



Compositional and functional analysis of freeze-dried bovine skim colostrum powders

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Abstract

In the early few days, the composition of colostrum changes significantly, which strongly impacts the functional characteristics of skimmed colostrum powders. This study evaluated the effect of compositional changes of colostrum and its functional characteristics in the first 5 days of post-partum milking. The protein content of samples from day-1 to day-5 reduced from 13.5 to 3.5% (w/v). The lactose content increased while fat, ash, and solid not fat content decreased significantly ($p < 0.05$) during this period. The foaming capacity changed slightly, while the emulsifying capacity significantly decreased from day-1 to day-5 ($50.8 \pm 1.35\%$ to 32.6 ± 1.8). The X-Ray diffraction (XRD) showed that the first 2 days' samples were amorphous, and the later samples showed slight peaks due to an increase in lactose content. Differential scanning calorimetry (DSC) found that the thermal stability increased (109°C on day-1 to day-5 (109 – 132.4°C) with the increasing lactose and casein content. Since freeze-drying is a non-thermal process, no changes in secondary structures (α -helix ~ 1655 , random coil ~ 1646 , and β -sheets $\sim 1632\text{ cm}^{-1}$) were observed in Fourier transforms infrared spectroscopy (FTIR). The FTIR indicates that the bioactive proteins were intact, which can be used to incorporate health-promoting effects. The in vitro digestibility of the day-1 sample showed a higher amount of recovered IgG ($55.28 \pm 1.11\%$), IgM ($18.28 \pm 0.93\%$), and IgA ($11.42 \pm 0.58\%$) as compared to the number of recovered immunoglobulins in later days samples. The recovered IgG for day-5 was $27.02 \pm 0.82\%$, while the IgM and IgA could not be detected after the first 2 days. These colostrum powders showed good functional properties and can be incorporated into functional foods, nutraceuticals, and infant formulas to obtain the immune-boosting benefits of their bioactive proteins.

Keywords Colostrum · Digestibility · Freeze drying · Functional properties · Immunoglobulins · Physicochemical · Powders

Introduction

The increasing interest in promoting good health through diet has sparked a trend toward “functional foods” and “nutraceuticals.” This trend is driven by food processing industries that aim to high market-value food ingredients with added health benefits. Developed countries such as the USA and European countries are experiencing rapid growth

in demand for these natural food sources [1]. Colostrum, the first 3–7 days of milk after parturition, is a rich source of potent bioactive, which makes it a suitable candidate for such markets [2]. In developing nations, feeding milk and other food preparations fortified with colostrum powder could help to reduce infant mortality rates [3]. Although organized dairy farms produce an abundance of colostrum daily, nutraceutical properties remain underutilized in many countries due to insufficient preservation and processing technologies.

Bovine colostrum contains various immune-building constituents, including lysozymes, lactoferrin, immunoglobulins (Igs), with the most abundant being IgG (about 70% of Igs) and lactoperoxidase, etc. [4]. The expedient concentration of IgG in bovine colostrum is more than 50 g/L. IgG, a large globular protein with a molecular weight of about 150 kDa, is the most discussed immunoglobulin in calf-raising and is

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measured in calf serum around 24-h post-colostrum feeding to evaluate passive transfer of immunity (PTI) [5]. Igs are a major immune component of colostrum, having a high potential for utilization in infant formulae, health beverages, specialized dietary supplements, and innovative functional foods [2]. However, during the 1st week of lactation, instantaneous or sometimes gradual changes occur in colostrum composition according to the prerequisite of the neonate, including total solids, Igs, and protein content showing a reduction in their concentration. The pH, lactose content, casein fraction, and fat escalate significantly in early lactations [4]. The functional properties of powders are salient to incorporating the protein powders into other food supplements or developing a functional product from protein constituents. The compositional variation of colostrum in the early days affects its functional characteristics significantly, which include emulsifying capacity, foaming capacity, thermal stability, wettability, solubility, hygroscopicity, and buffering capacity [7].

Dairy liquids are generally converted into powders due to their stability and easy storage. Although many drying methods are available to convert protein dispersions into powders, freeze-drying still seems a promising technique for sensitive products [6]. Previous studies reported extreme changes in physical and functional parameters of early lactations of bovine colostrum [8]. The compositional and conformational changes that occur in colostrum during different stages of lactation and how these changes impact the functional properties of colostrum powders have not been comprehensively studied yet. Thus, the objective of this study was to conduct a comprehensive functional and structural analysis to evaluate the effect of compositional changes of colostrum on the functional properties of freeze-dried skim colostrum powders. These powders were further characterized for their thermal stability by differential scanning calorimetry (DSC), and the conformational changes were evaluated through Fourier transform infrared spectroscopy (FTIR) and X-ray crystallography (XRD). The major Igs (IgG, IgM, and IgA) were quantified using enzyme-linked immunosorbent analysis to evaluate the bioaccessibility of these Igs through *in vitro* study.

Materials and methods

Materials and reagents

Holstein Friesian cow was milked after 3-h post-partum (1st parturition) by machine milking at David Enterprises Ltd., Thailand. The cow was milked by machine twice a day immediately after suckling by the calf for first 5 days. The colostrum samples were immediately stored at freezing condition ($-20\text{ }^{\circ}\text{C}$). The enzyme linked immunosorbent

analysis (ELISA, Pink-One) kits with all the required reagents including wash buffer and stop solution were purchased from LABISKOMA, South Korea. All the analytical grade chemicals and reagents including HCl, NaOH, NaCl, KCl, CaCl_2 , NaHCO_3 , and enzymes (Pepsin, trypsin, lipase, and bile extract) were procured from CTi & Science Co. Ltd, Thailand.

Sample preparation and physicochemical analysis

After the collection of all samples, frozen samples of each day lactations (two lactations/day) were thawed at $40\text{ }^{\circ}\text{C}$ using hot water bath and both lactations were mixed to obtain the homogeneous sample. To avoid the thermal effect on the physiological properties of samples, liquid (at around $28\text{ }^{\circ}\text{C}$) was immediately separated from solid sample at different intervals. The samples were diluted by adding distilled water into 1:1 ratio. The cream was separated from diluted samples using laboratory scale semi-open type of cream separator at $45\text{ }^{\circ}\text{C}$. The skimmed samples were lyophilized after physicochemical analysis using a pilot scale freeze dryer (Scanvac, Coolsafe 55-4, Labogene, Denmark). Total solids, protein, fat, and ash percentage were estimated using standard AOAC methods number 923.10, 979.09, 920.39C, and 925.03 respectively [9]. Lactose concentration was estimated by subtracting the sum of protein, ash, and fat content from total solid content. Casein content was calculated by decreasing the pH of the 50 mL sample (liquid colostrum) to 4.6 using HCl at $40\text{ }^{\circ}\text{C}$, resulting the precipitation of casein. The percentage of lactic acid (titratable acidity) of a 10 mL sample was estimated by titrating the sample against 0.1N NaOH solution, and the pH of raw colostrum was measured using a pH meter (Hanna, USA). The electrical conductivity of liquid colostrum was determined by dipping a digital electrical conductivity meter (RCYAGO, EC meter, China) in 100 mL sample at room temperature. Ethanol stability of samples was estimated by recording the maximum concentration of ethanol that did not cause coagulation in the sample. The 2 mL of sample was mixed with 2 mL of aqueous ethanol solutions at various concentrations (40–100% v/v).

Immunoglobulins analysis

Commercially available ELISA kits (Pink-One, Labiskoma, Seoul, Korea) were used to quantify the IgG, IgA, and IgM concentrations. Standards for antibody concentrations were prepared as instructed by the manufacturer, using serial dilutions in phosphate buffer (pH 7.4). The samples were added to cuvettes after washing with wash buffer at $25\text{ }^{\circ}\text{C}$ (0.05 M Tris, 0.138 M NaCl, 0.05% Tween 20 and pH 8.0) and incubated for 1.5 h. The cuvettes were washed and incubated again for an hour after adding the detection antibody. The results were obtained at 450 nm wavelength by a micro plate reader (Tecan

Infinite F-50, Fornax Technologies, Austria) for all respective antibodies and data was assessed using Magellan software.

Functional characterization of skim colostrum powders

The emulsifying capacity and foaming properties were estimated at room temperature and neutral pH (7.0) using a method described by [10]. The 1% w/v of skim colostrum powder in 100 mL of distilled water, and 20 mL of corn oil were homogenized by blending the mixture at 2000 rpm for 2 min in a high-speed blender (IKA, Germany). The emulsion was centrifuged for 5 min at 1100×g.

$$\text{Emulsifying capacity(\%)} = \frac{\text{Height of emulsified layer}}{\text{Height of Total sample in tube}} \times 100$$

The stability of emulsion was determined by heating it at 80 °C for 30 min and centrifuging it at 1100×g for 5 min.

$$\text{Emulsifying Stability(\%)} = \frac{\text{Height of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \times 100$$

The sample (2 mg/mL) was poured into a beaker to evaluate the foaming characteristics, and the volume was recorded as A_1 . The sample was homogenized using a homogenizer (Model 50000-25, USA) at 900 rpm for 5 min, and the volume was recorded as A_2 ; after 30 min, the volume was recorded as A_3 .

$$\text{Foaming capacity(\%)} = \frac{A_2 - A_1}{A_1} \times 100$$

$$\text{Foaming stability(\%)} = \frac{A_3 - A_1}{A_1} \times 100$$

The water activity (a_w) of the powder samples (1.5 g) was measured at 25 °C using a water activity meter (Freund EZ-200, Japan). The sample (1 g) was placed in a desiccator with saturated NaCl solution (28% w/v) to maintain the relative humidity (RH) of 75% at 25 °C. The hygroscopicity was estimated using sample weight after equilibrium with the salt solution [11]. The solubility of powder samples was determined by dispersing the sample (5 g) in 100 mL of distilled water at 25 °C and neutral pH (7.0). The solution was centrifuged at 1000×g for 10 min and protein concentration was estimated in supernatant [10].

$$\text{Solubility(\%)} = \frac{\text{Protein concentration in supernatant}}{\text{Total Protein Content}} \times 100$$

The buffering capacity of powders was evaluated depending on pH changes within 15 min, due to addition of 1 mL of 0.1 M HCl/10 mL of colostrum solution (2% w/v) [10].

The color of the samples were analyzed using a Hunter lab colorimeter (CX-1075, Colorflex, USA) in terms of L^* (lightness), a^* (red-green component) and b^* (yellow-blue components) parameters and whiteness was calculated using the following equation.

$$\text{Whiteness} = 100 - \left[(100 - L^*)^2 + (a^*)^2 + (b^*)^2 \right]^{1/2}$$

Thermal denaturation analysis of powders

A sample of colostrum powder (2 mg) was hermetically sealed in an aluminum pan and heated from 40 to 150 °C, with a heating rate of 10 °C/min with a nitrogen purge rate of 50 mL/min using a DSC instrument (Model-214, NETZSCH, Germany). An empty aluminum pan was used as reference and each experiment was conducted in triplicates. The temperature of maximum deflection was recorded

as denaturation temperature of sample [12].

Fourier transform infrared spectroscopic (FTIR) analysis

The FTIR analysis was performed to evaluate the secondary structure of protein content in skimmed colostrum powders. The powder samples (2 mg) were pressed into a pellet on the surface of optical crystal cell, and spectra were recorded over the frequency range of 4000–500 cm^{-1} by a Nicolet iS50 FT-IR Spectrometer (Thermo Scientific, USA) at 25 °C [13].

X-ray diffractometry (XRD)

X-ray diffractograms were obtained at room temperature (25 °C) using an X-ray Diffractometer (Model 10190376, Bruker, Germany). The diffractometer was operated at 40 kV, and 40 mA with Cu K α radiation ($k = 1.5406 \text{ \AA}$) and scans were carried out over a range of 3–40 (2 θ) with step size and step time of 0.05 and 1.0 s, respectively [13].

In vitro digestibility and bio accessibility

The in vitro digestion study was conducted following the method described by [14, 15] with slight modifications. The skimmed colostrum powder (1 g) was dispersed in 10 mL distilled water. Enzyme A (pepsin 120 mg/30 mL of 0.1 M HCl) and saline A (0.31 g NaCl, 0.11 g KCl, 0.025 g

CaCl₂ and 0.06 g NaHCO₃ in 100 mL distilled water) were added in a beaker and pH was adjusted to 2.5 by 0.1 M HCl solution. The simulated small intestinal fluid (SSIF) was prepared by adding 0.54 g NaCl, 0.065 g KCl and 0.033 g CaCl₂, 200 mg pancreatin, lipase 5 mg, trypsin 6.5 mg and 1.2 g bile extract in 100 mL of 0.1 M NaHCO₃ and pH was adjusted to 7.0 using 0.1 M NaOH solution. In gastric digestion, 5 mL of sample was mixed with 27 mL of saline A and 2 mL of enzyme A, and the pH was adjusted to 2.5. The samples were incubated at 37 °C for 1.5 h and monitored pH. The gastric digestion was stopped by adjusting the pH to 5.3 using a 0.9 M sodium bicarbonate solution. The 3.0 mL of SSIF was mixed with 10.0 mL of gastric digested solution and the pH was adjusted to 7.0 for 1.5 h at 37 °C to complete the small intestinal digestion. At the end of in vitro digestion, the supernatant was carefully collected after centrifugation at 4000×g for 5 min at 25 °C, and ELISA analysis was done immediately to measure the Igs. Since the day-1 sample contained high amount of Igs, the Igs release profile of day-1 sample was evaluated taking 1 mL of sample after half hour for 5 consecutive hours from small intestine digestion.

Results and discussion

Proximate analysis of raw colostrum and skim colostrum powders

The day-1 sample of skim colostrum contained a higher amount of total solids (23.7% w/v), including total protein and lactose. The concentration of protein contents decreased significantly ($p < 0.05$) within these 5 days (Fig. 1). The protein content of samples from the day-1 to day-5 reduced from 13.5% (w/v) to 3.5% (w/v). The early lactations contain high amount of whey protein consisting of globulins and high amount of Igs (as observed by ELISA results) to provide passive immunity to calf against infection or diseases [16]. The concentration of casein was also significantly higher in the day-1 milking (Fig. 1) and decreased with each milking day. Colostrum contains a high amount of κ -casein, which decreased with time while the amount of α s-casein increased with time, and the β -casein was constant in all samples. The lactose content increased while fat, ash and solid not fat content decreased significantly ($p < 0.05$) during this period. The concentration of lactose

$$\text{Bio accessibility(\%)} = \frac{\text{Amount of IgG after digestion}}{\text{Total Amount of IgG in samples}} \times 100.$$

Statistical analysis

The SPSS software (SPSS Version 16, Chicago, IL, USA) was employed for conducting the statistical analysis where one-way ANOVA was carried out. The significant differences among the samples were determined using the post hoc Tukey's test, having a confidence interval of 95%.

content was found to double (2% (w/v) on the day-1 to 4% (w/v) on the day-5). Previous studies have also reported similar trends in compositional change of colostrum [7, 17] (Table 1).

Fig. 1 Immunoglobulins content (mg/mL) in the raw colostrum samples (Mean \pm SD)

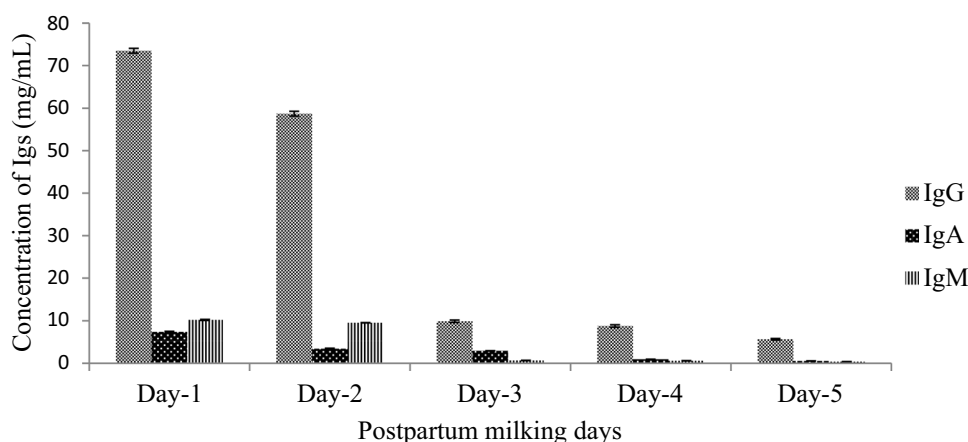
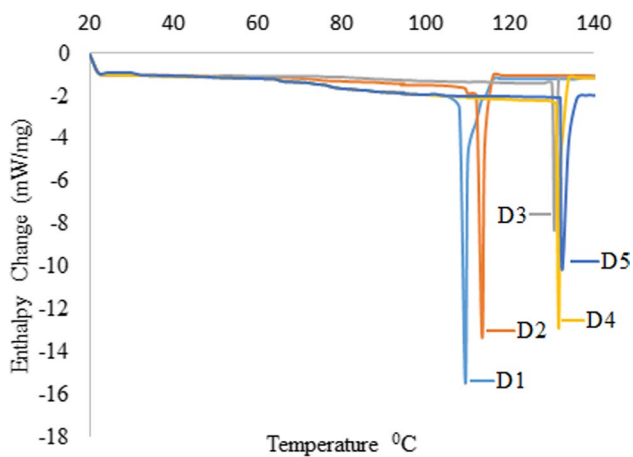


Table 1 Proximate analysis of raw colostrum and skim colostrum powders for 5 days (mean percentage \pm SD)

	Day-1	Day-2	Day-3	Day-4	Day-5
Raw colostrum					
Total solids	23.7 \pm 0.22 _a	18.3 \pm 0.09 _b	14.1 \pm 0.07 _c	13.5 \pm 0.05 _d	12.8 \pm 0.07 _e
Total protein	13.5 \pm 0.05 _a	9.6 \pm 0.12 _b	6.8 \pm 0.13 _c	4.8 \pm 0.12 _d	3.5 \pm 0.09 _e
Fat	6.8 \pm 0.15 _a	5.7 \pm 0.09 _b	4.2 \pm 0.19 _c	4.3 \pm 0.17 _c	4.2 \pm 0.03 _c
Lactose	2.2 \pm 0.16 _a	2.1 \pm 0.14 _a	2.1 \pm 0.12 _a	3.5 \pm 0.50 _b	4.3 \pm 0.19 _b
Ash	1.2 \pm 0.02 _a	1.1 \pm 0.02 _b	1.0 \pm 0.01 _c	0.9 \pm 0.02 _d	0.8 \pm 0.01 _e
SNF	16.9 \pm 0.3 _a	12.6 \pm 0.14 _b	9.8 \pm 0.14 _c	9.2 \pm 0.12 _{cd}	8.6 \pm 0.09 _d
Casein	4.8 \pm 0.12 _a	4.4 \pm 0.03 _b	3.6 \pm 0.12 _c	3.3 \pm 0.03 _{de}	2.8 \pm 0.07 _e
Whey protein	8.7 \pm 0.11 _a	5.2 \pm 0.01 _b	3.2 \pm 0.12 _c	1.6 \pm 0.13 _d	0.7 \pm 0.18 _e
Skim colostrum powder					
Total solids	95.2 \pm 0.32 _a	95.3 \pm 0.08 _a	94.8 \pm 0.12 _a	94.9 \pm 0.2 _a	95.3 \pm 0.2 _a
Total protein	75.2 \pm 0.30 _a	72.5 \pm 0.10 _a	65.1 \pm 0.15 _b	49.6 \pm 0.2 _c	40.6 \pm 0.16 _d
Casein	26.8 \pm 0.21 _a	33.3 \pm 0.20 _b	34.6 \pm 0.19 _b	34.1 \pm 0.15 _b	32.5 \pm 0.21 _c
Whey	48.5 \pm 0.15 _a	39.3 \pm 0.25 _b	30.6 \pm 0.16 _c	16.5 \pm 0.12 _d	8.2 \pm 0.15 _e
Lactose	12.3 \pm 0.2 _a	15.9 \pm 0.22 _b	20.1 \pm 0.21 _c	36.2 \pm 0.13 _d	41.9 \pm 0.10 _e
Ash	6.7 \pm 0.24 _a	8.3 \pm 0.09 _b	9.6 \pm 0.22 _c	9.2 \pm 0.3 _c	9.3 \pm 0.30 _c

The different superscripts in the same row indicate the significant differences ($p < 0.05$)

**Fig. 2** Thermal denaturation illustration of skim colostrum powders during the first 5 days of lactation

Estimation of immunoglobulin content

The Igs remain higher in the first 2 days of lactation and decrease significantly in later lactations. It was found that the IgG decreased from 73.5 ± 0.56 to 5.7 ± 0.19 mg/mL (Fig. 2), indicating a statistically significant difference ($p < 0.05$), while the amount of IgA was higher in the day-1 sample (7.4 ± 0.14 mg/mL) and suddenly decreased from the day-2 (3.4 ± 0.1 mg/mL) to onwards. The amount of IgM also has shown a similar trend in the 5 days lactations, decreasing from 10.2 ± 0.12 to 0.4 ± 0.02 mg/mL. The concentrations of Igs decreased in colostrum with the number of milking at varying rates in different cow species. In a previous study, the amount of IgG (around 93 mg/mL) decreased from 20 to 30% after 10 h, while the amount of IgA (1.6 mg/mL) and IgM (4.5 mg/mL) decreased by around 50% during first 10 h [18].

Table 2 Physicochemical characteristics of raw colostrum samples (Mean \pm SD)

Colostrum	Day-1	Day-2	Day-3	Day-4	Day-5
pH	6.11 \pm 0.03 _a	6.18 \pm 0.03 _a	6.33 \pm 0.04 _b	6.36 \pm 0.02 _{bc}	6.42 \pm 0.02 _c
TA (%LA)	0.64 \pm 0.01 _a	0.40 \pm 0.02 _b	0.31 \pm 0.01 _c	0.20 \pm 0.01 _d	0.17 \pm 0.01 _d
Specific gravity	1.075 \pm 0.01 _a	1.064 \pm 0.00 _b	1.044 \pm 0.01 _c	1.039 \pm 0.00 _d	1.035 \pm 0.01 _d
Ethanol stability (%E)	46.0 \pm 2.0 _a	52.0 \pm 2.0 _b	56.0 \pm 2.0 _{bc}	60.0 \pm 2.0 _{cd}	62.0 \pm 1.20 _e
Electrical conductivity (μ S/cm)	5130 \pm 20 _a	4946 \pm 25.2 _b	4813 \pm 30.6 _c	4650 \pm 26.5 _d	4410 \pm 20 _e

*The different superscripts in the same row indicate the significant differences ($p < 0.05$)

Physicochemical properties of raw colostrum

The specific gravity of colostrum was significantly higher on the day-1 (1.075 ± 0.01) and decreased substantially in later days up to 1.04 ± 0.01 on the 5th day of parturition, which was near to specific gravity of normal milk (1.03). This change in density was due to higher amount of total solids on day-1 ($23.7 \pm 0.22\%$) compared to the day-5 sample's total solids ($12.8 \pm 0.07\%$). Previous studies reported that the specific gravity of milk varied from 1.074 to 1.054 during the early 2 days and gradually decreased to 1.03 in the 1st week of parturition [4].

Similarly, titratable acidity was higher (0.64 ± 0.01) on the day-1 and decreased significantly up to the day-5 (0.17 ± 0.01) as the pH of colostrum was also lower than that of the normal milk (Table 2). It was reported that the titratable acidity had a strong relationship with the protein concentration of colostrum, and when protein concentration decreased, the titratable acidity of the milk also decreased. Since the titratable acidity is a measurement of buffering capacity to lactic acid due to proteins, CO_2 , phosphates and citrates, the low pH and high titratable acidity of colostrum were related to increased concentration of protein fragments, phosphate, citrates, and CO_2 due to increased permeability of the mammary gland during the prepartum period, allowing more blood elements to enter the milk.

Electrical conductivity (EC) is one of the suitable ways to monitor microbial growth, metabolic activity, and changes in salt concentration. In milk, lactose is not a conductor, and fat content hinders conductivity, while protein have minor importance. The samples have shown a decreasing trend in electrical conductivity as colostrum contains a higher concentration of free ions (e.g., chlorides, phosphates, citrates etc.), responsible for electrical conductivity. The high concentration of calcium, phosphate, magnesium, and sodium were reported in colostrum which decreased rapidly in later parturition [4].

Ethanol stability is defined as the minimum concentration of aqueous ethanol that results in milk coagulation. The ethanol stability of colostrum was found to be significantly increasing up to the day-5 (Table 2). In a previous study, it was reported that ethanol stability of colostrum was low in the first 4 days, around 54% on average, as observed in our study for the 4th day ($60 \pm 2\%$). The samples with poor ethanol stability also had a low value of pH; for day-1, it was significantly ($p < 0.05$) acidic (6.1 ± 0.03) as compared to that of day-5 (6.4 ± 0.02), which was nearly equal to the normal pH of milk (6.7). The minerals including calcium and magnesium play a crucial role in milk and colostrum ethanol stability. The less ethanol stability of colostrum in the early days indicates the high concentration of minerals as the excess of calcium and magnesium ions causes the coagulation over phosphate and citrate. The ethanol stability of milk was reported to be around 70%, which contains fewer minerals than colostrum [19].

Functional characterization of skimmed colostrum powder

The emulsifying capacity for the first 2 days of skimmed colostrum powder remained similar ($50.8 \pm 1.35\%$ and $50.5 \pm 1.83\%$, respectively), while there was a significant decrease in emulsifying capacity for the samples of day-3 and onwards (Table 3). The possible decrease is due to the reduction in the globular protein content of samples, which could emulsify the samples [20]. There was a significant decrease in emulsifying stability of skim colostrum from the first ($39.6 \pm 3.4\%$) to the 5th day ($26.1 \pm 1.2\%$). This trend can also be related to less whey protein in the sample on the 5th day compared to the 1st-day sample. Since the globular proteins increases the emulsifying ability as well as the stability of emulsion, the less amount of these proteins caused less stability.

Table 3 Functional characteristics of freeze-dried skimmed colostrum powder (Mean \pm SD)

Colostrum	Day-1	Day-2	Day-3	Day-4	Day-5
Emulsifying capacity (%)	$50.8 \pm 1.4_a$	$50.5 \pm 1.8_a$	$41.4 \pm 1.4_b$	$32.9 \pm 1.4_c$	$32.6 \pm 1.8_c$
Emulsifying stability (%)	$39.6 \pm 1.9_a$	$36.5 \pm 1.4_{ab}$	$30.6 \pm 1.0_{bc}$	$26.0 \pm 0.4_c$	$26.1 \pm 1.2_c$
Foaming capacity (%)	$19.3 \pm 1.2_{ab}$	$15.5 \pm 1.8_a$	$13.8 \pm 1.1_a$	$13.0 \pm 2.3_a$	$12.1 \pm 1.3_a$
Buffering capacity	$0.043 \pm 0.005_a$	$0.038 \pm 0.002_{ab}$	$0.035 \pm 0.003_{bc}$	$0.031 \pm 0.004_c$	$0.024 \pm 0.001_d$
Solubility (%)	$96.9 \pm 1.5_a$	$95.5 \pm 0.7_{ab}$	$95.0 \pm 1.3_{ab}$	$94.1 \pm 0.7_b$	$92.8 \pm 0.8_b$
Wettability (min)	$12.4 \pm 1.1_a$	$10.2 \pm 0.4_b$	$9.2 \pm 0.3_{bc}$	$8.4 \pm 0.3_d$	$8.0 \pm 0.2_d$
Hygroscopicity (%)	$23.0 \pm 0.42_a$	$21.4 \pm 1.16_b$	$20.9 \pm 0.78_b$	$19.1 \pm 1.52_c$	$17.1 \pm 0.21_d$
Whiteness	$83.3 \pm 0.32_a$	$84.4 \pm 0.33_b$	$87.2 \pm 0.45_c$	$87.7 \pm 0.27_c$	$88.5 \pm 0.20_d$
Water activity	$0.23 \pm 0.01_a$	$0.28 \pm 0.02_b$	$0.31 \pm 0.01_c$	$0.33 \pm 0.01_c$	$0.31 \pm 0.02_c$

*The different superscripts in the same row indicate significant differences ($p < 0.05$)

The ability of proteins in the sample to generate stabilized foams by reducing the interfacial tension at the interface between the air and water phases governed their functional properties. The foaming capacity for day-1 was $18.3 \pm 2.9\%$, which changed slightly during the 5 days, but statistically, the change was non-significant ($p > 0.05$). High mixing speed up-surfed the foam formation and stability, but the foam was generated only for a short interval of time, indicating the instability of foams produced by colostrum proteins (data not shown). The presence of flexible and random macromolecules like caseins formed less viscoelastic film due to loosen dynamic structure, while the globular proteins form the films with higher rigidity because of strong interactions and high packing density. Due to the lower whey to casein ratio in the day-5 sample, it was concluded that casein decreased the foaming and emulsifying properties compared to the day-1 colostrum containing a high concentration of globular proteins [12].

The buffering capacity is explicated as the resisting ability of milk to change its pH due to the addition of a base or acid due to its buffering integrant (proteins, soluble phosphate, carbonate, calcium phosphate and citrate). The colostrum maintains its buffering capacity due to higher concentrations of these integrant [4]. The higher buffering capacity of colostrum from day-1 (0.043 ± 0.005) was observed due to high protein content and salt, as compared to day-5 (0.024 ± 0.001) containing less concentration of protein, salt, citrate, and soluble phosphate. The significantly dwindle trend ($p < 0.05$) with advancing lactation was interjected for buffering capacity, which could be related to a reduction in protein and salt contents. The higher buffering capacity of colostrum compared to milk with a gradual decrease in its later milking was also indicated in a previous study [21].

The higher solubility of samples ($> 90\%$) was observed as the samples were freeze-dried, which had a non-significant effect on protein structures. There was slight decline in the solubility of day-1 (96.9%) samples to day-5 (92.8%) due to higher amount of whey proteins in day-1 samples which decreased significantly up to day-5 sample. It was found that the wettability for the day-1 sample was 12.4 ± 1.07 min and decreased significantly ($p < 0.05$) to 8.0 ± 0.22 min on the day-5. This decline in wettability time was also due to less concentration of whey protein, precisely the decrease in globular protein concentration. The globular proteins have strong interactions within their molecules that hold the molecules at the water–air interface, increasing the time to absorb at the water surface. In later samples, the amount of casein increases, which has a loose structure and can interrupt the interactions of globular proteins [12].

During the storage of proteins, the capacity to absorb moisture is termed as hygroscopicity. The hygroscopicity decreased significantly from 23 ± 0.4 to $17.1 \pm 0.2\%$, while

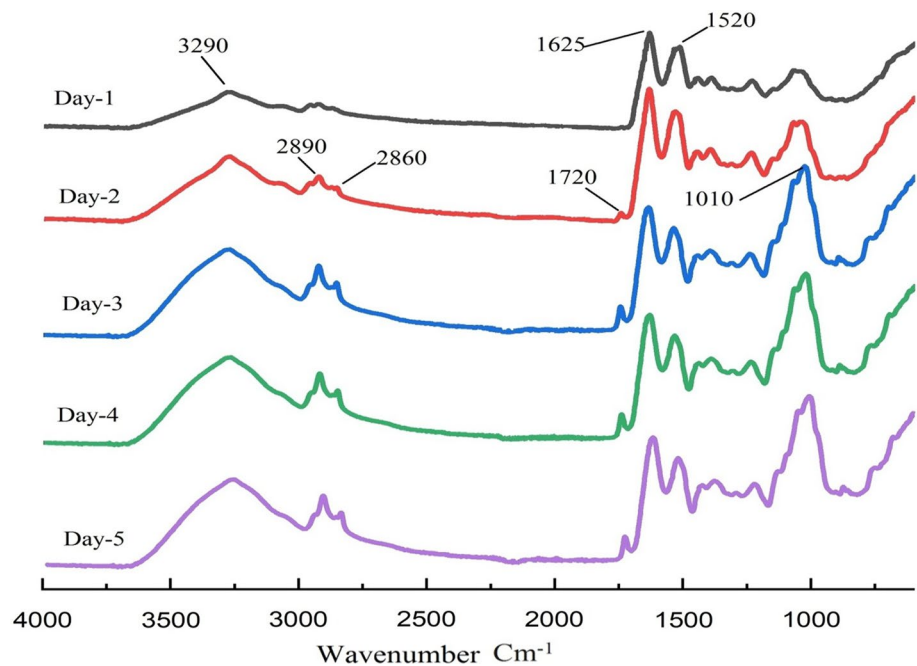
the water activity of the samples changed from 0.23 ± 0.01 to 0.34 ± 0.02 . These results were found in good agreement with a previous study reporting that the hygroscopicity of cow milk powders was between 21.6 and 18.8% [22]. The higher value of hygroscopicity and low water activity of colostrum on the day-1 were due to higher protein content. In later milking, the protein content decreased, and lactose content increased, which was responsible for opposite results.

The whiteness of colostrum increased significantly from 83.3 ± 0.32 to 88.5 ± 0.19 from day-1 to day-5. In early lactations, colostrum contains slightly higher concentration of the lutein, all-trans β -carotene and cis-13 β -carotene due leakage of blood in the milk after parturition, which decreased sharply in later lactations. The colostrum had slight reddish yellow color in the early 2 days due to higher concentrations of carotenoids, which decreased speedily as the mammary secretions turned to normal milk. A recent study on color changes of colostrum in same species of bovine also reported considerable decrease in b^* values (yellowness) from day-1 to day-3. samples postpartum [17].

Thermal denaturation analysis of colostrum proteins

The samples were analyzed using DSC to obtain the thermograms; results indicated the higher denaturation in early 2 days samples, melting starts from 106°C (T_o = onset temperature) and 108°C (T_o). The proteins denatured completely at 109.4°C (T_p = peak temperature) and 113.4°C (T_p) while the conclusion temperature (T_c) and enthalpy changes were 114°C , -15.542 mW/mg and 116°C , -13.35 mW/mg for 1st and 2nd days respectively (Fig. 3). Proximate analysis showed that the amount of whey protein (higher amount of Igs observed through ELISA) was higher in the colostrum during the first 2 days. The samples showed less stability than lactations of the later 3 days since the whey was more prone to heat denaturation. The compositional variation and concentration of each protein component affected the whey protein's heat stability and functional properties. In previous studies, it was reported that the denaturation temperature of cow milk whey in liquid form was 106°C and 74°C for pure serum albumin, 81°C for pure β -lactoglobulin (β -Lg), 61°C for pure α -lactalbumin (α -La) and Igs completely denture up to 89°C [12]. The melting of proteins starts at 128°C , 128.5 and 130°C (T_o) and denatures completely at the peak temperature of 130.5°C (ΔH , -8.15 mW/mg), 131.6°C (ΔH , 12.91 mW/mg) and 132.4°C (ΔH , 10.15 mW/mg) for day-3, day-4 and day-5 respectively. The improvement of heat stability can be related to a higher amount of casein as compared to whey in these samples, since the native casein showed

Fig. 3 The FTIR spectrum for freeze-dried skimmed colostrum powders for 5 days of lactations



thermal denaturation at 103.8 °C [23]. The amount of lactose was also increased in later days of colostrum which further improved the heat resistance of β -lactoglobulin, resulting in the overall stability of skim colostrum in later days [24].

Fourier transform infrared spectroscopy

FTIR analysis aimed to study the structural changes of colostrum proteins influenced by the changes in concentration and their interactions with other components of colostrum. The peak at 3600–2970 cm^{-1} corresponds to hydroxyl (OH) bond and NH– bond stretch from proteins, which increased the absorption intensity in later lactations (Fig. 3). The absorption peaks of 2970–2810 cm^{-1} were from fatty acids in the samples, allocated to CH_2 antisymmetric and symmetric stretching, and the absorption peak at 1743 cm^{-1} shows C=O stretching from milk fats. The absorption at 1560–1510 cm^{-1} was due to N–H and C–H bending vibration of amide II from proteins. The amide groups (C=O termed as amide I, N–H, and C–N related to amide II) absorb the spectrum at 1800–800 cm^{-1} , commonly linked to proteinaceous components. Spectral regions between 1685–1560 and 1250–910 cm^{-1} correspond to amide I, mainly C=O stretching of proteins and the absorption bands at 1200–1110, 990–905 and 900–725 cm^{-1} were associated with carbohydrates [25, 26].

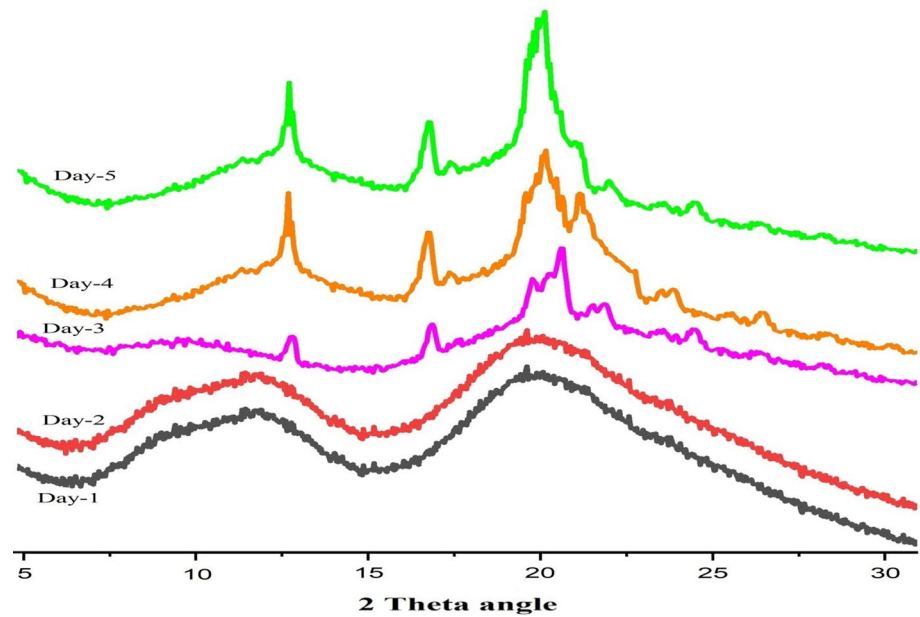
Since freeze drying is a non-thermal process, no changes in secondary structures (α -helix \sim 1655, random coil \sim 1646 and β -sheets \sim 1632 cm^{-1}) and peak shifts were observed

[27]. However, the absorption intensity changes were observed for some peak regions. With a decrease in the concentration of proteins in later lactation, an increase in the intensity of the peaks at 3290–2860, 1720 and 1010 cm^{-1} were observed, which were linked to proteins, fatty acids, and polysaccharides respectively, while no change observed at 1685–1620 cm^{-1} (antiparallel β sheets) [25, 28].

X-ray diffractometry analysis

XRD is based on X-ray scattering from samples, and in the dairy industry, pharmaceutical and food products, it is commonly utilized to study lactose crystallization. The X-ray diffractograms of freeze-dried bovine skimmed colostrum powders were shown in Fig. 4. The major fraction of samples were an amorphous structure and the samples of the first 2 days showed no crystallinity due to less lactose content ($12.3 \pm 0.2\%$). Some peaks were observed at two theta angles of 13, 17 and 21 in later 3-day samples due to high lactose content as observed in proximate analysis of powders (Table 1). The powder samples were stored at room temperature (25 °C) which can be a reason of crystallinity in later 3 days samples. The amorphous colostrum samples were thermodynamically in a non-equilibrium state which could easily convert the lactose into crystalline form. Since the freeze-drying is a non-thermal process and took 24 h to complete, so the overall evaporation rate was not high. Also, the samples were freezing; therefore, the proteins and other components were not allowed to rearrange in ordered structures [29].

Fig. 4 XRD diffractograms of freeze-dried skimmed colostrum powders at 5 days of lactations



The bio accessibility of immunoglobulins

The Igs are important in avoiding pathogenic infections to protect the gut system and develop the immune system. The colostrum contains a high amount of Igs, which help to develop the immune system of neonates and protect the child from infections at an early age [30]. The in vitro study of the skimmed colostrum powders examined the stability and bio accessibility of Igs (IgG, IgA, and IgM) after stomach and small intestinal digestions. The recovered Igs were estimated using the ELISA technique before and after the complete digestion, while the activity of recovered Igs was not estimated. The results showed that the Igs partially resist severe pH conditions and enzymatic degradation. The recovered

IgG content was higher than IgM and IgA, and more prone to digestion. The recovered IgG content decreased from 55.28 ± 1.11 to $27.02 \pm 0.82\%$ of their actual concentrations from the day-1 to day-5 (Fig. 5a). In later days, the samples show high digestibility due to fewer amounts of other glycoproteins and glycosylated components, which protect Igs in the early 2 days as IgG glycosylation is a variable characteristic of the Fab domain [31]. Various justifications have been presented to explain the proteolytic stability of these immunoglobulins in the gut. The colostrum IgG contained a small amount of O-linked glycans in the heavy chain that reduced the pepsin hydrolysis, and colostrum also contained a high amount of trypsin inhibitor and other co-factors to inhibit the proteolysis. These components may be responsible for

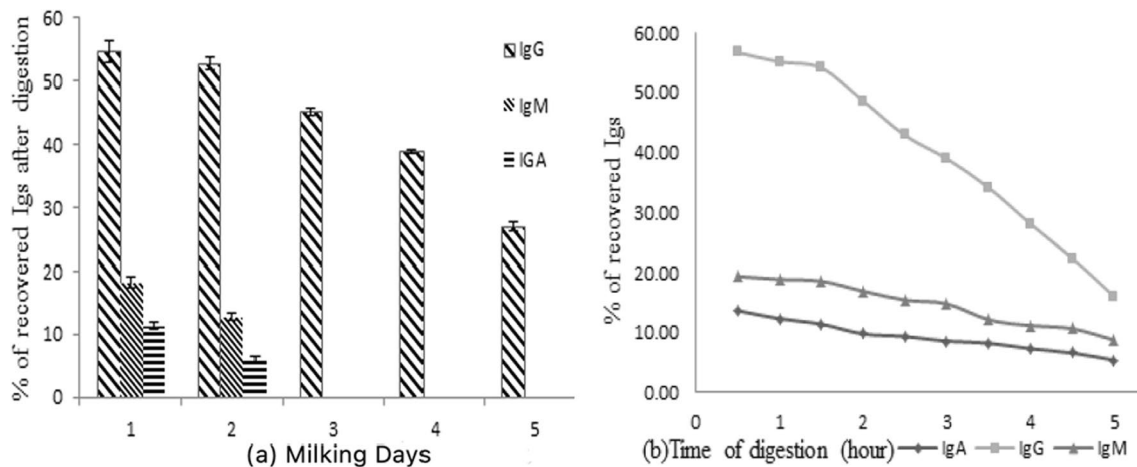


Fig. 5 Digestibility of immunoglobulins in skimmed colostrum powders. **a** The percentage of Igs recovered after digestion for 5 days sample. **b** The digestion profile for 5 h of day-1 skimmed colostrum powder

improving the digestion stability of bovine colostrum Igs in early lactations [32].

In a study, the amount of bio accessible IgG content was reported as around 59%, while the amount of recovered IgM was around 19% [32]. That study also illustrated that the amount of recovered IgG before entering the colon could be around 50–19% in active form during oral administration of adults. Furthermore, the Fab dimers and monomer of IgG retain the binding and antigen-neutralizing activity even after digestion until they are not denatured [31]. The amount of bio accessible IgM was $18.28 \pm 0.93\%$ and $12.79 \pm 0.52\%$, while the amount of IgA was $11.42 \pm 0.58\%$ and $6.11 \pm 0.51\%$ for the day-1 and day-2, respectively. Both Igs (IgM and IgA) could not be detected after digestion for 3 days since the initial amount of these Igs were quite low in the samples. Due to enhanced digestion stability, the day-1 sample showed a higher amount of recovered Igs, so the digestion profile was also studied for 5 h only on the day-1 sample. The results of the digestion profile showed that during the first half hour, the amount of recovered Igs was higher (56.74% of IgG, 19.38% of IgM and 13.69% of IgA), which decreased significantly within the 5-h interval (16.03% of IgG, 8.79% of IgM and 5.45% of IgA) (Fig. 5b). Similar results were presented in a previous study in which the bovine colostrum powder was orally administered to infants that showed around 13% recovery of IgG [31].

Conclusion

The composition of colostrum changes significantly from day-1 to day-5 after parturition, which showed a high impact on the functional properties of powders. The day-1 sample shows a higher amount of total solids and protein compared to later days. During these 5 days period, lactose content increased while fat, ash, and solid not fat content decreased significantly ($p < 0.05$). The higher solubility, hygroscopicity, buffering capacity, emulsifying ability, and foaming capacity were observed for day-1 samples due to a higher protein concentration, which decreased significantly with a decrease in protein content on day-5. In samples the ability of proteins to generate stabilized foams by reducing the interfacial tension at the interface between the air and water phases governed their functional properties. The FTIR indicated that the bioactive components (including Igs) were intact (no changes in secondary structures) due to freeze-drying, which highlights the feasibility of freeze-drying over other techniques using heat. The Igs contents were also higher in the first 2 days, which showed a sudden decrease in later days samples. Additionally, the in vitro digestibility of the first 2 days' samples revealed a high quantity of bio-accessible Igs content,

indicating that these powders can be used as a good source of Igs to strengthen the immune system and prevent numerous gut-related illnesses. Powders can impart the health advantages of their bioactive components into functional beverages, infant formulas, food items, and nutraceuticals.

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Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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