26 ± 0.07 ⁱ	6.24 ± 0.06
BC _{lyoph}	ND	ND	7.38 ± 0.09 ^e	7.43 ± 0.11 ^h	ND
BC _{vacdried}	ND	ND	6.00 ± 0.09 ^a	6.02 ± 0.04 ^c	ND
BC _{L.plant}	ND	ND	8.36 ± 0.11 ⁱ	5.27 ± 0.08 ^b	ND
BC _{L.par.}	ND	ND	8.15 ± 0.08 ^h	5.00 ± 0.05 ^a	ND
BC _{L.plant lyoph}	ND	ND	7.82 ± 0.09 ^g	7.10 ± 0.10 ^g	ND
BC _{L.par lyoph}	ND	ND	7.11 ± 0.06 ^d	6.65 ± 0.09 ^e	ND
BC _{L.plant vacdried}	ND	ND	7.58 ± 0.06 ^f	6.96 ± 0.10 ^f	ND
BC _{L.par vacdried}	ND	ND	6.86 ± 0.11 ^c	6.98 ± 0.10 ^f	ND
BC _{ultr lyoph}	ND	ND	6.92 ± 0.13 ^c	7.01 ± 0.07 ^g	ND
BC _{ultr vacdried}	ND	ND	6.30 ± 0.09 ^b	6.45 ± 0.04 ^d	ND

^{a-i}Mean values with different superscripts are significantly different ($P \leq 0.05$).

¹LAB = lactic acid bacteria; TBC = number of aerobic mesophilic sporeforming bacteria; ND = not detected; BC = bovine colostrum; BC_{lyoph} = freeze-dried (−40°C) BC; BC_{vacdried} = vacuum dried (+45°C) BC; BC_{L.plant} = BC fermented with *Lactobacillus plantarum*; BC_{L.par.} = BC fermented with *Lactobacillus paracasei*; BC_{L.plant lyoph} = BC fermented with *L. plantarum* and freeze dried (−40°C); BC_{L.par lyoph} = BC fermented with *L. paracasei* and freeze dried (−40°C); BC_{L.plant vacdried} = BC fermented with *L. plantarum* and vacuum dried (+45°C); BC_{L.par vacdried} = BC fermented with *L. paracasei* and vacuum dried (+45°C); BC_{ultr lyoph} = BC after ultrasonication and freeze drying (−40°C); BC_{ultr vacdried} = BC after ultrasonication and vacuum drying (+45°C). Data expressed as mean values (n = 5) ± SD.

fermentation, and dehydration could be used to reduce microbial contamination of BC; however, more investigations are needed to evaluate the influence of these treatment methods on sensitive biologically active compounds in BC.

CONCLUSIONS

Ultrasonication and fermentation, as well as different methods of dehydration, can be promising technologies for BC treatment. The treatments significantly affected the concentrations of most FA, and we found higher total content of UFA, n-6 FA, and n-9 FA in treated BC samples; however, content of *trans* FA were not increased. An increase of FAA content in BC as a result of different treatments (with ultrasonication being the most effective) can be very promising because it leads to an increase in BC functionality. All of the treatments reduced the presence of cadaverine, histamine, and tyramine in BC. The concentrations of macro- and microelements in BC were in the order of Ca > Na > K > Mg and Zn > Fe > Sr > Ba > Mn > Cu > Al > Se > Mo > Cr > Ni > Sn > Co > Pb > Cd, respectively. The concentrations of nucleotide monophosphates in BC were significantly increased by using a combination of fermentation with LUHS135 and vacuum drying. All of the treatments reduced the microbial contamination of BC, as LAB count ranged from 8.36 to 6.00 log₁₀ cfu/mL. Finally, we concluded that a combination of ultrasonication, fermentation, and dehydration could be used to reduce microbial contamination of BC; however, more investigations are needed to evaluate the influence of these treatment methods on sensitive biologically active compounds in BC.

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