



Effects of Spray-Drying, Freeze-Drying and Pasteurization on Microbiological Quality and IgG Level of Bovine Colostrum

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HIGHLIGHTS

- Spray-drying and freeze-drying methods were more effective than pasteurization to reduce microbial loads of colostrum.
- Freeze-drying treatment showed a lower reducing effect on the IgG level comparing to the other ones.
- Freeze and spray-drying methods could be more effective than pasteurization to enhance quality of colostrum.

Article type

Original article

Keywords

Colostrum
Immunoglobulin G
Food Microbiology
Food Preservation

Article history

Received: 14 Mar 2018

Revised: 21 Apr 2018

Accepted: 16 May 2018

Acronyms and abbreviations

IgG=Immunoglobulin G
SPC=Standard Plate Count
TCC= Total Coliform Count
CFU= Colony Forming Unit

ABSTRACT

Background: Nowadays, colostrum has been known as a considerable and valuable by-product of large-scale dairy production in the world. The main objective of this study was to evaluate the effects of pasteurization, spray-drying and freeze-drying methods on bacterial loads and Immunoglobulin G (IgG) level of bovine colostrums.

Methods: Colostrum samples were collected from the first milking postpartum of Iranian Holstein dairy cattle farms. The samples were treated by pasteurization (60 °C for 30 min and 55 °C for 60 min), spray-drying and freeze-drying methods. Standard Plate Counts (SPC), *Escherichia coli* count, and Total Coliform Count (TCC) were analyzed at days 1, 10, 20, and 30 of storage. Also, IgG level were assessed at the end of 30-day storage. Statistical analysis was performed using SPSS 17.0 (Chicago, IL, USA) software.

Results: Although all four treatments showed direct impact on reduction of SPC, TCC, and *E. coli* count in colostrum stored at 1, 10, 20, and 30 days, but the spray-drying and freeze-drying methods were significantly ($p<0.05$) more effective to reduce microbial loads. The mean IgG levels of the samples were 60.35 mg/ml for untreated samples (control); 30.65±6.95 mg/ml for spray-dried treatment; 36.97±6.79 mg/ml for freeze-dried treatment; 28.12±6.53 mg/ml for heated treatment at 60 °C/30 min; and 34.97±9.80 mg/ml for heated treatment at 55 °C/60 min. All four treatments resulted in significant ($p<0.05$) reduction of the IgG levels.

Conclusion: Considering the obtained results, it seems that freeze-drying and spray-drying methods could be more effective than pasteurization ones to enhance quality and shelf life of bovine colostrum for a long time.

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Introduction

Colostrum, as a nutritious liquid, is secreted during 48 to 72 h after mammalian parturition and supplies immunoglobulins, growth factors, antimicrobial peptides, including lactoferrin, lactoperoxidase and a perfect com-

bination of vitamins and minerals to insure the health, vitality, and growth of the new-born (Playford et al., 2000). The role of colostrum for a new born calf during first few days of life is critical due to providing passive immunity (Lilius and Marnila, 2001; Santos et al., 2017).

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To cite: Sotudeh Sh., Rabbani Khorasgani M., Etemadifar Z., Zarkesh-Esfahani S.H. (2018). Effects of spray-drying, freeze-drying and pasteurization on microbiological quality and IgG level of bovine colostrum. *Journal of Food Quality and Hazards Control*. 5: 54-60.

It has been historically accepted that bovine colostrum could be served as a human healthy food due to its nutritional and clinical importance such as usage of colostrum for various illnesses in India for thousands of years, especially for treatment of rheumatoid arthritis (Solomons, 2002). Also, usage of colostrum products have been growing due to an increased demand for functional foods and dietary supplements as an alternative to traditional drug therapy (Gapper et al., 2007). Hyper immune bovine colostrums containing anti-lipopolysaccharide immunoglobulin obtained from cow vaccinated against particular pathogens may play a significant role in healthcare such as a supplement for medical treatment diet (Brinkworth and Buckley, 2003; Byakwaga et al., 2011; Dominguez et al., 1997; Marnila and Gill, 2000).

Dairy cows produce far more colostrum than their calves need; hence, maintenance the excess colostrum is necessary for later usage of bovine colostrum. Nowadays, colostrum has been known as a considerable and valuable by-product of large-scale dairy production in the world (Yurchenko et al., 2016). Also, one of the main challenges in dairy production is to store high-quality colostrum in order to feed the newborn heifer calves (Phipps et al., 2016). However, microbial contamination of colostrum is a concern because it seems that bacteria in colostrum may interfere with passive absorption of colostral antibodies specially Immunoglobulin G (IgG). The microbial contamination could transfer to colostrum during its collection, handing, and storage (Fecteau et al., 2002; Godden et al., 2012; McGuirk and Collins, 2004; Stewart et al., 2005). Bacteriological loads of colostrum may highly influence the bioactive protein quality of finished colostrum-based products marketed for human consumption (Houser et al., 2008), thereby high quality colostrum should be used for the commercial production.

There are different methods have been proposed for storage of colostrum. For suitable storage of bovine colostrum without reducing its hygienic quality and IgG level, it seems that we need the best treatment protocol. Pasteurization and drying are more common than the other methods. Pasteurization successfully leads to reduce or complete elimination of *Mycobacterium avium*, mold and yeast, *Salmonella* spp., and the other pathogens (Elizondo-Salazar and Heinrichs, 2008; Stabel, 2008). There are two different methods for pasteurization of colostrums: 1) pasteurization at 60 °C for 30 min, and 2) pasteurization at 56 °C for 60 min (Argüello et al., 2003). However, overheating during pasteurization as a common method can cause denaturation of the proteins in the colostrum and destroy the efficacy of the end product. On the other hand, two other methods have been recognized for drying: 1) use of freeze drying which means drying during vacuum conditions and cooling to -40 °C, and 2)

spray drying which means drying under the hot conditions and in spray form in vacuum (Chelack et al., 1993).

There are some published researches in the literature about preservation methods of bovine colostrums; however, based on our knowledge, no comprehensive paper has been published yet on comparative evaluation of the effects of different treatments on the various microorganisms and IgG stability during shelf life of colostrum. Thereby, the first objective of this study was to evaluate the effects of pasteurization (60 °C for 30 min and 55 °C for 60 min), spray-drying and freeze-drying on bacterial population of bovine colostrum includes *Escherichia coli* count, Total Coliform Count (TCC), and Standard Plate Count (SPC) during 1, 10, 20, and 30 days after each treatment. The second aim of this study was to describe the effect of the mentioned treatments on IgG levels in treated samples at the end of day 30.

Materials and methods

Preparation of colostrum samples

Colostrum samples were collected from the first milking postpartum of Iranian Holstein dairy cattle farms within 0-72 h in Yazd province, Central Iran. Five individual samples were placed in sterile pots and transported in ice box to laboratory for *in vitro* experiments. IgG concentration levels were immediately measured in fresh samples. Each colostrum samples were then divided into 4 identical aliquots of approximately 250 ml each. These aliquots were then allocated to 1 of 4 groups for different treatments, including Group 1: freeze-drying, Group 2: spray-drying, Group 3: pasteurization at 55 °C for 60 min, and Group 4: pasteurization at 60 °C for 30 min.

Treatments

Samples were immersed in water bath (Parsian Teb, Iran) and heated at a constant temperature of 55 °C and 60 °C for 60 min and 30 min, respectively and held for the prescribed time in order to pasteurization while their containers were shaken to equalize the temperature inside them. Temperature was constantly monitored by thermometer. For rapid cooling after heat treatments, the samples were transferred to ice water. Then, the samples were stored at refrigerator until the microbiological and IgG level analyses (Trujillo et al., 2007).

Before lyophilisation, colostrum was stored in freezer at -20 °C; then, the frozen bovine colostrum was lyophilized (Castro et al., 2005; Klobasa et al., 1998). Lyophilisation treatment was performed using freeze-dryer VUCO5 (ZIRBUS, Germany). Spray-drying of colostrum was performed using spray-dryer SD-05 (LabPlant, UK). The maximum applied temperature was 50 °C.

IgG quantification

Radial Immune Diffusion (RID) kit was used for IgG quantification which was previously prepared at Faculty of Veterinary Medicine, Tehran University, Tehran, Iran. The circular wells were punched in the gel, using a 3 mm bore needle. Five wells received antigen solution for the preparation of the standard curve. An amount of 10 μ l standard samples with two repetitions and 10 μ l whey of each treated sample with a 1:50 dilution were poured in the wells, respectively. Then, the plates were incubated at 37 °C for 24 h and formation of antigen-antibody complex was expressed as sedimentary circle diameter (R) that is proportional to the IgG level. The standard curve was traced based on R^2 values of standard samples. Then, IgG antibody concentration in colostrum samples were obtained by R^2 determination from standard curve in mg/ml (Ameri and Wilkerson, 2008; Hadorn and Blum, 1997). The concentration of IgG was measured in fresh colostrum and also treated colostrums after 30 days of storage.

Microbiological analysis

The fresh colostrum samples were tested for microbiological quality before the treatments. The samples were tested for SPC, TCC, and *E. coli* count according to Institute of Standards and Industrial Research of Iran (ISIRI, 2008; 2015; 2016). The treated colostrums for temperature control were preserved at refrigerator. Then, during 1, 10, 20, and 30 days after treatment, each sample was analyzed for microbial counts.

Microbiological analysis was performed with the preparation of serial dilution of bovine colostrum in sterile ringer solution (1:10 ratio). After that, the appropriate dilution was used for the tests. The enumeration of SPC was performed on Plate Count Skim Milk agar (Q-LAB, UK) and incubated at 30 °C for 72 h.

Total coliforms and *E. coli* count were done by most probable number procedure. *E. coli* count was carried out in Lauryl Sulphate broth (Q-LAB, UK), and incubated at 37 °C for 24-48 h, EC broth (Q-LAB, UK) at 44 °C for 24-48 h, and tryptone water (Q-LAB, UK) at 44 °C for 24 h. TCC was carried out in Lauryl Sulphate broth, and incubated at 37 °C for 24-48 h, and Brilliant Green Bile broth (Q-LAB, UK) at 37 °C for 24 h.

Statistical analysis

Statistical analyses were performed using SPSS 17.0 (Chicago, IL, USA) software in order to evaluate the effects of different treatments on microbiological parameters and colostrum IgG with final significance declared at $p < 0.05$. The paired sample T test was done for microbial changes in different days of each treatment and between

each treatment with fresh colostrum, General Linear Model procedure with Repeated Measures was performed for the evaluation of the microbial changes among treatments. Fisher's Least Significant Difference (LSD) method was used for comparison between treatments. One Way ANOVA with LSD method was performed to test the effect of treatment on IgG levels.

Results

The SPC of fresh colostrum samples (control) varied from 2.6×10^4 to 7.9×10^6 Colony Forming Unit (CFU)/ml with average bacterial count about 5.5×10^5 CFU/ml. TCC for five fresh colostrum ranged from 4.8×10 to 7.90×10^2 CFU/ml with a mean of 1.5×10^2 CFU/ml. The Mean of *E. coli* count of fresh colostrum for five samples was 4.5×10 CFU/ml ranged from 2.0×10 to 1.1×10^2 CFU/ml. Moreover, the IgG concentrations at the first milking were 54.72 to 65.63 mg/ml with a mean of 60.35 mg/ml.

The mean IgG levels of the samples were 30.65 ± 6.95 mg/ml for spray-dried treatment ranged from 21.20 to 38.70 mg/ml; 36.97 ± 6.79 mg/ml for freeze-dried treatment ranged from 25.70 to 42.20 mg/ml; 28.12 ± 6.53 mg/ml for heated treatment at 60 °C/30 min ranged from 21.20 to 36.20 mg/ml; and 34.97 ± 9.80 mg/ml for heated treatment at 55 °C/60 min ranged from 20.56 to 45.20 mg/ml. All four treatments resulted in significant ($p < 0.05$) reduction of the IgG levels. Although there was no significant difference ($p > 0.05$) between IgG levels among the various treatments, but freeze-drying treatment showed a lower reducing effect on the IgG level comparing to the other ones.

Although all four treatments showed direct impact on reduction of SPC, TCC, and *E. coli* count in colostrum stored at 1, 10, 20, and 30 days, but the spray-drying and freeze-drying methods were significantly ($p < 0.05$) more effective than reduction of microbial loads (Tables 1 to 3).

Discussion

First milking bovine colostrum is an important source of immunoglobulins, nutrients, and other important immune factors while high bacterial contamination in colostrum can reduce the efficiency of colostrum absorption. Many species of bacteria found in colostrum have negative effect on colostrum composition especially protein component due to proteases production (Godden et al., 2006). The fresh colostrum should not be having high count of microorganisms and it must be free from pathogenic bacteria to be permitted for human consumption or making colostrum products. There are a few publications

describing the microbiological quality of colostrum and the effect of different treatments on the microbiological quality and IgG stability of colostrum during storage (Godden et al., 2006; McMartin et al., 2006; Stewart et

al., 2005). In this study, for monitoring the microbiological quality of bovine colostrum, SPC, TCC, and *E. coli* as hygiene indicators were selected for evaluation of the effects of treatments on microbiological quality.

Table 1: Standard plate count (\log_{10} CFU/ml) in colostrum treated with four different methods during 1, 10, 20, and 30 days of storage

Type of treatment	Storage time (day)							
	1		10		20		30	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Spray-dried	5.25±0.950	3.48-5.90	4.58±0.665	3.30-5.00	4.39±0.710	3.00-5.00	4.65±0.689	3.48-5.30
Freeze-dried	5.31±0.540	4.48-5.75	5.93±1.06	3.95-6.60	5.03±0.743	4.00-5.60	5.43±0.650	3.95-5.90
Pasteurization								
60 °C/30 min	4.08±0.622	3.90-5.47	5.24±0.65	3.95-5.60	6.30±0.719	4.85-6.60	7.60±0.523	6.72-7.90
55 °C/60 min	5.39±0.430	4.81-5.85	7.23±1.09	5.00-7.93	8.31±0.835	6.65-8.87	8.66±0.334	7.11-8.97

Table 2: Total coliforms count (\log_{10} CFU/ml) in colostrum treated with four different methods during 1, 10, 20, and 30 days of storage

Type of treatment	Storage time (day)							
	1		10		20		30	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Spray-dried	1.39±0.499	0.48-1.85	1.53±0.587	0.48-1.96	0.66±0.390	ND-1.04	0.96±0.346	ND-1.56
Freeze-dried	2.11±0.848	0.48-2.56	1.10±0.573	ND-1.48	0.71±0.414	ND-1.00	1.34±0.647	0.48-1.97
Pasteurization								
60 °C/30 min	1.03±0.327	ND-1.70	0.73±0.600	ND-1.38	0.79±0.647	ND-1.30	1.08±0.801	ND-1.60
55 °C/60 min	1.45±0.299	1.00-1.78	2.02±0.591	1.00-2.45	3.27±0.590	2.26-3.86	3.05±0.963	1.15-3.40

ND= Not Detected

Table 3: *Escherichia coli* count (\log_{10} CFU/ml) in colostrum treated with four different methods during 1, 10, 20, and 30 days of storage

Type of treatment	Storage time (day)							
	1		10		20		30	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Spray-dried	0.91±0.258	0.48-1.18	0.88±0.553	ND-1.48	0.23±0.384	ND-0.87	0.57±0.522	ND-1.18
Freeze-dried	1.36±0.543	0.3-1.66	0.94±0.559	ND-1.38	1.29 ±0.807	ND-1.96	1.20±0.821	ND-1.87
Pasteurization								
60 °C/30 min	0.99±0.719	ND-1.66	0.34±0.465	ND-1.04	0.47±0.523	ND-1.18	0.30±0.447	ND-1.00
55 °C/60 min	1.27±0.294	ND-1.69	1.55±0.556	0.79-2.15	2.59±0.332	1.04-1.30	1.09±0.172	1.04-1.30

ND= Not Detected

Our results showed that 60 °C treatment despite shorter period was more effective in relation to lower temperature and longer period; also, the 55 °C/60 min treatment could reduce TCC and *E. coli* count only for a short time period, whereas had no effective role in the reduction of SPC. Although several studies have been carried out about milk pasteurization, but studies in the field of colostrum pasteurization is negligible. Similarly, Elizondo-Salazar and Heinrichs (2009) reported that colostrum pasteurization at 60 °C/30 min is sufficiently appropriate for the elimination of pathogenic bacteria. In another research, Godden et al. (2006) showed elimination of inoculated bacteria in colostrum treated at 60 °C; these researchers showed that *Mycobacterium paratuberculosis* (1×10^3 CFU/ml), *S. Enteritidis* (1×10^6 CFU/ml) and *Listeria monocytogenes* (1×10^6 CFU/ml) have been eliminated during 30 min and for *E. coli* (1×10^6 CFU/ml) after 15 min of heat treatment. They reported that although the general pasteurization methods (High Temperature/Short Time), such as common pasteurization at 72 °C for 15 s, could be suitable; but high temperature causes viscosity increase and will have a negative impact on the immunoglobulin concentration. This problem can be solved with pasteurization at lower temperature with higher time (60 °C for 30 min). Meylan et al. (1996) reported that after heat treatment of colostrum at 63 °C for 30 min, the bacteria were reduced in an effective manner. McMartin et al. (2006) showed that colostrum at 60 °C for 120 min was completely pasteurized and its pathogenic bacteria were eliminated without increase of viscosity and reduction of IgG concentration. Elizondo-Salazar et al. (2010) showed the effectiveness of 57 °C for 30 min on reduction of SPC and TCC in colostrum. The findings of the previous studies are in agreement with our results indicating the perfect effectiveness of pasteurization with low temperature and long time for assuring the pathogens elimination of bovine colostrum. Moreover, 60 °C treatment could eliminate the microorganisms in a more effective manner, nonetheless this method is effective only to use the colostrum for a short period and time passage leads to increase of microbial counts and inappropriateness of colostrum taste. Therefore, it can be suggested that pasteurization at 60 °C along with other methods could be more effective for preservation of colostrum.

In the present research, both the treatment with spray-drying and freeze-drying methods had significant effects on the reduction of all studied microbial groups at least one log₁₀. So, these two methods may result in longer preservation time than that of pasteurization treatments. It should be indicated that spray-drying and freeze-drying are the commonly used methods in the industry for production of colostrum powder (Chelack et al., 1993; Houser et al., 2008).

Although we did not find any significant differences among IgG levels in colostrum treated by all four methods, however, freeze-drying had a lower reducing effect on the IgG level that is similar with findings of Castro et al. (2005). In this regard, Elfstrand et al. (2002) reported that lyophilization had no effect on the amount of IgG₁, but the amount of IgG₂ and IgA reduced up to 25%. Moreover, it was determined that heat treatment at 55 °C for 60 min could maintain antibody level from destruction, but treatment at 60 °C for 30 min may reduce the antibody level. McMartin et al. (2006) elucidated heat treatment at 60 °C for 120 min have no effect on the antibody content. However, 63 °C heat treatment resulted in 34% reduction of antibodies. Elizondo-Salazar et al. (2010) described that the IgG levels in untreated colostrum decreased with increasing temperature and holding time, while the lowest decline was observed in treated colostrum at 57 °C for 30 min about 66.8 g/l and most decline was done in treated colostrum at 63 °C for 90 min in comparison with untreated colostrum (71.6 g/l). Argüello et al. (2003) reported that colostrum pasteurization at two temperatures of 56 °C for 60 min and 57 °C for 10 min reduced IgG antibody by 37%. It was stated that pasteurization and spray-drying process has strongly negative effect on IgG levels (Ramya et al., 2016). A similar research exhibited that pasteurization of colostrum at 60 °C for 30 min with the assistance of steam system has a little effect on the reduction of IgG antibody but this reduction is not statistically significant (Elizondo-Salazar and Heinrichs, 2009). In this study a significant decline about 50% was observed in amount of IgG level in spray drying colostrum in comparison with untreated colostrum, while Chelack et al. (1993) showed that the dehydration of colostrum via the spray drying method causes only 5% reduction in the colostrum antibody level. Considering the variations in data reported in the previous investigations and also the current work, there are still some controversies about effect of different preserving treatments on antibody levels of colostrum.

Conclusion

It seems that freeze-drying and spray-drying methods could be more effective than pasteurization ones to enhance quality and shelf life of bovine colostrum for a long time.

Author contributions

Sh.S., M.R.K., and Z.E. designed the study and analyzed the data; Sh.S., M.R.K., Z.E., and S.H.Z. conducted the experimental work; Sh.S. and M.R.K. wrote the manuscript. All authors revised and approved the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgements

This research was ethically approved by the local institutional review board. This article is provided from a MSc thesis at Faculty of Science, University of Isfahan, Isfahan, Iran. The authors wish to thank authorities of University of Isfahan for financial support.

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