CHANGE OF NUTRIENT AND BIOACTIVE COMPONENTS IN BOVINE COLOSTRUM AFTER SPRAY DRYING

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ABSTRACT

Bovine colostrum powder (BCP) was obtained by using spray drying method with the inlet temperature at 120^{o}C and the outlet temperature at 60^{o}C. Nutrient and bioactive components such as protein, lipid, lactose, mineral, glycoprotein (e.g. lactoferrin) and the immunoglobulins IgG, IgA and IgM in BCP were analyzed before and after spray drying process.

Experimental results showed that the average values for total protein, lipid, lactose, mineral, lactoferrin in BCP were 25.31 %, 26.63 %, 33.92 %, 7.23 %, 1.497 %; and the average values for IgG, IgA and IgM in BCP were 136.31 mg/g, 27.31 mg/g and 4.22 mg/g respectively. The obtained results also illustrated that these compositions in BCP samples were decreased from 0.87 to 62.67 % compared with those in pasteurized colostrums samples, depending on the nature of each component in the colostrums. For instance, lactoferrin and total protein were lost 43.3 % and 34.76 %; IgG, IgA and IgM were decreased 22.57 %, 15.48 % and 45.41 %; while lactose and minerals were just lost 3.66 % and 0.87 %.

Keywords: Bioactive components, bovine colostrum, bovine colostrum powder, lactoferrin, immunoglobulins, nutrient compositions, spray drying.

1. INTRODUCTION

Colostrum is the first substance secreted by a mother's breast for her baby. It precedes milk and contains everything needed to start healthy growth of the young child. It not only contains fat, proteins, lactose, minerals, but also contains immune factors, vitamins such as A, E and B12, orotic acid and growth factors [1]. In other words, it supplies passive and active immunity and starts the growth of the baby. The colostrum in this study is from cows which contains four times as much immune factors as human colostrum. Colostrum contains immunoglobulins IgG, IgM, IgD, IgE, IgA and Secretory IgA. It also contains cytokines such as Interleukin 1 & 6, tumour necrosis factor, Gamma Interferon, leukocytes, lymphokines, lactalbumins, albumin, prealbumin, antibodies, lactoferrin and many others. Its like consuming a fully functioning immune system. It also contains transfer factors which can confer immunity from the cow to human beings, and mysterious informational peptides. An other substance in colostrum is Proline-rich Polypeptides (PRP.) PRP regulates the thymus and modulates the immune system. Glycoproteins (Protease inhibitors) are also found in colostrum [1, 2].

Immunoglobulins (Igs) are a family of globular proteins with antimicrobial and other protective bioactivities. The Igs are the principal agents that protect the gut mucosa against pathogenic microorganisms, and in colostrum they confer passive immunity to the ruminant until its own immune system is developed [3]. IgG antibodies express multifunctional activities, including complement activation, bacterial opsonisation and agglutination, and act by binding to specific sites on the surfaces of most infectious agents or products, either inactivating them or reducing infection. In bovine colostrum and milk, immunoglobulin G (IgG; subclasses IgG1 and IgG2) is the major immune component, although low levels of IgA and IgM are also present [4].

Lactoferrin is a glycoprotein existed in external secretions such as milk, especially colostrum. Lactoferrin has various bioactive functions such as broad-spectrum antimicrobial activity, promotion of iron transfer and absorption. Lactoferrin also has potent anti-viral effects against the replication of human HIV, cytomegalovirus, and herpes simplex viruses in various *in vitro* studies [5]. In 1999, the "Japanese Journal of Cancer Research" reported on a study that found lactoferrin significantly inhibited liver and lung cancer metastasis in mice. Later study using laboratory animals showed a large reduction in intestinal polyps after the animals were exposed to tumor-causing chemicals; another noted lactoferrin's ability to suppress the growth of human pancreatic cancer cells. Although it was not recommended for cancer therapy as of 2010, lactoferrin seems to hold promise as an anti-cancer agent. Lactoferrin also has therapeutic potential to treat inflammatory hepatitis, and possibly other inflammatory diseases, anti-osteoporosis and increase helpful microflora such as bifidus [6, 1]. Therefore, lactoferrin has great potential used widely in foods, pharmaceuticals, and cosmetics.

In the technological process to produce bovine colostrum powder, the first important point must be paid to prevent contamination during the harvest, storage, and feeding processes [7]. Management strategies to prevent bacterial proliferation in stored colostrum may include freezing, refrigeration, and the use of preservative agents such as potassium sorbate in refrigerated fresh colostrum [7]. One additional method of reducing or eliminating bacterial pathogens is to heat-treat fresh bovine colostrum. Currently in Vietnam there is no bovine colostrums processing industry. Two-thirds of bovine colostrum is consumed by calves, the rest one-thirds of colostrum is boiled and refrigerated at 4 ^{o}C to use for dairy households. However, some early studies showed that pasteurized colostrum at the same condition (time and temperature) recommended for milk resulted in denaturation of from 12 to 30 % of colostral IgG and sometimes caused significant increases in viscosity [8]. S. McMartin et al. – University of Minnesota, US identified the critical temperature at or below which heat treatment of bovine colostrum would produce no significant change in colostrum viscosity, IgG concentration, or Ig activity. Their research results showed that the reasonable pasteurization condition using for bovine colostrum was at temperature 60 °C for 120 minutes. This heat-treatment could preserve important colostral antibodies, immunoglobulins and prevent viscosity changes while still eliminate important pathogens [9].

Colostrum contains high levels of proteins, as well as many bioactive substances that are susceptible to be decomposed during heat-treatment processing. Currently, spray drying is commonly used to make colostrum powder because it produce a dried colostrum in which immunoglobulin quantity and function are preserved and is the most cost-effective [9]. In this study, the suitability of spray drying as a method for colostrum powder production was evaluated by analysing its effects on the content of IgG, IgA, IgM, lactoferrin, and others nutrients such as protein, lipid, lactose and mineral.

2. MATERIALS AND METHODS

2.1. Collection and pre-treatment of bovine colostrum

Colostrum samples within 0 - 72 h postpartum were collected from the LaiSind cows at the Ba Vi Research Centre for Cows and Pasture (Hanoi). LaiSind cow breed is a cross between cattle breeds Sind (India) with local yellow cattle breeds, is considered suitable for growing with Vietnam climate and for high milk production. Colostrum was collected from three time intervals postpartum: 0 - 24 h, 25 - 48 h and 49 - 72 h. Colostrum after milking was filtered through a cloth filter to remove impurities, and then refrigerated. Colostrum were then transported on ice to the laboratory, and stored immediately at 4 – 5 ^{o}C.

2.2. Processing of colostrum

Colostrum was pasteurized at a temperature 60 °C for 120 minutes [9]. This heat-treating can preserve important colostral antibodies, immunoglobulins and prevent viscosity changes while still eliminate important pathogens. After pasteurization, colostrum was stored at 4 ^{o}C between 4 days to spray dried; and about 50 ml of each sample were taken and stored at -20 ^{o}C between 4 weeks to analysed nutrient and bioactive components.

In this study, spray drying method was chosen with the inlet temperature at 120 ^{o}C and the outlet temperature at 60 ^{o}C to obtain colostrum powder [10].

Colostrum powder after spray drying is packaged. The Immunoglobulin IgG, IgA and IgM; lactoferrin and the nutrients such as protein, lipids, lactose, total mineral in colostrum after pasteurization and after spray drying were analysed. The process to obtain colostrum powder showed in Fig. 1.

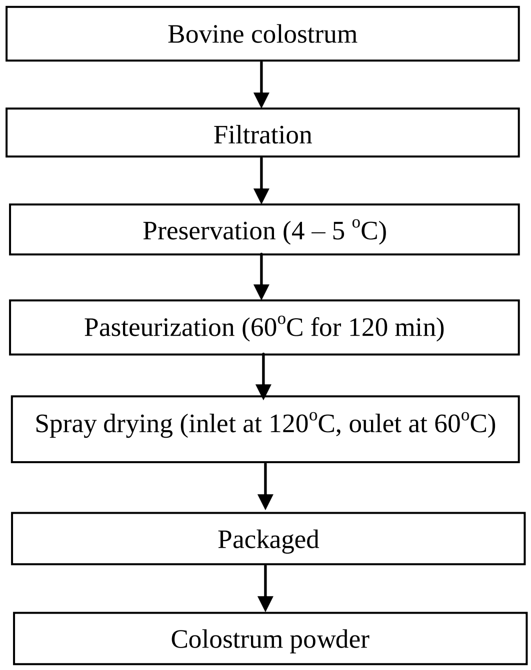

Figure 1. Processing scheme of colostrum.powder.

2.3. Determination of nutrient components

Total protein was measured by Kjeldahl method. ***Total fat*** was measured by Gerber method (using Butyrometer). ***Lactose*** was measured by Dinitrosalycilic acid method (DNS). ***Total mineral*** was measured by Calcine method [11, 12]. Each measurement was repeated 3 time.

2.4. Determination of bioactive components Igs and lactoferrin

The content of immunoglobulin IgG, IgA, IgM and lactoferrin in colostrum before spray drying (after pasteurization) and in colostrum powder (after spray drying) were determined by ELISA using Bovine Immunoglobulin ELISA Quantitation Kit E10-118, E10-121, E10-101 and Bovine Lactoferrin Elisa Quantitation Kit E10-126 respectively (Bethyl Laboratories, US). Analyses were performed following the manufacture's instructions. Briefly, 10 µg of capture antibody in 100 µl of 0.05 M $NaHCO_3$, pH 9.6 were coated to the wells of Nunc C bottom Immunoplate by incubating for 1 hour at room temperature. After 3 washes with 200 µl of PBS (50 mM Tris, 0.14 M NaCl, 0.05 % Tween 20, pH 8.0), wells were blocked with 200 µl of blocking solution (50 mM Tris, 0.14 M NaCl, 0.05 % Tween 20, pH 8.0). Samples and standards (100 µl) were loaded in duplicated and incubated for 1 hour at room temperature, followed by 3 washes. Subsequently, 100 µl of HRP conjugate antibody (dilution 1 : 20,000) were added to each well and incubated for 1 hour at room temperature. Wells were washed five times and the reaction was visualized by the addition of 100 µl chromogenic substrate (TMB) for 10 min. The reaction was stopped with 100 µl H_2SO_4 and absorbance at 450 nm was measured using the Model 680 Microplate Reader (Biorad). As a reference for quantification, a standard curve was established by a serial dilution of calibrators included in the kits [13, 1].

3. RESULTS AND DISCUSSION

3.1. Nutrient components

The content of total solids (dry matter) in colostrum was measured by method of drying to constant weight [12]. Dry matter concentration of pasteurized colostrum (%) corresponding with periods of time interval postpartum 0 - 24 h, 25 - 48 h and 49 - 72 h were 21.33 %, 11.67 % and 10.67 % respectively. Colostrum powder sample was diluted to obtain the same concentration with pasteurized colostrum sample. The measured results of total protein, lipid, lactose and total mineral in colostrum before and after spray drying are presented in table 1.

Table 1. Nutrient components in colostrum before and after spray drying at various intervals postpartum *(percent content comparing with dry matter concentration in colostrum).*

Interval postpartum (h)	Protein (%)	Lipid (%)	Lactose (%)	Mineral (%)
0 - 24	*62.12 ± 1.1* **28.14 ± 1.4**	*33.29 ± 2.2* **27.30 ± 2.1**	*18.24 ± 0.9* **17.28 ± 0.6**	*7.41 ± 1.0* **7.34 ± 1.4**
25 - 48	*78.83 ± 1.4* **24.12 ± 1.3**	*46.70 ± 2.0* **26.50 ± 2.3**	*39.33 ± 0.5* **38.87 ± 0.7**	*7.28 ± 1.4* **7.23 ± 1.0**
49 -7 2	*65.60 ± 1.5* **23.67 ± 1.2**	*45.83 ± 1.8* **26.10 ± 1.9**	*47.80 ± 0.8* **45.62 ± 0.8**	*7.22 ± 1.1* **7.15 ± 1.2**

(*Italic printed data*: before spray drying, *Bold printed data:* after spray drying).

The obtained result demonstrated that content of protein, fat, lactose and mineral in pasteurized colostrum before spray drying are significantly higher than that in mature milk [12]. Although breeding conditions were not the same, it was preliminarily estimated that the concentration of protein, fat and lactose in pasteurized colostrum of Vietnamese Laisind cows are significant higher than these compositions of Swedish Friesian cows [14] and are slightly higher than these compositions of Pennsylvania (USA) cows [15]. The protein, fat and mineral contents decreased with time, while the concentration of lactose increased.

The content of protein, fat, lactose and minerals in pasteurized colostrum were reduced after spray drying. The total mineral content was not significantly reduced (from 0.94 % in colostrum collected within 0 - 24 h postpartum to 0.97 % in colostrum collected within 49 - 72 h postpartum), but the protein content was reduced greatly from 54.7 % to 69.4 % respectively.

3.2. Bioactive components

3.2.1. Immunoglobulin IgG

Table 2. Concentration of immunoglobulin IgG in colostrum before and after spray drying at three time intervals postpartum.

Time interval postpartum (h)	Pasteurized colostrum	Diluted colostrum powder	Colostrum powder
	IgG (mg/ml)	IgG (mg/ml)	IgG (mg/g)
0 - 24	67.28 ± 0.04	55.45 ± 0.02 *(reduced 17.58 % comparing with before spray drying)*	260.05 ± 0.05
25 - 48	23.74 ± 0.03	11.80 ± 0.02 *(reduced 50.29 %)*	101.10 ± 0.03
49 - 72	7.42 ± 0.04	5.10 ± 0.01 *(reduced 31.26 %)*	47.77 ± 0.04
Average (0 – 72 h)	32.81 ± 0.037	24.12 ± 0.017	136.31 ± 0.04

The data in the table 2 indicated that IgG concentration was reduced from first 24 h postpartum (67.28 mg/ml) to first 72 h postpartum (7.42 mg/ml). IgG concentration was significantly reduced from 17.58 to 50.29 % by spray drying processing. We also estimated preliminarily that average IgG concentration in pasteurized colostrum in this study (32.81 mg/ml) was approximated with IgG concentration in report of Foley and Otterby [16] (32 mg/ml), and was approximated with average IgG concentration in Pennsylvania Bovine Colostrum (IgG = $IgG_1 + IgG_2$ = 36 mg/ml) [15].

3.2.2. Immunoglobulin IgA

The obtained result demonstated that IgA concentration was less reduced from the first 24 h postpartum (4.55 mg/ml) to first 72 h postpartum (4.30 mg/ml). IgA concentration was reduced from 9.0 to 21.16 % by spray drying processing. Average IgA concentration in pasteurized colostrum in this study (4.39 mg/ml) was higher significantly comparing to average IgA concentration in Pennsylvania Bovine Colostrum (1.66 mg/ml) [15].

Table 3. Concentration of immunoglobulin IgA in colostrum before and after spray drying at three time intervals postpartum.

Time interval postpartum (h)	Pasteurized colostrum	Diluted colostrum powder	Colostrum powder
	IgA (mg/ml)	IgA (mg/ml)	IgA (mg/g)
0-24	4.55 ± 0.02	4.14 ± 0.03	19.43 ± 0.05
25-48	4.31 ± 0.06	3.58 ± 0.04	30.72 ± 0.04
49-72	4.30 ± 0.05	3.39 ± 0.02	31.77 ± 0.01
Average	4.39 ± 0.043	3.70 ± 0.03	27.3 ± 0.03

3.2.3. Immunoglobulin IgM

Table 4. Concentration of immunoglobulin IgM in colostrum before and after spray drying at three time intervals postpartum

Time interval postpartum (h)	Pasteurized colostrum	Diluted colostrum powder	Colostrum powder
	IgM (mg/ml)	IgM (mg/ml)	IgM (mg/g)
0 - 24	2.05 ± 0.01	1.38 ± 0.04	6.45 ± 0.05
25 - 48	1.08 ± 0.03	0.35 ± 0.02	3.04 ± 0.03
49 - 72	0.66 ± 0.02	0.34 ± 0.01	3.17 ± 0.02
Average	1.26 ± 0.02	0.69 ± 0.023	4.22 ± 0.03

The data in the table 4 indicated that IgM concentration was reduced from first 24 h postpartum (2.05 mg/ml) to first 72 h postpartum (0.66 mg/ml). IgM concentration was reduced significantly from 32.68 to 67.59 % by spray drying processing. We also preliminary evaluated that average IgM concentration in pasteurized colostrum in this study (1.26 mg/ml) was lower significantly comparing to average IgM concentration in Pennsylvania Bovine Colostrum (4.32 mg/ml) [15], but nearly equal with IgM in report of Lidia Elfstrand et al. [14].

3.2.4. Lactoferrin

The obtained result demonstrated that lactoferrin concentration was reduced from first 24 h postpartum (0.42 mg/ml) to first 72 h postpartum (0.25 mg/ml). Lactoferrin concentration was significantly reduced (43 – 56 %) by spray drying processing. Average lactoferrin concentration in pasteurized colostrum in this study (0.33 mg/ml) is lower significant comparing with average lactoferrin in Pennsylvania Bovine Colostrum (0.82 mg/ml) [15].

Table 5. Concentration of lactoferrin in colostrum before and after spray drying at three time intervals postpartum.

Time interval postpartum (h)	Pasteurized colostrum	Diluted colostrum powder	Colostrum powder
	Lactoferrin (mg/ml)	Lactoferrin (mg/ml)	Lactoferrin (mg/g)
0 - 24	0.42 ± 0.01	0.28 ± 0.04	2.27 ± 0.03
25 - 48	0.31 ± 0.03	0.14 ± 0.01	1.19 ± 0.02
49 - 72	0.25 ± 0.02	0.11 ± 0.02	1.03 ± 0.02
Average	0.33 ± 0.02	0.18 ± 0.02	1.50 ± 0.02

The difference between the concentration of IgG, IgM, IgA and lactofferin in the colostrum sample of this research with the concentration of IgG, IgM, IgA and lactofferin in the colostrum sample of Pennsylvania Bovine Colostrum [15] that we temporarily compared as above caused by the difference of experimental conditions, of cow breed and of culture conditions. We also noted that difference with the research of S. I. Kehoe et al. [15] - the bioactive components IgG, IgA, IgM and lactoferrin were analysed from colostrum samples that still not been pasteurized, in this study - the bioactive components IgG, IgA, IgM and lactoferrin were analysed from pasteurized colostrum samples.

4. CONCLUSIONS

The protein, fat and mineral contents of bovine colostrum collected within 0 - 72 h postpartum was decreased by time, while the concentration of lactose increased. After spray drying, the content of protein, fat, lactose and minerals in colostrum were decreased; among them protein was the most decreased. The content of bioactive compounds such as immunoglobulin IgG, IgM and lactoferrin in bovine colostrum collected following three time interval postpartum 0 - 24 h, 25 - 48 h and 49 - 72 h were decreased significantly but the content of IgA was decreased at the lowest level following the above time interval postpartum. After spray drying, the content of IgG, IgM and lactoferrin were decreased significantly, however, among them IgA decreased at the lowest level.

In general, in colostrum powder obtained by spray drying, although the bioactive components were decreased, they were still remained at significant high concentrations. The average content of bioactive compounds IgG, IgA, IgM and lactoferrin in Bovine colostrum powder obtaining by spray drying were 136.31 mg/g, 27.31 mg/g, 4.22 mg/g and 1.50 mg/g.

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TÓM TẮT

SỰ THAY ĐỔI CÁC THÀNH PHẦN DINH DƯỠNG VÀ CÁC HOẠT CHẤT SINH HỌC TRONG SỮA BÒ NON SAU SẤY PHUN

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Sử dụng phương pháp sấy phun với nhiệt độ đầu vào là 120 ^oC và nhiệt độ đầu ra là 60 ^oCđể thu nhận sữa bò non dạng bột (BCP). Các thành phần dinh dưỡng và hoạt chất sinh học như protein, lipid, lactose, khoáng chất, glycoprotein (lactoferrin) và các globulin miễn dịch IgG, IgA và IgM trong BCP đã được phân tích trước và sau khi sấy. Kết quả nghiên cứu cho thấy rằng hàm lượng protein, lipid, lactose, khoáng chất và lactoferrin trung bình tương ứng trong BCP là 25,31 %; 26,63 %; 33,92 %; 7,23 %; 1,497 %; và hàm lượng trung bình của IgG, IgA và IgM trong BCP tương ứng là 136,31 mg/g; 27,31 mg/g và 4,22 mg/g. Kết quả nghiên cứu cũng chỉ ra rằng hàm lượng các thành phần nói trên trong BCP sau khi sấy phun giảm từ 0,87 đến 62,67 % so với hàm lượng của chúng trong sữa bò non sau khi thanh trùng, tùy thuộc vào bản chất của mỗi cấu tử. Chẳng hạn như, lactoferrin và protein bị giảm đi 43,3 % và 34,76 %; IgG, IgA và IgM giảm 22,57 %; 15,48 % và 45,41 %; trong khi đó lactose và khóang chất chỉ bị giảm 3,66 % và 0,87 %.

Từ khóa: hoạt chất sinh học, sữa bò non, bột sữa bò non, lactoferrin, các globulin miễn dịch, các thành phần dinh dưỡng, sấy phun.