Fermented, ultrasonicated, and dehydrated bovine colostrum: Changes in antimicrobial properties and immunoglobulin content

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ABSTRACT

This study evaluated the influence of fermentation with *Lactobacillus plantarum* LUHS135 and *Lactobacillus paracasei* LUHS244, ultrasonication, and different methods of dehydration on the content of IgG, IgA, and IgM in bovine colostrum (BC), as well as the antimicrobial activity of the treated and fresh BC samples [fresh = BC; freeze dried = BC_{lyoph}; vacuum dried (+45°C) = BC_{vacdried}; BC fermented with LUHS135 = BC_{LUHS135}; BC fermented with LUHS244 = BC_{LUHS244}; BC fermented with LUHS135 and freeze dried = BC_{LUHS135lyoph}; BC fermented with LUHS244 and freeze dried = BC_{LUHS244lyoph}; BC fermented with LUHS135 and vacuum dried = BC_{LUHS135vacdried}; BC fermented with LUHS244 and vacuum dried = BC_{LUHS244vacdried}; BC ultrasonicated and freeze dried = BC_{ultr lyoph}; BC ultrasonicated and vacuum dried = BC_{ultr vacdried}]. The antimicrobial activity was assessed against *Klebsiella pneumoniae*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Proteus mirabilis*, methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Bacillus cereus*, *Streptococcus mutans*, *Enterobacter cloacae*, *Citrobacter freundii*, *Staphylococcus epidermis*, *Staphylococcus haemolyticus*, and *Pasteurella multocida* using the agar

well diffusion method, as well as in liquid medium. In liquid medium analysis showed that the fermented BC samples had the broadest antimicrobial spectrum (of 15 tested pathogenic strains, BC_{LUHS135vacdried} and BC_{LUHS135lyoph} inhibited 13; BC_{LUHS244vacdried} inhibited 12; and BC_{LUHS135}, BC_{LUHS244}, and BC_{LUHS244lyoph} inhibited 11). Based on the inhibition zones, BC_{LUHS135lyoph} samples exhibited the broadest inhibition spectrum, inhibiting the growth of 12 of the 15 tested pathogenic strains). According to the lactic acid bacteria strain selected for BC fermentation, different properties of the BC will be obtained. To ensure a broad antimicrobial spectrum and high IgG content, fermentation with LUHS135 can be recommended (IgG concentration in BC_{LUHS135} was retained), whereas fermentation with LUHS244 will provide a high IgM concentration (IgM concentration increased by 48.8 and 21.6% in BC_{LUHS244} and BC_{LUHS244lyoph} samples, respectively). However, IgA is very sensitive for fermentation, and further studies are needed to increase IgA stability in BC. Finally, fermented BC can be recommended as a food/beverage ingredient, providing safety, as well as improved functionality through displaying a broad spectrum of antimicrobial activities.

Key words: bovine colostrum, fermentation, immunoglobulin, drying, ultrasonication

INTRODUCTION

Functional and personalized characteristics are preferred today in the healthy food and beverage sectors. The food and beverage industries have many opportunities to prepare healthy and functional products

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that help people to improve their overall wellness by including new ingredients in the main food and beverage formulations. Nowadays, bovine colostrum (BC) is popular as a functional ingredient because it is associated with many health benefits (Saad et al., 2016; Chae et al., 2017). Bovine colostrum is well known as an antimicrobial, toxin neutralizer (Støy et al., 2014), and epithelial cell proliferation promoting agent (Rathe et al., 2014). Consequently, BC is a highly promising compound for functional food/beverages, as well as for nutraceutical preparation. Our previous study showed that fermented BC (up to 3%) in combination with thyme essential oil (up to 0.2%), and with mandarin or grapefruit essential oil (up to 0.2%) for taste-masking, allowed obtaining good texture and high overall acceptability gummy candies containing desirable antimicrobials, and such antimicrobial candies could be a consumer-preferred form of nutraceuticals (Bartkiene et al., 2018c). However, the chemical composition of BC is very sensitive to technological treatment (McGrath et al., 2016), and the changes in chemical composition can lower the functionality of BC. Also, it is desirable that the ingredients used at an industrial scale will be convenient for usage and storage, and biological and chemical stability should be guaranteed.

We previously evaluated the influence of fermentation with *Lactobacillus plantarum* strain LUHS135 and *Lactobacillus paracasei* strain LUHS244, ultrasonication, and different methods of dehydration on the chemical composition of BC, including fatty acid and free amino acid (FAA) profiles, as well as the content of micro- and macroelements (Bartkiene et al., 2018a). Also, the lactic acid bacteria (LAB) count, microbial contamination (aerobic mesophilic spore-forming bacteria; enterobacteria, including *Escherichia coli*; fungi/yeasts) as well as the concentrations of biogenic amines (BA) and nucleotide monophosphates (NM) in BC samples were analyzed (Bartkiene et al., 2018a). Although the minimum requirements for BC are >50 g/L of IgG and a total plate count of <100,000 cfu/mL, up to 60% of BC produced in the United States fails to meet these standards (Morrill et al., 2012). Ensuring BC has the minimum quality requirements is not easy because of the specific composition of BC (high protein content, as well as the presence of sensitive and antimicrobial compounds, such as immunoglobulins).

Only a few research investigations have been published about the influence of various preservation methods on the detailed chemical composition of BC (Ramya et al., 2016; Saalfeld et al., 2016; Denholm et al., 2017). However, we have shown that the use of ultrasonication and fermentation, as well as different methods of dehydration, can be promising technologies for BC treatment (Bartkiene et al., 2018a) because the use of the above-

mentioned treatments significantly increased the total content of unsaturated, n-6, and n-9 fatty acids, as well as the FAA content in ultrasonicated BC samples. All of the treatments led to decreased microbial contamination in BC samples and lowered the presence of BA (cadaverine, histamine, tyramine). Conversely, the LAB count in all of the treated BC samples was >6.00 log₁₀ cfu/mL. Moreover, it was found that the functionality of the BC could be greatly improved by fermentation of BC with LUHS135 strain, as it increased the NM concentration in BC samples (Bartkiene et al., 2018a). The NM are involved in many essential physiological processes. For this reason, dietary supplements of NM can be beneficial for humans under certain conditions (Domínguez-Álvarez et al., 2017). The European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies recommends supplementation of adapted milk products with up to 5 mg/100 kcal of adenosine monophosphate, cytidine monophosphate, guanosine monophosphate, inosine monophosphate, and uridine monophosphate (EFSA NDA, 2014). Finally, it was concluded that a combination of fermentation, ultrasonication, and dehydration reduces the microbial contamination of BC and increases the NM concentration. Also, it was found that the technological LAB can reduce mycotoxin content in fermentable substrate (Bartkiene et al., 2018b,d), as well as in biological fluids (Ritieni et al., 2010). Nonetheless, more investigations are needed to evaluate the influence of these treatment methods on sensitive, biologically active compounds (e.g., immunoglobulins) in BC.

Immunoglobulins have potential as therapeutics in oncology, chronic inflammation, and cardiovascular, transplantation, and infectious diseases (Correia, 2010). For this reason, to ensure that they remain in BC after technological treatment would be very desirable because the inclusion of oral bovine immunoglobulins in the main food/beverage formulations may be a promising approach to support immune function in vulnerable groups, such as infants, children, elderly, and immunocompromised patients (Ulfman et al., 2018). The present study aimed to evaluate the influence of the treatments mentioned above (fermentation, ultrasound, and dehydration) on IgG, IgA, and IgM contents in BC and, additionally, assess the antimicrobial activity of the treated BC samples against a range of pathogenic and opportunistic bacterial strains.

MATERIALS AND METHODS

Materials

The BC was obtained from “Linus Agro” agricultural company (Luksiai, Lithuania), within 2 h of calf deliv-

ery. Samples were taken from 20 Lithuanian black-and-white (Holstein) dairy cows in the winter (Bartkiene et al., 2018a). *Lactobacillus plantarum* LUHS135 and *L. paracasei* LUHS244 strains, for BC fermentation, were selected according to their good technological and antimicrobial properties (Bartkiene et al., 2018c). Before the experiment, LUHS135 and LUHS244 were stored at -80°C in a Microbank system (Pro-Lab Diagnostics, Wirral, UK) and grown in de Man, Rogosa, and Sharpe (MRS) broth (CM 0359, Oxoid, Hampshire, UK) at 30°C for 48 h before use.

Fermentation, Ultrasonication, and Dehydration of BC

The LUHS135 and LUHS244 strains were grown in MRS medium (Biolife, Milan, Italy) at 30°C . Two percent of the MRS solution (vol/vol), in which the strains were multiplied, was inoculated into fresh medium and propagated for 18 h at 30°C . Further multiplied strains (average cell concentration $9.2 \log_{10} \text{ cfu/mL}$) were used for BC fermentation. The LUHS135 and LUHS244 strains were added to the BC (3%, vol/vol), followed by fermentation in a CO_2 incubator (Memmert GmbH + Co. KG, Schwabach, Germany) for 24 h at 30°C .

The BC was ultrasonicated at low frequency (37 kHz, 160 W) in a Proclean 3.0DSP apparatus (Ulsonix, Berlin, Germany). Each 20-g sample of BC was processed at 40°C for 20 min, and each experiment was performed at least twice.

After fermentation with LUHS135 and LUHS244 strains, the BC samples were ultrasonicated, and as well as the nonfermented BC samples, were then dried: (1) freeze dried at -40°C for 72 h (condenser temperature -85°C , pressure $2 \times 10^{-6} \text{ mPa}$; Sublimator $3 \times 4 \times 5$, Zirbus Technology, Bad Grund/Harz, Germany), and (2) vacuum dried (temperature $45 \pm 2.0^{\circ}\text{C}$ and pressure $6 \times 10^{-3} \text{ mPa}$; XF020 vacuum dryer, France Etuves, Chelles, France).

In total, 11 samples were obtained: fresh = BC; freeze dried (-40°C) = BC_{lyoph} ; vacuum dried ($+45^{\circ}\text{C}$) = $\text{BC}_{\text{vacdried}}$; BC fermented with LUHS135 = $\text{BC}_{\text{LUHS135}}$; BC fermented with LUHS244 = $\text{BC}_{\text{LUHS244}}$; BC fermented with LUHS135 and freeze dried (-40°C) = $\text{BC}_{\text{LUHS135 lyoph}}$; BC fermented with LUHS244 and freeze dried (-40°C) = $\text{BC}_{\text{LUHS244 lyoph}}$; BC fermented with LUHS135 and vacuum dried ($+45^{\circ}\text{C}$) = $\text{BC}_{\text{LUHS135 vacdried}}$; BC fermented with LUHS244 and vacuum dried ($+45^{\circ}\text{C}$) = $\text{BC}_{\text{LUHS244 vacdried}}$; BC ultrasonicated and freeze dried (-40°C) = $\text{BC}_{\text{ultr lyoph}}$; BC ultrasonicated and vacuum dried ($+45^{\circ}\text{C}$) = $\text{BC}_{\text{ultr vacdried}}$.

Determination of BC Antimicrobial Activities by the Agar Well Diffusion Method and in Liquid Medium

All 11 BC samples were assessed for their antimicrobial activities against a variety of pathogenic and opportunistic bacterial strains (*Klebsiella pneumoniae*, *Salmonella enterica* 24 SPn06, *Pseudomonas aeruginosa* 17–331, *Acinetobacter baumannii* 17–380, *Proteus mirabilis*, methicillin-resistant *Staphylococcus aureus* (MRSA) M87fox, *Enterococcus faecalis* 86, *Enterococcus faecium* 103, *Bacillus cereus* 18 01, *Streptococcus mutans*, *Enterobacter cloacae*, *Citrobacter freundii*, *Staphylococcus epidermis*, *Staphylococcus haemolyticus*, and *Pasteurella multocida*) by the agar well diffusion method and in liquid medium.

For the agar well diffusion assay, suspensions of 0.5 McFarland standard of each pathogenic bacteria strain were inoculated onto the surface of cooled Mueller–Hinton agar (Oxoid, Basingstoke, UK) using sterile cotton swabs. Wells of 6 mm in diameter were punched in the agar and filled with 50 μL of the BC. The antimicrobial activities against the tested bacteria were established by measuring the inhibition zone diameters (mm). The experiments were repeated 3 times, and the average of the inhibition zones was calculated.

To evaluate antimicrobial activity of BC in liquid medium, the BC was diluted 1:3 (vol/vol) with physiological solution. Then, to the 1 mL of the diluted BC, 10 μL of the pathogenic and opportunistic bacterial strain, multiplied in a selective medium, was added and incubated at 35°C for 24 h. After incubation, the viable pathogenic and opportunistic bacterial strains in BC were controlled, by plating them on selective medium. The results were interpreted as negative if the pathogens did not grow on selective medium, and positive if the pathogens grew on selective medium. Experiments were performed in triplicate.

Determination of IgG, IgA, and IgM Concentrations in BC Samples Using an ELISA Method

For evaluation of IgG, IgA, and IgM concentrations, commercially available, 2-site ELISA kits (Abcam, Cambridge, UK) were used. The results were interpreted by spectrophotometric readings at 450 nm wavelength for all respective antibodies. Standards for antibody concentrations were prepared as instructed by the manufacturer, using serial dilutions. For quantitative analysis, 4-parameter logistic curves were constructed to calculate the antibody concentrations. For optimal quantification before the experiment, the

samples were diluted 1/800,000 for IgG, 1/20,000 for IgM, and 1/10,000 for IgA testing.

Statistical Analysis

The results were expressed as the mean value of at least 3 measurements $\pm$ standard deviation. To evaluate the effects of the different treatments on the antibody concentrations in BC samples, the data were analyzed by one-way ANOVA (SPSS version 22.0, IBM Corp., Armonk, NY). The results were deemed as statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Influence of Fermentation, Ultrasonication, and Dehydration on Antimicrobial Properties of BC

Table 1 provides the inhibition zones of the fresh and treated BC samples against the pathogenic and opportunistic bacterial strains. The BC_{LUHS135 lyoph} samples displayed the broadest inhibition spectrum, as they inhibited the growth of 12 out of the 15 tested pathogenic and opportunistic bacterial strains, including the highest inhibition zone against *K. pneumoniae* (22.0 $\pm$ 0.8 mm). Similar inhibition zones against *K. pneumoniae* were established by BC_{LUHS135 vacdried} (22.0 $\pm$ 1.2 mm) and BC_{LUHS244 lyoph} (23.0 $\pm$ 1.3 mm) samples, which also inhibited a broad spectrum of the tested pathogenic and opportunistic bacterial strains (both samples inhibited 10 out of the 15 tested strains). The BC_{LUHS135} and BC_{LUHS244 vacdried} inhibited 9 out of the 15 tested pathogenic and opportunistic bacterial strains and the inhibition zones ranged from 16.0 $\pm$ 1.3 and 18.0 $\pm$ 0.7 mm (BC_{LUHS135} and BC_{LUHS244 vacdried} against *E. faecium* 103 and *S. epidermis*, respectively) to 7.0 $\pm$ 0.6 and 9.0 $\pm$ 0.4 mm (BC_{LUHS135} against *A. baumannii* 17–380 and BC_{LUHS244 vacdried} against *A. baumannii* 17–380 and MRSA M87fox), respectively. Five out of the 15 tested pathogenic and opportunistic bacterial strains were inhibited by BC_{vacdried}, BC_{ultr vacdried}, and BC_{ultr lyoph}, and the ultrasonicated samples (vacuum dried and lyophilized) showed similar antimicrobial activities against the same pathogenic strains (*K. pneumoniae*, *S. enterica* 24 SPn06, MRSA M87fox, *E. faecium* 103, *C. freundii*).

Although the BC_{vacdried} samples did not demonstrate antimicrobial activities against *E. faecium*, they inhibited the growth of *B. cereus* 18 01 (this strain was not inhibited by ultrasonicated BC samples). Fresh BC and BC_{lyoph} samples possessed the lowest inhibition properties, only inhibiting the growth of 2/15 and 3/15 of the tested pathogenic and opportunistic bacterial strains (fresh BC inhibited *K. pneumoniae* and MRSA M87fox, and BC_{lyoph} inhibited MRSA M87fox, *E. faecium* 103,

Table 1. Inhibition of the pathogenic and opportunistic bacterial strains with bovine colostrum (BC) evaluated using the agar well diffusion method and in a liquid medium¹

BC sample	Liquid medium															Agar well diffusion method		
	Pathogenic and opportunistic bacterial strain															Total number of pathogenic and opportunistic bacterial strains inhibited	Number of tested strains inhibited by BC (out of 15 tested)	Range of inhibition zones (mm)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15			
BC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	2	11–25
BC _{LUHS135}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	11	9	7–16
BC _{LUHS244}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	11	8	7–15
BC _{vacdried}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8	5	8–20
BC _{lyoph}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	3	9–20
BC _{LUHS135 vacdried}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	13	10	7–22
BC _{LUHS135 lyoph}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	13	12	7–22
BC _{LUHS244 vacdried}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	11	9	9–18
BC _{LUHS244 lyoph}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	11	10	8–23
BC _{ultr vacdried}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6	5	8–12
BC _{ultr lyoph}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6	5	9–12

¹Three replicate analyses were performed. (–) The pathogens did not grow; (+) the pathogens grew. BC = fresh BC; BC_{lyoph} = freeze dried (–40°C) BC; BC_{vacdried} = vacuum dried (+45°C) BC; BC_{LUHS135} = BC fermented with LUHS135; BC_{LUHS244} = BC fermented with LUHS244; BC_{LUHS135 lyoph} = BC fermented with LUHS135 and freeze dried (–40°C); BC_{LUHS244 lyoph} = BC fermented with LUHS244 and freeze dried (–40°C); BC_{LUHS135 vacdried} = BC fermented with LUHS135 and vacuum dried (+45°C); BC_{LUHS244 vacdried} = BC fermented with LUHS244 and vacuum dried (+45°C); BC_{ultr vacdried} = BC after ultrasonication and freeze drying (–40°C); BC_{ultr lyoph} = BC after ultrasonication and vacuum drying (+45°C). 1 = *Klebsiella pneumoniae*; 2 = *Salmonella enterica* 24 SPn06; 3 = *Pseudomonas aeruginosa* 17–331; 4 = *Acinetobacter baumannii* 17–380; 5 = *Proteus mirabilis*; 6 = methicillin-resistant *Staphylococcus aureus* M87fox; 7 = *Enterococcus faecalis* 86; 8 = *Enterococcus faecium* 103; 9 = *Bacillus cereus* 18 01; 10 = *Streptococcus mutans*; 11 = *Enterobacter cloacae*; 12 = *Citrobacter freundii*; 13 = *Staphylococcus epidermidis*; 14 = *Staphylococcus haemolyticus*; 15 = *Pasteurella multocida*.

and *P. multocida*). The antimicrobial properties of the BC samples were affected by the fermentation with the different LAB strains: BC_{LUHS135} inhibited *S. enterica* 24 SPn06, *A. baumannii* 17–380, *P. mirabilis*, MRSA M87fox, *E. faecalis* 86, *E. faecium* 103, *B. cereus* 18 01, *C. freundii*, and *S. epidermis*, whereas BC_{LUHS244} inhibited *S. enterica* 24 SPn06, *P. mirabilis*, MRSA M87fox, *E. faecalis* 86, *E. faecium* 103, *B. cereus* 18 01, *C. freundii*, and *P. aeruginosa* 17–331.

Similar tendencies of the antimicrobial activity were observed in liquid medium (Table 1). Fresh BC and BC_{lyoph} samples did not inhibit the growth of the tested pathogenic and opportunistic bacterial strains. However, all of the other treated BC samples inhibited the growth of *P. aeruginosa* 17, MRSA M87fox, *C. freundii*, and *S. epidermis*. It should be mentioned that BC did not inhibit *P. multocida* in the liquid medium. The highest antimicrobial activity was displayed by the fermented BC samples (out of the 15 tested pathogenic and opportunistic bacterial strains, BC_{LUHS135 vacdried} and BC_{LUHS135 lyoph} inhibited 13, BC_{LUHS244 vacdried} inhibited 12, and BC_{LUHS135}, BC_{LUHS244}, and BC_{LUHS244 lyoph} inhibited 11). From the results obtained, it can be stated that the combination of BC with the selected LAB strains is very promising, as they can improve the BC antimicrobial activity.

It is known that the immunoglobulins found in BC bind to many human pathogens and allergens and can neutralize infection of human cells, as well as treat gastrointestinal inflammation (Ulfman et al., 2018). Also, passive oral administration of BC has been experimentally evaluated as a preventive or therapeutic modality for a variety of enteric infections (Tacket et al., 1988; Davidson et al., 1989; Tacket et al., 1992; Greenberg and Cello, 1996; Freedman et al., 1998; Savarino et al., 2017). Food formulations with BC can compensate or have a beneficial effect on health due to the addition of specific components if those components are lacking in the daily diet (Santini and Novellino, 2018). Furthermore, our results agree with Shahbazi et al. (2016) that the antimicrobial activity of ingredients can be increased by the combination of components with different antimicrobial properties, as well as by fermentation with LAB showing antimicrobial properties, and this technology might allow reduction of the dose of each compound. In addition, synergism of the different antimicrobials can be adapted in an attempt to prevent or delay the emergence of resistant populations of the pathogens (Mathur et al., 2017), and our results showed that after fermentation and ultrasonication, BC inhibited MRSA M87fox (established by both methods: inhibition zones and MIC). Bovine colostrum is a safe ingredient since there are no contraindications regarding high dose levels (Menchetti et al., 2016). Reducing

the content of pathogenic bacteria is very important for public health because these microorganisms can cause diseases (Özogul and Hamed, 2018). For this reason, LAB are included in many technologies (as a technological or functional ingredient). However, LAB can decarboxylate FAA in fermentable substrates, which leads to BA formation. Conversely, BA can also be formed by foodborne pathogens, and our previous work showed that the use of LUHS135 and LUHS244 strains for BC fermentation reduced the concentrations of the BA and the pathogenic bacteria strains in BC (Bartkiene et al., 2018c). Lactic acid bacteria are associated with natural, healthy, as well as functional products, primarily because fermentation, as a natural preservation method, is more desirable by consumers, compared with chemical processes. Lactic acid bacteria produce a wide spectrum of antimicrobial compounds and are capable of inhibiting a variety of microorganisms in food, with a strong influence on safety.

Influence of Fermentation, Ultrasonication, and Dehydration on IgG, IgA, and IgM Concentrations in BC

Table 2 reveals the concentrations of IgG, IgA, and IgM in the BC samples. In comparing the IgG concentration between fresh and treated BC samples, the IgG concentration was retained in BC_{LUHS135} and BC_{ultr vacdried}. In BC_{LUHS244} and BC_{LUHS244 vacdried}, the IgG concentration was 21.5 and 32.1%, respectively, lower than that in fresh BC. In comparison, relatively higher IgG concentration decreases occurred in the fermented (with both strains) and lyophilized samples (41.8 and 38.2% in BC_{LUHS135 lyoph} and BC_{LUHS244 lyoph}, respectively). By combining the fermentation with LUHS135 strain and vacuum drying, the IgG loss in BC samples was 54.0%, which was similar to that in the ultrasonicated and lyophilized sample (51.3% lower in BC_{ultr lyoph} compared with fresh BC). The highest IgG losses were found in BC_{lyoph} and BC_{vacdried}, in which the IgG was reduced 4.2- and 3.0-fold, respectively, relative to the IgG content in fresh BC.

Different tendencies of the IgA concentration in BC samples were found. Among all the BC samples, the highest IgA concentration was established in BC_{lyoph} (108.8 ± 3.1 ng/mL), whereas in fresh BC, the concentration was 15.5% lower. When compared with fresh BC, BC_{ultr lyoph} had 18.9% less IgA, and in BC fermented with LUHS135 and LUHS244 strains, the IgA concentration was reduced by 51.5 and 96.1%, respectively. The combination of ultrasonication and vacuum drying (BC_{ultr vacdried}) decreased the IgA concentration by 2.3 times relative to fresh BC, but in vacuum-dried samples (BC_{vacdried}) this increased to 12.8

times. These findings indicate that IgA is highly sensitive to the vacuum drying technique, as well as fermentation because, in fermented and lyophilized samples, very high losses of the IgA were detected compared with the IgA concentration in fresh BC (97.2 and 96.4% lower in BC_{LUHS135 lyoph} and BC_{LUHS244 lyoph}, respectively). The fermented and vacuum-dried samples had slightly more IgA than the fermented and lyophilized samples, but compared with the IgA concentration in fresh BC, the amounts were 83.7% (BC_{LUHS135 vacdried}) and 91.0% lower (BC_{LUHS244 vacdried}).

A comparison of the IgM concentrations among the BC samples showed that lyophilization, fermentation with LUHS244 strain, and their combination increased the IgM concentration by 51.2% (BC_{lyoph}), 48.8% (BC_{LUHS244}), and 21.6% (BC_{LUHS244 lyoph}). Also, lower IgM contents were noticed in all BC fermented with LUHS135 compared with LUHS244 (58.3, 74.9, and 83.1% less in BC_{LUHS135} vs. BC_{LUHS244}, BC_{LUHS135 lyoph} vs. BC_{LUHS244 lyoph}, and BC_{LUHS135 vacdried} vs. BC_{LUHS244 vacdried}, respectively). Vacuum drying of fresh BC also reduced the IgM level (7.8-fold). The BC_{ultr lyoph} had more than double the amount of IgM than in BC_{ultr vacdried}, but both had less than in fresh BC (74.4% less in BC_{ultr vacdried} and 39.4% less in BC_{ultr lyoph}).

Chemical preservation methods are not desirable for consumers and cannot preserve BC satisfactorily. For this reason, heating and freezing are the most preferred methods, and freeze drying is the more suitable technique because a higher concentration of active immunoglobulins can be saved (Borad and Singh, 2018). Also, spray drying, as well as high-pressure processing can be promising techniques to retain active immunoglobulins.

Nowadays, membrane processing is often employed to manipulate the composition of BC formulations, although new antimicrobial and functional properties for BC can be provided by fermentation with selected LAB strains. However, LAB can excrete proteolytic enzymes, which can lead to a highly proteinaceous substrate, as well as biodegradation of immunoglobulins. Biogenic amines can be formed from the FAA, but our previous investigation showed that fermentation, ultrasonication, and dehydration can be used to reduce the microbial contamination of BC (aerobic mesophilic spore-forming bacteria, enterobacteria, *E. coli*, and fungi/yeasts), which can also decarboxylate FAA, and in treated BC samples, lower concentrations of cadaverine, histamine, and tyramine were recorded compared with fresh BC (Bartkiene et al., 2018a). From another perspective, an increase in free essential, as well as non-essential AA in BC after fermentation and ultrasonication, or either technique, can be very promising, as it can lead to increased functionality of BC, as a food/beverage component.

Also, oxidation of immunoglobulins can occur during the cell culture process, purification, formulation, and storage of the molecules. Studies reveal that oxidation of methionine residues not only results in loss of activity but also reduced stability of the molecule, potential immunogenicity, and decreased in vivo half-life (Teh et al., 1987; Lam et al., 1997; Hermeling et al., 2004; Chumsae et al., 2007; Liu et al., 2008).

Oxidation of immunoglobulins can take place on several different AA residues in the molecule, although methionine residues exposed to solvent tend to be the most susceptible (Kroon et al., 1992; Liu et al., 2008;

Table 2. Concentrations (ng/mL) of IgG, IgA, and IgM in fresh, fermented, ultrasonicated, and dehydrated bovine colostrum (BC) samples¹

BC sample	IgG	IgA	IgM
BC	155.7 ± 2.3 ^h	108.8 ± 3.1 ^h	181.1 ± 3.1 ^h
BC _{LUHS135}	157.2 ± 1.9 ^h	44.6 ± 2.4 ^e	74.4 ± 2.4 ^e
BC _{LUHS244}	122.3 ± 3.0 ^g	3.6 ± 0.5 ^a	178.2 ± 3.9 ^h
BC _{lyoph}	36.7 ± 2.1 ^a	91.9 ± 1.6 ^g	119.8 ± 3.6 ^f
BC _{vacdried}	52.3 ± 2.7 ^b	7.2 ± 1.1 ^b	15.4 ± 1.0 ^b
BC _{LUHS135 lyoph}	90.6 ± 3.4 ^d	2.6 ± 0.4 ^a	36.6 ± 1.7 ^d
BC _{LUHS244 lyoph}	96.3 ± 2.9 ^e	3.3 ± 0.5 ^a	145.6 ± 3.8 ^g
BC _{LUHS135 vacdried}	71.6 ± 1.7 ^c	15.0 ± 1.3 ^c	5.0 ± 0.6 ^a
BC _{LUHS244 vacdried}	105.7 ± 3.5 ^f	8.3 ± 0.9 ^b	29.6 ± 1.4 ^c
BC _{ultr vacdried}	154.1 ± 3.1 ^h	39.4 ± 1.2 ^d	30.7 ± 1.6 ^c
BC _{ultr lyoph}	75.8 ± 2.7 ^c	74.5 ± 2.8 ^f	72.6 ± 2.0 ^e

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

¹ Values are mean ± SD of 3 replicate analyses (n = 3). BC = fresh BC; BC_{lyoph} = freeze dried (−40°C) BC; BC_{vacdried} = vacuum dried (+45°C) BC; BC_{LUHS135} = BC fermented with LUHS135; BC_{LUHS244} = BC fermented with LUHS244; BC_{LUHS135 lyoph} = BC fermented with LUHS135 and freeze dried (−40°C); BC_{LUHS244 lyoph} = BC fermented with LUHS244 and freeze dried (−40°C); BC_{LUHS135 vacdried} = BC fermented with LUHS135 and vacuum dried (+45°C); BC_{LUHS244 vacdried} = BC fermented with LUHS244 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).

Table 3. Correlations between immunoglobulin concentration in bovine colostrum samples and their antimicrobial activity assessed using the agar diffusion method

Pathogenic strain	IgG	IgA	IgM
<i>Klebsiella pneumoniae</i>	−0.3174	−0.3185	−0.3147
<i>Salmonella enterica</i> 24 SPn06	0.3696	0.0849	0.2642
<i>Pseudomonas aeruginosa</i> 17–331	−0.1070	−0.7258	0.0035
<i>Acinetobacter baumannii</i> 17–380	−0.0049	−0.5654	−0.3165
<i>Proteus mirabilis</i>	0.4833	−0.0826	0.2318
Methicillin-resistant <i>Staphylococcus aureus</i> M87fox	−0.1581	0.6765	0.3363
<i>Enterococcus faecalis</i> 86	0.3888	−0.3012	0.5154
<i>Enterococcus faecium</i> 103	0.1682	−0.7132	−0.4182
<i>Bacillus cereus</i>	−0.0361	−0.5130	0.0784
<i>Streptococcus mutans</i>	−0.0052	−0.3838	0.0305
<i>Enterobacter cloacae</i>	−0.2228	−0.5577	−0.3910
<i>Citrobacter freundii</i>	−0.1838	−0.3916	−0.3675
<i>Staphylococcus epidermis</i>	0.2752	−0.2573	0.0321
<i>Staphylococcus haemolyticus</i>	—	—	—
<i>Pasteurella multocida</i>	−0.3905	−0.2470	−0.3329

Bertolotti-Ciarlet et al., 2009; Pan et al., 2009). We previously showed that the lowest concentration of free methionine was in freeze-dried BC samples, and ultrasonication increased the content of methionine (the highest level of methionine was established in ultrasonicated and vacuum-dried BC; Bartkiene et al., 2018a). Increasing the methionine can be desirable, as this AA has an essential role in protein synthesis (Xue et al., 2015).

Variations in the influence on various immunoglobulins by fermentation, ultrasonication, and different drying methods can be observed, but to increase the functionality and antimicrobial properties of BC, fermentation with LUHS135 and LUHS244 can be recommended. According to the selected strain, different characteristics of the BC will be obtained. Correlations between immunoglobulin concentration in BC samples and their antimicrobial activity assessed by the agar diffusion method are presented in Table 3. Weak negative correlations between *K. pneumoniae* and all the tested immunoglobulins, between *A. baumannii* 17–380 and IgM, between *E. faecalis* 86 and IgA, between *S. mutans* and IgA, between *E. cloacae* and IgM, between *C. freundii* and IgA, as well as IgM, and between *P. multocida* and IgG, as well as IgM were found. Moderate negative correlations between *A. baumannii* 17–380 and IgA, between *E. faecium* 103 and IgM, between *B. cereus* and IgA, and between *E. cloacae* and IgA were established. Also, negative strong correlations between *A. baumannii* 17–380 and IgA, as well as between *E. faecium* 103 and IgA were found. These results showed that immunoglobulins are not responsible for the inhibition of the above mentioned pathogenic strains. However, weak positive correlations between IgG and *S. enterica* 24 SPn06, as well as *E. faecalis* 86 were found, and moderate positive correlations between IgA and MRSA M87fox, as well as between IgM and *E.*

faecalis 86 were established. According to Polonelli et al. (2017), the antimicrobial, antiviral, antitumor, and immunomodulatory activities are exerted through different mechanisms of action, and the immunoglobulin peptides are active as antibodies, independently from their specificity and isotype. The antimicrobial activity of BC can also depend on the concentration used. Chae et al. (2017) noted that increasing the BC concentration reduced *E. coli* bacterial adherence to the cells, and showed direct antibacterial activity.

To ensure a broad antimicrobial spectrum and high IgG content, fermentation with LUHS135 should be chosen, whereas fermentation with LUHS244 will provide a high IgM concentration. It should be mentioned that IgA is very sensitive to fermentation and further studies should aim to increase IgA stability in BC during the technological processes.

CONCLUSIONS

It was established that the broadest antimicrobial spectrum was displayed by the fermented BC samples (out of 15 tested pathogens, BC_{LUHS135 vacdried} and BC_{LUHS135 lyoph} inhibited 13, BC_{LUHS244 vacdried} inhibited 12, and BC_{LUHS135}, BC_{LUHS244}, and BC_{LUHS244 lyoph} inhibited 11), indicating fermentation of BC with selected LAB strains can improve the functionality of this ingredient. The properties of the BC depend on the LAB strain selected for BC fermentation. To ensure a broad antimicrobial activity and high IgG content of BC, fermentation with LUHS135 strain can be recommended. To provide a high IgM concentration, fermentation with LUHS244 should be selected (IgM concentration increased by 48.8 and 21.6% in BC_{LUHS244} and BC_{LUHS244 lyoph}, respectively). Conversely, IgA is very sensitive to fermentation, and further studies are needed to increase IgA stability in BC. Finally, the fer-

mentation of BC with LUHS135 and LUHS244 strains is a promising technology for BC, as a food/beverage ingredient due to its safety and functionality, which includes providing a broad spectrum of antimicrobial activities, as well as active immunoglobulins.

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