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EC NUTRITION
Research Article

Effect of Thermal and Non-Thermal Treatments on Safety and Quality of Calostro Bovino Audero

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Received: February 06, 2020; **Published:** November 28, 2020

Abstract

When evaluating bovine colostrum, it is interesting to consider its use as a matrix of technological processes for its transformation into unfunctional food, it is important to highlight the importance of the nutritional and immunological value it presents. The chemical composition of bovine colostrum is rich in nutrients of high biological value (serum proteins), has growth factors, immune factors (immunoglobulins, mainly the G variant) and tissue antimicrobials and repair [1]. Regardless of the zootechnical and therapeutic use in livestock species, due to its nutritional status, colostrum can be used in humans, since it has a promising field of development of alternatives for the relief of human infections associated with immunodeficient states. In addition, the use is proposed as a strategy to prevent or control epidemic outbreaks of diseases [2].

Keywords: *Bovine Colostrum; Serum Proteins; Milk*

Introduction

Serum proteins are heat-sensitive proteins and are denatured by heat at temperatures above pasteurization treatments. In milk, 80% of them are α-lactoalbumin, but in colostrum, β-lactoglobulins are the majority. In the nutritional aspect, these proteins are richer than caseins in essential amino acids [3]. The β-lactoglobulins are not found in human milk, they have a wide functionality and nutritional characteristics that make it a multifunctional ingredient to be used in many foods and for biochemical applications. With respect to α-lactalbumin, they are at a considerable level in colostrum and stand out for having broad benefits, such as promoting the absorption of minerals, antibacterial, immunomodulatory and antitumor effects. The immunoglobulins present in colostrum are proteins that are part of the defense system against pathogenic microorganisms. Immunoglobulin G (IgG) is the most abundant. The amount of this immunoglobulin is one of the quantified parameters that guarantee its nutritional quality.

The thermosensitive nature of bovine colostrum influences the processing of colostrum-based products when it comes to achieving safety and stability for human consumption. At present, a potential consumption of colostrum-based products is observed, occupying a fundamental role in specific consumers [4]. They are marketed as nutraceutical products, that is, foods that have a beneficial effect on human health [5]. The application of a thermal treatment such as low discontinuous pasteurization, has as main objective to reduce the banal bacteria by 99% and eliminate 100% of the pathogenic bacteria that can affect the health of the consumer and alter the food; modifying to a lesser extent the physical structure, the chemical components and the organoleptic properties initially present, at a minimal cost. In addition, it allows prolonging the shelf life of the food by giving it a longer shelf life at refrigeration temperatures [6-8]. On the

other hand, the use of a non-thermal treatment that is equivalent to a "cold pasteurization", the High Hydrostatic Pressure (APH) technology, is available. This type of technology offers the possibility of having products that were not subjected to temperature, with more natural characteristics, with minimal effects on their sensory and nutritional properties, free of pathogenic microorganisms and with low counts of altering microbiota [9].

Spray drying technology as a heat treatment to achieve product preservation complements the aforementioned processes. Spray drying is characterized by being a fast, highly reproducible technique, suitable for industrial applications and whose processing costs are relatively low. The main disadvantage is the use of high temperatures necessary in the process of evaporation of the liquid, which can affect the nutritional quality of bovine colostrum. The objective of this work was to study how thermal and non-thermal preservation treatments (low pasteurization and APH) and conservation (spray drying) affect the Physicochemical, microbiological and nutritional quality of bovine colostrum.

Materials and Methods

The test was divided into two stages: in the first, control samples (CC) were compared with colostrum samples treated by low pasteurization (64°C for 3 min). The second stage consisted of a comparison of control colostrum with colostrum treated by high hydrostatic pressures, at two levels of pressure and pressurization time (305 MPa for 15 min and 505 MPa for 5 min). After the application of both preservation treatments, all samples (controls and treated) were dehydrated by spray drying.

Sample collection

The first colostrum (2 liters) of 7 fresh cows from the experimental farm of the Rafaela Agricultural Experimental Station of INTA was used. The pH and fat content measurement was performed on each sample to determine the initial chemical-physical quality. Samples were then standardized to prevent grease from interfering in subsequent analyzes and spray drying technology. For this, a skimming process was applied by centrifugal force. Subsequently, the measurement of fat was carried out again to determine the effectiveness of the process and the pH to guarantee the initial conditions of the control samples.

Thermal and non-thermal treatment of samples

From the control samples (skimmed colostrum without any treatment) aliquots were generated for the following treatments: low pasteurization at 63°C for 3 min (T1), corresponding to stage 1 and high hydrostatic pressures at two pressures (305 and 505 Mpa) and pressurization times (15 and 5 min), corresponding to stage 2. In both stages the treatments were controlled with control samples to which no treatment was applied. Subsequently, both control and treated samples were dehydrated by spray drying (T3), using standardized drying parameters (Table 1).

Process	Variables Results
Inlet temperature	60°C
Outlet temperature	39°C
Spray Air Flow (60mm)	742 l h^{-1}
Peristaltic Pump (7%)	3,5 ml/min
Aspiration (100%)	37,50 m^3/h

Table 1: *Conditions used for spray drying of the samples.*

Sample analysis

The analyses performed on the control samples (CC), on the liquid and dehydrated treated samples (T1, T2 and T3) were the following:

- **Chemical physicists:** pH (standard potentiometric method of APHA Bradley., *et al.* 1993), humidity (thermogravimetric principle by means of halogen moisture analyzer HB43-S from Mettler Toledo), fat material according to Gerber (IRAM 14003-2: 1990), total protein and non-protein nitrogen (Kjeldahl method, according to ISO FDIS 8968-3 IDF 20-3: 2004).
- **Microbiological:** Total aerobic bacteria (official AOAC method (986.33 and 989.10) ISO 4833: 2003), coliform bacteria (official AOAC method (986.33 and 989.10) ISO 4833: 2003), *Escherichia coli* (official AOAC method (986.33 and 989.10) ISO 4833: 2003) and *Staphylococcus aureus* coagulase positive (Fil 145A: 1997)
- Inhibitor test (Delvotest commercial kit).
- **Nutritional quality:** Quantification of soluble proteins (IgG, α-lactalbumin and β-lactoglobulin) by liquid chromatography by UPLC.

Results and Discussion

The Chemical physical composition of colostrum without skimming.

Table 2 shows the initial characterization of colostrum received.

Parameter	Colostrum Stage 1	Colostrum Stage 2
Fat % (gr/100ml)	6,33 ± 1,04	7,27 ± 0,12
Total Solids % (gr/100ml)	24,20 ± 5,68	25,33 ± 0,58
pH	6,49 ± 0,07	6,70 ± 0,02

Table 2: *Initial characterization of colostrum.*

For each variable analyzed, the values correspond to the average and standard deviation of the samples used in each stage.

Characterization of skimmed colostrum.

After skimming to obtain the control colostrum (CC) samples used in stages 1 and 2, their characterization was performed (Table 3).

Parameter	CC Stage 1	CC Stage 2
Fat % (gr/100ml)	0,45 ± 0,05	0,15 ± 0,05
Total Protein % (gr/100ml)	13,59 ± 1,53	15,97 ± 3,89
Total Solids % (gr/100ml)	16,82 ± 1,70	20,70 ± 0,57
PH	6,49 ± 0,07	6,69 ± 0,02

Table 3: *Initial characterization of skimmed control colostrum.*

For each variable analyzed, the values correspond to the average and standard deviation of the 3 CC samples used in each stage.

Microbiological composition

The results of the analyzes in the CC samples of *E. coli* and *Staphylococcus aureus* coagulase count positive gave no pathogens and the antibiotic test was negative for the antibiotics evaluated. The presence of a low coliform load was observed at 30°C.

Nutritional quality

Skimmed colostrum controls were initially quantified in the values of Immunoglobulin G, β-lactoglobulin and α-lactoalbumin. The values obtained are specified in table 4.

Parameter mg/L	CC Step 1	CC Step 2
IgG	83.532 ± 10.031	108.076 ± 36.315
β-lactoglobulina	12.252 ± 1.670	11.052 ± 5.874
α-lactoalbúmina	4.089 ± 140	2.393 ± 1.800

Table 4: *Initial nutritional characterization of CC.*

For each variable analyzed, the values expressed in milligrams per liter correspond to the average and standard deviation of the samples analyzed at each stage.

In the Argentine Food Code (CAA, Chapter 7 diet or dietary foods, Article 1382, 2010) it is defined that colostrum must meet the following microbiological requirements: value of mesophilic bacteria less than 10,000 CFU/ml, absence of coliforms and pathogens (*Escherichia coli* and *Staphylococcus aureus* coagulase-positive) must also be negative for the antibiotic test. From the nutritional point of view it must have IgG values greater than or equal to 50,000 mg/L, and from the chemical-physical point of view it must respond to the following values expressed in grams per 100 milliliters: for 6% fat, total solids of 23 - 26% and total proteins of 14%. These values established in comparison with those obtained in the characterization of the samples used for stage 1 and stage 2 of the test, show that, from the point of view of safety, nutritional and compositional quality, the initial quality of colostrum control used for the trials was adequate.

Stage 1: Low pasteurization and spray drying

Low pasteurization achieved the reduction of the RBT (total mesophilic aerobic bacteria count) and total coliforms after application and with respect to nutritional quality did not affect the levels of β-lactoglobulin, α-lactoalbumin and IgG (Table 5).

Para meter mg/L	CC	CP	Treatment Effect
IgG	83.532 ± 10.031	84.453 ± 20.510	Not significant
β-lactoglobulina	12.252 ± 1.670	12.064 ± 2.181	Not significant
α-lactoalbúmina	4.089 ± 140	3.846 ± 318	Not significant

Table 5: *Nutritional quality of control colostrum (CC) and pasteurized colostrum (CP).*

The values expressed in milligrams per liter correspond to the average and standard deviation of the analyzed samples.

The β-lactoglobulin was significantly affected by the dehydration treatment (losses of 58 - 61%), with dehydrated colostrum (both CC and CP) presenting lower values than those presented before spray drying (Table 6).

Parameter			% loss		
mg/L	CCD	CPD	CCD	CPD	Treatment Effect
IgG	80.914 ± 14.639	65.813 ± 13.351	3	22	Not significant
β-lactoglobulina	5.193 ± 1.709	4.670 ± 1.787	58	61	Not significant
α-lactoalbúmina	2.996 ± 153	2.934 ± 75	27	24	Not significant

Table 6: *Nutritional quality of dehydrated control colostrum (CCD) and dehydrated pasteurized colostrum (CPD).*

The values expressed in milligrams per liter correspond to the average and standard deviation of the analyzed samples.

Stage 2: High Hydrostatic Pressures and spray drying

It was observed that treatments with APH (CA: 305 MPa for 15 minutes and CB: 505 MPa for 5 minutes), were not effective in achieving the reduction of total aerobic mesophilic bacteria. With respect to nutritional quality, after the application of both treatments of high hydrostatic pressures, significant losses (47-66%) were observed in the IgG levels for the CA and CB treatments respectively (Table 7).

Parameter				% loss		
mg/L	CC	CA	CB	CA	CB	Treatment Effect
IgG	108.0876 ± 36.315	57.678 ± 8.678	36.606 ± 3.883	47	66	p>0,05
β-lactoglobulina	11.052 ± 5.874	7.486 ± 3.296	4.922 ± 4.750	32	55	Not significant
α-lactoalbúmina	2.393 ± 1.800	3.161 ± 1.531		-	-	Not significant

Table 7: *Nutritional quality of control colostrum (CC), colostrum treated by APH 305 MPa for 15 minutes (CA) and colostrum treated by APH 505 MPa for 5 minutes (CB).*

The values expressed in milligrams per liter correspond to the average and standard deviation of the analyzed samples.

No significant differences were observed between serum protein and IgG levels after the dehydration process. The samples treated by APH at higher pressure gelled, for this reason they could not be dehydrated.

Characteristics of dehydrated colostrum obtained in stage 2.

Dehydrated colostrum was characterized by assessing humidity, the percentage of total protein, soluble protein, IgG, β-lactoglobulin and α-lactoalbumin (Table 8). The results are expressed in gr % (grams of the specified component every 100gr of colostrum). In general, the skimmed powder colostrum obtained has a high composition of IgG and serum proteins, giving them a functional characteristic.

Parameters gr/100gr	Dehydrated Colostrum
Humidity	5,00
Total protein	63,00
Soluble protein	30,08
Ig G	28,00
β-lactoglobulin	3,70
α-lactalbumin	1,55

Table 8: *Characterization of dehydrated colostrum every 100gr.*

Average values and standard deviation of the parameters evaluated for the characterization of dehydrated colostrum (CAD) of stage 2.

Conclusion

In this work, different technological strategies applied to bovine colostrum were compared, aimed at ensuring its safety, minimizing the loss of its high nutritional value. The first one consisted of traditional techniques such as low pasteurization. The second strategy was studied in an innovative technological intervention, the application of high hydrostatic pressure technology. After the application of both technologies, spray drying was evaluated as alternative preservation of the final product.

Low pasteurization allowed reducing the number of microorganisms present in the initial matrix, preserving soluble protein levels and maintaining IgG levels within established values in the CAA. Dehydration of this product affected the levels of β-lactoglobulin., It was

achieved obtain a colostrum powder, low in fat, without antibiotics, harmless, and with a high biological value, due to the high content of serum proteins and immunoglobulins.

Regarding the effect of APH technology, it was evident that the application of both combinations of pressure and time were insufficient to reduce the initial microbiological load present in it. In addition, in its different treatments applied, it affected IgG levels, the effect being higher at the higher pressure. However, IgG levels remained within the optimal range established by the CAA for colostrum for the treatment in which a pressure of 305 MPa was applied for 15 min, while, for the most intense treatment, the reduction led to values per below the acceptable lower limit of this immunoglobulin for this type of milk matrix.

Considering the results obtained, and taking into account the industrial acceptance of conventional treatment, such as low pasteurization and at its disadvantage the high cost of APH technology, it is concluded that low pasteurization was the most appropriate treatment to achieve the objectives. However, more studies should be carried out using the treatment of high hydrostatic pressures, with different combinations of pressure and time, in order to establish the appropriate combinations that allow reducing the microbial load, while maintaining the concentrations of the components of nutritional interest in colostrum.

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Volume 15 Issue 12 December 2020