Hindawi
Journal of Food Quality
Volume 2024, Article ID 2608059, 10 pages
https://doi.org/10.1155/2024/2608059

*Research Article*

# Changes in Techno-Functional Characteristics of Cow Colostrum Powder Prepared by Freeze Drying

**Anamika Das**,[1] **Raman Seth**,[2] **Ayon Tarafdar**,[3] **Swarnima Dey**,[4] **Yogesh Kumar**,[4] **and Ranjna Sirohi**[5]

[1]*Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj 211007, India*
[2]*Dairy Chemistry Division, National Dairy Research Institute, Karnal 132001, India*
[3]*Livestock Production and Management Section, ICAR-Indian Veterinary Research Institute, Izatnagar 243122, Bareilly, Uttar Pradesh, India*
[4]*Department of Food Technology, SRM University, Sonepat, Haryana 131029, India*
[5]*Sri Karan Narendra Agriculture University, Jobner 303329, Rajasthan, India*

Correspondence should be addressed to Anamika Das; anamikandri06@gmail.com

Received 12 September 2023; Revised 30 January 2024; Accepted 6 February 2024; Published 15 February 2024

Academic Editor: Vicente M. G mez L pez



The present study investigates the characteristics of freeze-dried bovine colostrum powder prepared from the first three milkings of Karan fries cattle at different intervals. Bioactive components of bovine colostrum are heat sensitive, and therefore, in order to retain the biological activity of different components, it should be subjected to minimal nonthermal treatment. In this study, the optimum temperature/time combination of 60°C/45 min was used for the processing of cow colostrum. At this temperature, the microbial count significantly decreased as compared to raw colostrum. In freeze-dried bovine skimmed colostrum powder (BSCP) and bovine colostrum whey powder (BCWP), IgG emerged as the highest fraction among immune factors, whereas in growth factors, insulin-like growth factor 1(IGF1) and transforming growth factor $\beta$2 (TGF$\beta$2) were found in large proportion as compared to insulin-like growth factor 2 (IGF2) and transforming growth factor $\beta$1 (TGF$\beta$1). The IgG content (per 100 g) of BSCP prepared from 1st, 2nd, and 3rd milking was found to be 36.62, 27.87, and 20.51 g, respectively, whereas IgG content (per 100 g) of BCWP prepared from 1st, 2nd, and 3rd milking was found to be 33.32, 24.53, and 16.81 g, respectively. The IGF1 content (per 100 g) of BSCP prepared from 1st, 2nd, and 3rd milking was found to be 921.6, 741.2, and 617.2 $\mu$g, respectively, whereas IGF1 content (per 100 g) of BCWP prepared from 1st, 2nd, and 3rd milking was found to be 869.8, 688, and 454.4 $\mu$g, respectively. The TGF$\beta$2 content (per 100 g) of BSCP prepared from 1st, 2nd, and 3rd milking was found to be 1278, 1076, and 856.8 $\mu$g, respectively, whereas TGF$\beta$2 content (per 100 g) of BCWP prepared from 1st, 2nd, and 3rd milking was found to be 1167, 950.2, and 769.2 $\mu$g, respectively. The microstructure of freeze-dried cow colostrum whey powder revealed that its pore size was more than that of skimmed colostrum powder. BCWP exhibited lower phagocytic activity as well as lymphocyte proliferation index as compared to BSCP which was carried out *in vitro*. The observations of the study provide an insight about the components present in BCWP and BSCP apart from the physical characteristics of the products which might pave the way for its utilization in various food formats.

## 1. Introduction

Nowadays, consumers are more cognizant of their diet, and post COVID, the general masses are focusing more on strengthening their immune system. The ongoing trend reveals that consumers are interested in immune boosting ingredients and have designed their diet in such a way that it modulates the immune system of the individuals. The consumption of different bioactive ingredients in a balanced manner plays an important role in conferring immunity against viral infections [1]. In fact, bovine colostrum either in liquid form or in powdered form has become the most traded functional ingredient that has been used in medicines for improving the immune system, in baby food, health

drinks, pharmaceuticals, and cosmetics [2–4]. Colostrum is the first lacteal secretion obtained during the first 72 hours after the birth of newborn. The composition of colostrum is determined by the requirement of the newborn and usually it contains numerous immune factors and growth factors that will provide the much necessary immunity to the newborn. These components are a part of biological processes, such as GI tract maturation, immunological function, and energy balance and provides defense against pathogens [5]. The bioactive components of colostrum include immunoglobulins (Ig), lactoferrin, growth factors, and other compounds that not only possess antioxidant and anti-inflammatory properties but also they are essential for strengthening the immune system. During the COVID period, interest in the colostrum-based components and the possibility to utilize them in various recipes have increased. The concentration of these bioactive compounds is the highest in colostrum up to 72 hours after birth of the new born, and therefore the excess secretion can be utilized to harness the components. The shelf-life of raw colostrum is limited, and the available thermal processing treatments use high temperature/time combination which results in significant denaturation of bioactive proteins, and subsequently, a loss of biological activity occurs.

Cows and buffaloes produce 40–44 kg of colostrum, and consumption of colostrum depends upon calf's body weight. Colostrum optimum requirement is nearly 7–10% by weight of the calf's body weight, and the immunoglobulins from the colostrum should be ingested within 6–12 hours of birth as the gut permeability of the new born decreases rapidly within 24 hrs of birth. One percent of a healthy cow's annual milk supply is made up of colostrum which is significantly more than the calves' requirements [6]. Therefore, excess colostrum may be preserved, processed, and utilized for value addition of human foods. However, the high content of heat labile protein leads to poor heat stability of colostrum, and therefore the cost incurred in processing becomes high. Since powders can be stored at room temperature with more stable and preserved characteristics of products, dairy liquids are generally converted into powders on a commercial scale.

Freeze drying of colostrum is an obvious choice to retain the biofunctionality of colostrum components as compared to spray drying technique, but the issue of heat-induced denaturation of colostrum's bioactive components, particularly immunoglobulins (Igs), is still a significant challenge in the production of spray-dried colostrum powder which has also been reported by Borad and Singh [3]. The process of manufacturing spray-dried skim milk powder involves vacuum evaporation (55–75°C) of skim milk in order to get 45–48% total solid in concentrate which is then subjected to a spray drier where air inlet temperature is 180–185°C and outlet air temperature is 80–90°C. Keeping in view such processing conditions, spray drying of colostrum has not been considered to be a very good option as such a higher temperature might cause complete denaturation of immunoglobulins which has also been reported by Singh et al. [7] who observed that at 72°C, unfolding of immunoglobulins start and complete denaturation might occur when they are heated at a temperature of 89°C. Every method of drying whether it is spray drying or freeze drying results in some degree of denaturation of immune factors but the extent of loss of biological activity of immune factors and growth factors is more in spray drying as compared to freeze drying but spray drying has been the choice of commercial traders due to the low cost of the spray drying process [8–10]. However, where the final product is intended for obtaining some functionality, then the commercialized form must not only be safe for human consumption but also contains maximized quantity of bioactive molecules, regardless of the technique that was used to dry bovine colostrum into powder. Over the past one decade, extensive research has been conducted on the nutraceutical properties of bovine colostrum, and in the early 2000s, a number of clinical trials were conducted that demonstrated the beneficial impact of colostrum on the athletic performance, muscle mass, and bone health of adults. Several studies reported that athletes who consumed bovine colostrum experienced noticeable benefits, including increased resistance to fatigue, greater lean mass, reduced body fat, improved gut health, and enhanced immune system and could recover faster from injuries which can be attributed to the presence of antimicrobial compounds, immune factors, and certain growth-promoting factors, like insulin-like growth factor-I and -II in colostrum. However, many researchers have reported that further research is needed to fully understand the role of each compound [11–18].

For thousands of years, Ayurvedic doctors in India have employed bovine colostrum for therapeutic purposes. Colostrum can also be used therapeutically to treat conditions such as AIDS, cancer, heart disease, diabetes, autoimmune illnesses, allergies, herpes, bacterial, viral, and parasite infections, gingivitis, and the flu [19]. Prior to the invention of sulfa medicines and penicillin, conventional doctors in the United States and other countries used it as an antibiotic. Colostrum was widely recommended for the treatment of rheumatoid arthritis and as a source of antipolio antibodies in the early 1950s [20].

Compared to blood-derived analogues, colostrum is an alternative source for industrial-scale production of immunoglobulins because of its ready availability and because it is comparatively safe in nature. Colostrum fractions or specific peptides may be helpful for treating a wide range of gastrointestinal infections, such as inflammatory bowel disease, gut damage brought on by nonsteroidal anti-inflammatory drugs (NSAIDs), chemotherapy-induced mucositis, and Alzheimer's disease, according to clinical studies reported by Playford et al. [21]. Over the past ten years, colostrum-based products have been more popular as nutritional supplements, while research on colostrum processing has been less active. Commercially available colostrum products are available in the form of capsules, lozenges, chewing gum, whole-colostrum drinks, and powder which can be for both veterinary and human use. Leading commercial companies operating in this area include PanTheryx, Colostrum BioTec, Immuno-Dynamics, Ingredia Nutritional, New Image, Biostrum Nutritech, Imu-Tek, and Good Health NZ Products (https://www.orianresearch.com). To increase the bioactive content of

dairy products such as yogurt, kefir, fermented milk, ice cream, cheese, and butter, whole colostrum has also been used. Several traditional preparations such as Khees (India), Kalvdans (Scandinavia), Abrystir (Iceland), Rmelk (Norway), leipäjuusto (Finland), Molozyvo (Ukraine), and Groosniuys are produced for local market [22]. To preserve the biological activity of the bioactive components, colostrum should be processed at minimal time-temperature combination. In the present study, freeze drying have been adopted to prepare powder with the purpose that such powder can be preserved and further used in different food product preparations. Despite the existence of various drying methods for converting protein solutions into powders, freeze drying remains a highly promising technique for preserving sensitive products [23]. The functional characteristics of colostrum powders, such as emulsifying capacity, foaming capacity, thermal stability, wettability, solubility, and buffering capacity, are significantly influenced by the compositional variation of colostrum during the early days [3, 24]. In some studies, it has been reported that in early lactations of cow colostrum, physical and functional parameters show extreme changes [25]. The present study is focused on investigating the techno-functional characteristics of freeze-dried bovine skimmed colostrum powder and colostrum whey powder prepared from early lactation milkings obtained at different intervals from Karan fries cattle (indigenously developed at the ICAR-National Dairy Research Institute (NDRI), Karnal, India). Further, effect of heat treatment on major components and other biomolecules was also investigated.

## 2. Materials and Methods

*2.1. Procurement of Raw Bovine Colostrum.* Bovine colostrum was collected from Karan fries within 0–48 hours (first three milkings) from the ICAR-NDRI cattle yard.

*2.2. Processing and Freeze Drying of Colostrum.* The colostrum samples were defatted by centrifugation at 8000 g for 20 min at 2°C using a refrigerated centrifuge (Kubota, model 6800, Japan). The defatted samples were further used for processing. The skimmed bovine colostrum samples were heat-treated at different temperatures, viz., 72°C/15 sec, 63°C/30 min, and 60°C/45 min. Heat-treated defatted colostrum samples were divided into two equal parts. First part was kept at −20°C and later on freeze-dried at −40°C at 5 torr vacuum in a freeze dryer (LabTech, Japan). Second part was utilized for whey preparation, wherein 1.5% rennet was added at 32°C and was held for 1 h. The coagulum was cut and the curd cubes were cooked at 40°C for 30 min for expulsion of whey. The whey was extracted and frozen at −20°C and finally freeze-dried under the conditions mentioned above. The dried mass was ground to obtain a homogenous powder.

*2.3. Compositional Analysis of Raw Colostrum and Colostral-Dried Products.* Raw colostrum was analysed for TS (total solids), fat, ash, and lactose as per the procedure described for milk in SP:18, Part XI [26]. Total protein was determined by the Kjeldahl method as described in AOAC [27]. Powdered Colostrum samples were analysed for moisture, fat, ash, and lactose as per the procedure described for skim milk powder in SP:18, Part XI [26]. Total protein was determined by the Kjeldahl method as described in AOAC [27]. IgG and IgA content for raw colostrum and powdered colostrum samples was estimated by ELISA kit supplied by KOMA BIOTECH, Korea. TGF$\beta$1, TGF$\beta$2, IGF1, and IGF2 for raw colostrum and powdered colostrum samples were determined by the ELISA kit procured from USCN Life Science Inc., Wuhan, China.

*2.4. Microbiological Analysis.* SPC, coliform count, yeast and mold count, *Salmonella* count, and *S. aureus* count of raw bovine colostrum samples and heated colostrum samples were carried out according to the method described by Houghtby and coworkers [28].

*2.5. Physical Properties of Colostral-Dried Products.* Flowability, bulk density (loose and packed), and porosity were determined by the procedure described by Suleiman et al. [29]. Color values of bovine skimmed colostrum powder (BSCP) and bovine colostrum whey powder (BCWP) were measured using a colorflex colorimeter (Hunter Associates Laboratory, VA, USA), and the results were expressed through software version 4.1, and it is expressed in terms of $L^*$, $a^*$, and $b^*$ values. Microstructure of BSCP and BCWP was investigated using scanning electron microscopy (SEM) which was carried out as per the procedure laid down by Caric and Ibrahim [30]. The wettability of the powder samples was assessed as per the procedure given by Naik et al. [31]. The time required for 0.1 g of powder to get completely wet was recorded.

*2.6. In Vitro Immunomodulatory Activity.* Lymphocyte proliferation index and phagocytosis were carried out *in vitro* as per the method described by Hay and Westwood [32].

*2.7. Statistical Analysis.* All the observations were recorded as mean ± SE. MS-Excel, version 2010, was used for graphical representations. One-way analysis of variance (ANOVA) at a confidence level of 95% and other statistical parameters were calculated using GraphPad Prism version 5.01, and the significance of the data was reported using the Tukey post hoc test for comparison of means.

## 3. Results and Discussion

*3.1. Standardisation of Time-Temperature Combination for Heat Treatment of Colostrum Samples before Freeze Drying.* Bovine colostrum samples collected during the first 0–48 h of milking were subjected to heat treatment using batch method at different temperatures at 72°C/15 sec, 63°C/30 min, and 60°C/45 min, and it was visually observed for gel formation. The samples from all the milkings did not

coagulate at time-temperature combination of 63°C/30 min and 60°C/45 min, whereas at 72°C/15 sec, samples were found to form gel. Also, a significant ($P < 0.05$) reduction in IgG and IgA content was observed at all time-temperature combinations, but the loss of IgG and IgA content was significantly less ($P<0.05$) at 60°C/45 min as compared to 63°C/30 min (Table 1). When colostrum samples from different milkings were heat-treated at 60°C/45 min, IgG concentration decreased in the range of 14.63–11.68% and IgA concentration reduced in the range of 12.18–10.11%. The findings of the current investigation were concomitant to the work of Saldana and coworkers [33], who reported that immunoglobulins concentration decreased by 9% and 12% when the raw colostrum was heat-treated for 60°C/30 min and 60°C/60 min, respectively.

There was no significant ($P > 0.05$) decrease in the quantity of growth factors when colostrum was subjected to heat treatment at the abovementioned time-temperature combinations. Hence, heat treatment at 60°C/45 min was selected for colostrum processing. Also, standard plate count (SPC), coliform count, yeast and mold count, and *S. aureus* and *Salmonella* count were performed before and after heat treatment at 60°C/45 min to assess the reduction in different counts at this time-temperature combination. It was found that as compared to raw colostrum, there was a significant reduction in microbial count after the heat treatment at 60°C/45 min. and the same has been presented in Figures 1–3. The SPC of raw colostrum was found to be $6.17 \times 10^5$ (cfu/mL) which reduced to $5.75 \times 10^4$ (cfu/mL) after heat treatment at 60°C/45 min. in samples of 1st milking. In samples of 2nd milking, the SPC of raw colostrum was found to be $5.30 \times 10^5$ (cfu/mL) which reduced to $5.74 \times 10^4$ (cfu/mL) after heat treatment at 60°C/45 min. The SPC of raw colostrum in samples of 3rd milking was found to be $4.05 \times 10^5$ (cfu/ml) which decreased to $3.36 \times 10^4$ (cfu/ml) after heat treatment at 60°C/45 min. Similarly, the coliform count of raw colostrum samples of 1st, 2nd, and 3rd milking was found to be 312.7, 282.7, and 45 (cfu/mL) which reduced to 24.6, 17.3, and 18, respectively (cfu/mL) after heat treatment at 60°C/45 min. Yeast and mold count of raw colostrum samples of 1st, 2nd, and 3rd milking was found to be 25, 32.5, and 30.4 (cfu/mL) which reduced to 1.13, 2.66, and 1.53, respectively (cfu/mL), after heat treatment at 60°C/45 min. There was a 94% decrease in *Salmonella* count and 96% reduction in *S. aureus* count when the samples were heated at 60°C/45 min. Thus, the temperature time combination not only retains the activity of immune factors and growth factors in substantial proportion but also reduces the microbial count considerably. Godden et al. [34] reported that the viable count of *M. bovis*, *L. monocytogenes*, *E. coli O157:H7*, *Salmonella enteritidis*, and *Mycobacterium avium* subspecies *paratuberculosis* decreased below detectable limits when colostrum was heated at 60°C for 120 min.

*3.2. Effect of Freeze Drying on Proximate Composition of Skimmed Colostrum Powder and Colostrum Whey Powder.* The samples of raw colostrum that were collected during the first three milkings (0–48 h) after calving were analysed for constituents like total solids, protein, fat, lactose, ash, IgG, IgA, IGF1, IGF2, and TGF$\beta$1, TGF$\beta$2, and the values are presented in Table 2. The mean values of total solids, fat, protein, and ash in the first milking, second milking, and third milking showed a significant ($P < 0.05$) decreasing trend as the time period increased after calving. Puppel and coworkers [35] reported that colostrum has two to ten times more minerals (except for potassium) than milk, and compositional changes in colostrum occur with each hour. This study also reports similar results as observed by them, wherein protein content ranges from 16.8 to 4.8%, fat content ranges from 6.7 to 3.9%, and lactose content ranges from 2.9 to 4.2% during first 48 hrs after calving. McGrath et al. [36] reported that lactose content, casein fraction, and fat increase significantly in secretions of early lactation. In this study, average values of lactose content in first milking, second milking, and third milking showed a significantly increasing trend as the time period increased after calving. The average values of IgG in first milking, second milking, and third milking were observed to be 76.5 g/L, 63 g/L, and 43.5 g/L, respectively. The average values of IgA in first milking, second milking, and third milking were found to be 3.4 g/L, 2.7 g/L, and 2.3 g/L, respectively. The average values of IGF1 in first milking, second milking, and third milking were observed to be 465 ng/mL, 390 ng/mL, and 280 ng/mL, respectively. The average values of IGF2 in first milking, second milking, and third milking were found to be 175 ng/mL, 145 ng/mL, and 92.5 ng/mL, respectively. The average values of TGF$\beta$1 in first milking, second milking, and third milking were found to be 26.7 ng/mL, 20.7 ng/mL, and 19.8 ng/mL, respectively. The average values of TGF$\beta$2 in first milking, second milking, and third milking were observed to be 625 ng/mL, 482 ng/mL, and 280.5 ng/mL, respectively. Thus, immune factors and growth factors showed a significant decreasing trend as the time period increased after calving. Compositional analysis of raw colostrum revealed that except lactose all other constituents significantly decreased ($P < 0.05$) as the time after calving increased.

For obtaining 25 g of freeze-dried skimmed colostrum powder, it took 28 hours, and for obtaining 25 g of freeze-dried colostrum whey powder, it took 32.5 hours. The freeze-dried samples were analysed for proximate composition, immune factors, and growth factors, and the values are presented in Table 3.

There is no significant ($P > 0.05$) difference in total solids content between freeze-dried bovine skimmed colostrum powder and bovine colostrum whey powder. However, significant difference ($P < 0.05$) exists between the fat, lactose, protein, and ash content of bovine skimmed colostrum powder and bovine colostrum whey powder. Immune factors like IgG and IgA were significantly higher ($P < 0.05$) in bovine skimmed colostrum powder as compared to bovine colostrum whey powder. Similar observations were noted by Elfstrand et al. [9], wherein the overall reduction of immune factors and growth factors was due to the entrapment of these components in the casein matrix which was removed during colostrum-based preparation.

Growth factors like IGF1, IGF2, TGF$\beta$1, and TGF$\beta$2 were significantly higher ($P < 0.05$) in bovine skimmed colostrum powder as compared to bovine colostrum whey powder. The reduction in growth factors in bovine

Table 1: Observations related to heat treatment of colostrum samples at selected time-temperature combination.

| Milking intervals | 1st milking | | | 2nd milking | | | 3rd milking | | |
|---|---|---|---|---|---|---|---|---|---|
| Time/temperature combination for heat treatment | 72°C/15 sec | 63°C/30 min | 60°C/45 min | 72°C/15 sec | 63°C/30 min | 60°C/45 min | 72°C/15 sec | 63°C/30 min | 60°C/45 min |
| Gel formation | Yes | No | No | Yes | No | No | Yes | No | No |
| % reduction in IgG | $67.41^{a} \pm 0.19$ | $45.81^{b} \pm 0.16$ | $14.63^{c} \pm 0.18$ | $62.48^{d} \pm 0.19$ | $37.71^{e} \pm 0.16$ | $12.13^{f} \pm 0.18$ | $35.41^{g} \pm 0.19$ | $25.81^{h} \pm 0.16$ | $11.68^{i} \pm 0.18$ |
| % reduction in IgA | $69.12^{a} \pm 0.27$ | $22.54^{b} \pm 0.21$ | $12.18^{c} \pm 0.16$ | $49.22^{d} \pm 0.27$ | $19.84^{e} \pm 0.21$ | $11.18^{f} \pm 0.16$ | $29.12^{g} \pm 0.27$ | $15.14^{h} \pm 0.21$ | $10.11^{i} \pm 0.16$ |

$n = 15$, mean + SD; values followed by different alphabets a–i in superscript are significantly different ($P < 0.05$).

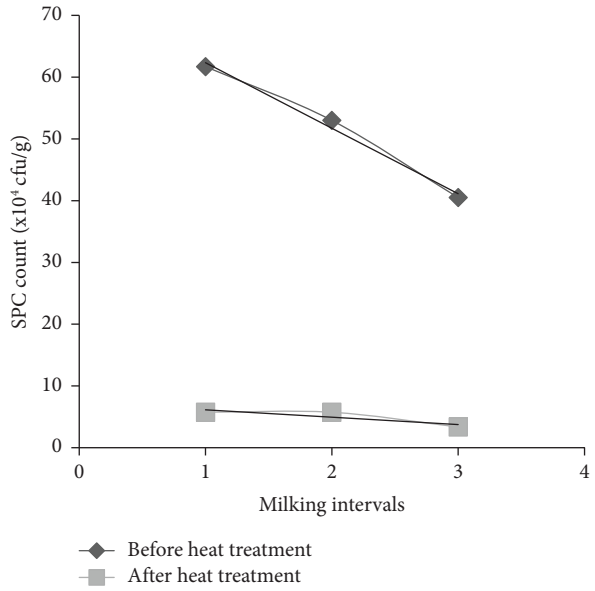



Figure 1: SPC count of colostrum samples before and after heat treatment at 60°C/45 min.

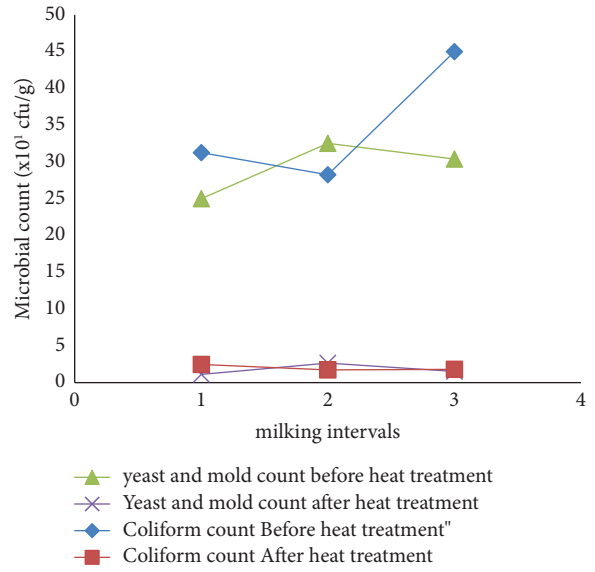



Figure 2: Coliform count and yeast and mold count of colostrum samples before and after heat treatment at 60°C/45 min.

colostrum whey powder may be due to removal of casein matrix which might have entrapped these molecules and got removed with the casein complex.

*3.3. Effect of Freeze Drying on Physical Properties of Dried Colostrum Products.* Some of the selected physical properties of dried colostrum products were studied and are presented in Table 4. Flowability (expressed in terms of Hausner ratio) of skimmed bovine colostrum powder from different milkings was found to be in the range of 1.37–1.23. For bovine colostrum whey powder prepared using colostrum from different milkings, flowability values were

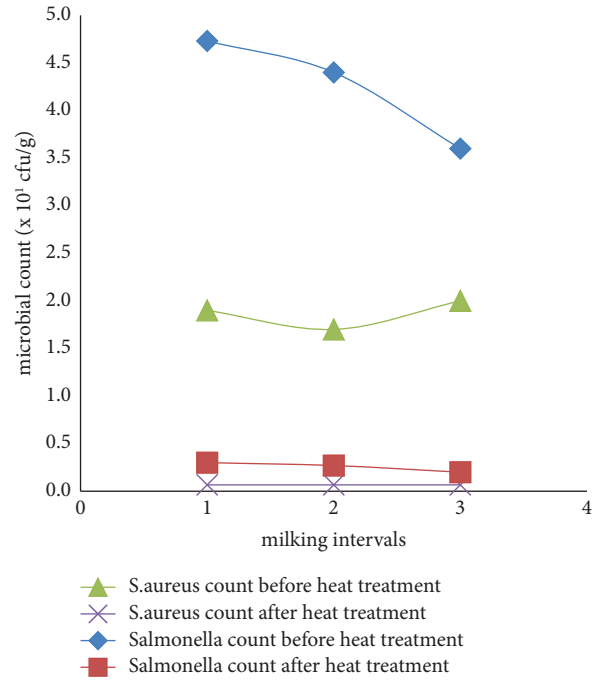



Figure 3: *Salmonella* count as well as *S. aureus* count of colostrum samples before and after heat treatment at 60°C/45 min.

Table 2: Composition of raw bovine colostrum collected during the first three milkings (0–48 hours).

| Constituents of raw colostrum | 1st milking | 2nd milking | 3rd milking |
|---|---|---|---|
| Total solids (%) | $22.93^{a} \pm 1.63$ | $17.69^{b} \pm 2.34$ | $15.88^{c} \pm 2.30$ |
| Fat (%) | $5.07^{a} \pm 0.4$ | $4.96^{b} \pm 0.37$ | $4.11^{c} \pm 0.11$ |
| Protein (%) | $14.33^{a} \pm 0.61$ | $13.57^{b} \pm 0.48$ | $10.57^{c} \pm 0.43$ |
| Lactose (%) | $2.75^{a} \pm 0.16$ | $2.91^{b} \pm 0.2$ | $3.6^{c} \pm 0.24$ |
| Ash (%) | $2.11^{a} \pm 0.27$ | $1.72^{b} \pm 0.24$ | $1.008^{c} \pm 0.22$ |
| IgG (g/L) | $76.5^{a} \pm 8.89$ | $63^{b} \pm 6.12$ | $43.5^{c} \pm 6.30$ |
| IgA (g/L) | $3.4^{a} \pm 0.36$ | $2.7^{b} \pm 0.25$ | $2.3^{c} \pm 0.15$ |
| IGF1 (ng/ml) | $465^{a} \pm 36.87$ | $390^{b} \pm 74.36$ | $280^{c} \pm 59.68$ |
| IGF2 (ng/ml) | $175^{a} \pm 11.83$ | $145^{b} \pm 9.05$ | $92.5^{c} \pm 3.75$ |
| TGF$\beta$1 (ng/ml) | $26.7^{a} \pm 8.31$ | $20.7^{b} \pm 5.95$ | $17.8^{c} \pm 1.62$ |
| TGF$\beta$2 (ng/ml) | $625^{a} \pm 36.78$ | $482^{b} \pm 48.01$ | $280.5^{c} \pm 18.99$ |

$n = 40$, mean ± SD; values followed by different superscript column wise are significantly different ($P < 0.05$).

found to be in the range of 1.43–1.23. A study reported by Rezende et al. [37] showed that freeze-dried powders had better flowability, wettability, and solubility. Upadhyay [38] reported that for skim milk powder, flowability was found to be 0.97, whereas for instant skim milk powder, it was found to be around 0.75. Turchiuli et al. [39] reported that the Hausner ratio has been used for the determination of flow characteristics of the powder samples which measures the powder's cohesiveness and has been used as the index of flow for dry powders. Dry substances possessing a Hausner ratio greater than 1.34 are regarded to be cohesive and consequently less free to flow [40]. High Hausner ratio indicates high cohesiveness between the particles that results in aggregation and exhibits decreased flowability [41].

Table 3: Composition of freeze-dried bovine skimmed colostrum powder and colostrum whey powder.

| Type of powder | Bovine skimmed colostrum powder | | | Bovine colostrum whey powder | | |
|---|---|---|---|---|---|---|
| Constituents | 1st milking | 2nd milking | 3rd milking | 1st milking | 2nd milking | 3rd milking |
| Total solids (%) | $94.88^{a} \pm 0.148$ | $94.89^{a} \pm 0.14$ | $94.83^{a} \pm 0.14$ | $94.73^{a} \pm 0.13$ | $94.65^{a} \pm 0.147$ | $94.60^{a} \pm 0.155$ |
| Fat (%) | $5.2^{a} \pm 0.15$ | $5.5^{b} \pm 0.23$ | $5.7^{c} \pm 0.21$ | $2.6^{d} \pm 0.026$ | $2.4^{e} \pm 0.026$ | $2.2^{f} \pm 0.027$ |
| Protein (%) | $69.91^{a} \pm 0.15$ | $64.93^{b} \pm 0.13$ | $61.94^{c} \pm 0.14$ | $67.22^{d} \pm 0.139$ | $61.06^{e} \pm 0.025$ | $57.79^{f} \pm 0.158$ |
| Lactose (%) | $12.71^{a} \pm 0.07$ | $20.21^{b} \pm 0.11$ | $24.44^{c} \pm 0.089$ | $15.23^{d} \pm 0.102$ | $23.23^{e} \pm 0.19$ | $26.91^{f} \pm 0.15$ |
| Ash (%) | $7.39^{a} \pm 0.018$ | $6.94^{b} \pm 0.04$ | $6.49^{c} \pm 0.019$ | $6.10^{d} \pm 0.05$ | $4.75^{e} \pm 0.03$ | $3.8^{f} \pm 0.04$ |
| IgG (g/100 g) | $36.62^{a} \pm 0.56$ | $27.87^{b} \pm 1.2$ | $20.51^{c} \pm 0.27$ | $33.33^{d} \pm 0.65$ | $24.53^{e} \pm 0.638$ | $16.8^{f} \pm 0.42$ |
| IgA (g/100 g) | $1.75^{a} \pm 0.024$ | $1.40^{b} \pm 0.019$ | $1.29^{c} \pm 0.009$ | $1.58^{d} \pm 0.054$ | $1.17^{e} \pm 0.010$ | $1.057^{f} \pm 0.013$ |
| IGF1 ($\mu$g/100 g) | $921.6^{a} \pm 11.68$ | $741.2^{b} \pm 4.86$ | $617.2^{c} \pm 7.28$ | $869.8^{d} \pm 13.45$ | $688^{e} \pm 5.96$ | $454.4^{f} \pm 6.34$ |
| IGF2 ($\mu$g/100 g) | $351.5^{a} \pm 3.96$ | $311.5^{b} \pm 3.86$ | $243.3^{c} \pm 4.16$ | $328.7^{d} \pm 2.20$ | $276.6^{e} \pm 2.17$ | $216.6^{f} \pm 3.08$ |
| TGF$\beta$1 ($\mu$g/100 g) | $6.64^{a} \pm 0.084$ | $5.40^{b} \pm 0.029$ | $4.17^{c} \pm 0.182$ | $5.93^{d} \pm 0.033$ | $4.94^{e} \pm 0.086$ | $3.32^{f} \pm 0.1420$ |
| TGF$\beta$2 ($\mu$g/100 g) | $1278^{a} \pm 20.15$ | $1076^{b} \pm 22$ | $856.8^{c} \pm 15.36$ | $1167^{d} \pm 19.33$ | $950.2^{e} \pm 31.19$ | $769.2^{f} \pm 4.22$ |

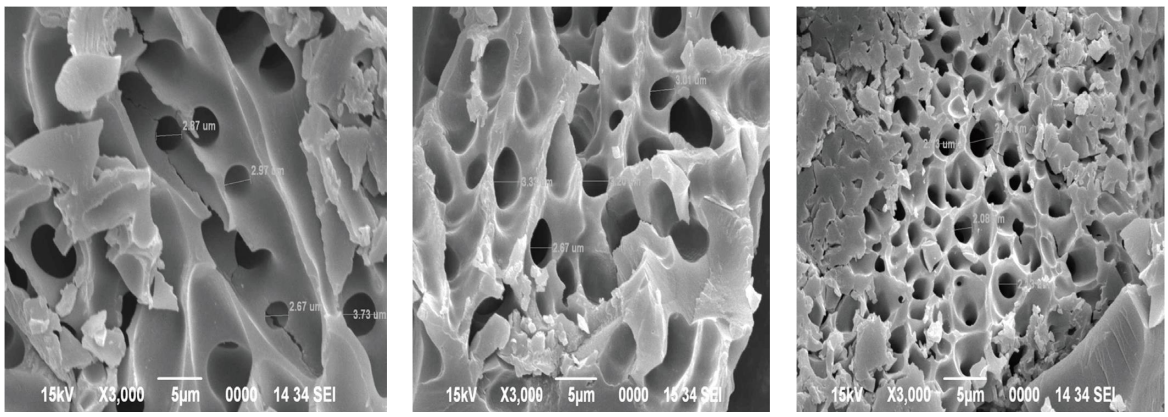
$n = 15$, mean $\pm$ SE; values followed by different superscript are significantly different ($P < 0.05$).

Table 4: Physical characteristics of freeze-dried bovine skimmed colostrum powder and colostrum whey powder.

| Type of powder | Bovine skimmed colostrum powder | | | Bovine colostrum whey powder | | |
|---|---|---|---|---|---|---|
| Constituents | 1st milking | 2nd milking | 3rd milking | 1st milking | 2nd milking | 3rd milking |
| Loose bulk density (g/ml) | $0.497^{a} \pm 0.007$ | $0.617^{b} \pm 0.009$ | $0.696^{c} \pm 0.007$ | $0.271^{d} \pm 0.009$ | $0.310^{e} \pm 0.007$ | $0.423^{f} \pm 0.010$ |
| Packed bulk density (g/ml) | $0.625^{a} \pm 0.008$ | $0.714^{b} \pm 0.008$ | $0.785^{c} \pm 0.010$ | $0.357^{d} \pm 0.006$ | $0.448^{e} \pm 0.007$ | $0.53^{f} \pm 0.011$ |
| Hausner ratio | $1.37^{a} \pm 0.019$ | $1.24^{b} \pm 0.009$ | $1.14^{c} \pm 0.017$ | $1.43^{d} \pm 0.028$ | $1.36^{e} \pm 0.018$ | $1.23^{f} \pm 0.006$ |
| Porosity | $100.2^{a} \pm 0.019$ | $100.1^{a} \pm 0.013$ | $100.1^{a} \pm 0.011$ | $100.1^{a} \pm 0.013$ | $100.1^{a} \pm 0.014$ | $100.1^{a} \pm 0.012$ |
| Color | | | | | | |
| $L^*$ value | $91.8^{a} \pm 0.155$ | $94.62^{b} \pm 0.145$ | $93.21^{c} \pm 0.091$ | $92.52^{e} \pm 0.052$ | $93.41^{d} \pm 0.135$ | $95.06^{f} \pm 0.096$ |
| $a^*$ value | $1.80^{a} \pm 0.031$ | $2.76^{d} \pm 0.031$ | $5.1^{b} \pm 0.037$ | $-7.32^{e} \pm 0.023$ | $-4.82^{c} \pm 0.015$ | $-3.8^{f} \pm 0.014$ |
| $b^*$ value | $25.64^{a} \pm 0.064$ | $23.42^{d} \pm 0.055$ | $21.39^{b} \pm 0.59$ | $22.68^{e} \pm 0.054$ | $20.70^{c} \pm 0.044$ | $16.35^{f} \pm 0.455$ |
| Wettability (sec) | $150.1^{a} \pm 3.03$ | $79.80^{b} \pm 1.19$ | $64.87^{c} \pm 0.98$ | $35.80^{d} \pm 0.54$ | $25.40^{e} \pm 0.57$ | $18.20^{f} \pm 0.89$ |

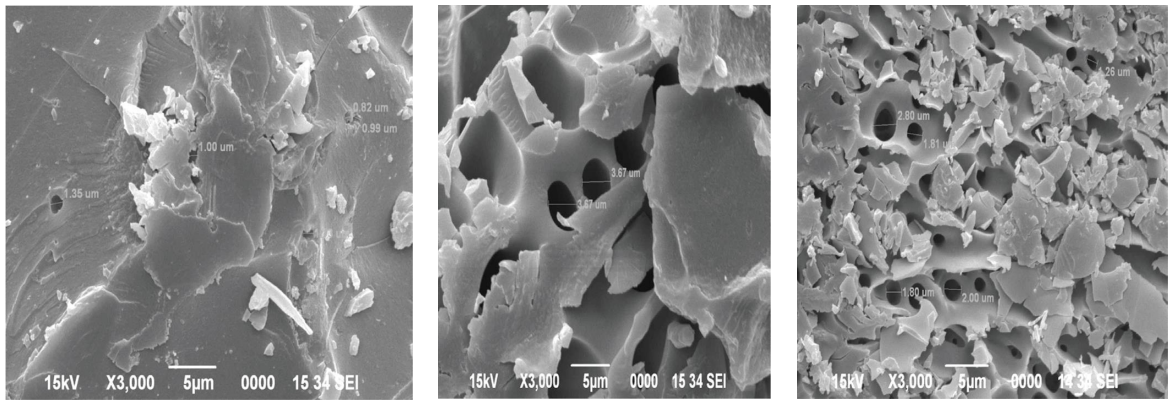
$n = 15$, mean $\pm$ SE; values followed by different superscripts are significantly different ($P < 0.05$).

As observed from Table 4, the loose bulk density of skimmed bovine colostrum powder prepared from different milkings was found to be in the range of 0.69–0.49 g/cm$^3$. The values of loose bulk density of bovine colostrum whey powder samples were found to be in the range of 0. 0.42–0.27 g/cm$^3$. The packed bulk density for skimmed bovine colostrum powder samples prepared from different milkings was found to be in the range of 0.78–0.62 g/cm$^3$, whereas the values of bovine colostrum whey powder samples, prepared from different milkings, were found to be in the range of 0.35–0.53 g/cm$^3$. Hols and van Mil [42] reported that for spray-dried powders, loose bulk density has been reported to be 0.38 g/cm3 and packed bulk density has been reported to be 0.41–0.43 g/cm$^3$. The importance of high bulk density lies in the fact that it significantly reduces packaging, storage, and transport cost of powder [43]. Porosity values for all the freeze-dried powder samples were found to be 100.1, and therefore, there was no significant difference ($P > 0.05$) among the samples of different treatments. The color values were expressed in terms of $L^*$, $a^*$, and $b^*$ wherein $L^*$ values indicate black to white and ranges from 0 to 100. Redness and green hue of any sample is indicated by $a^*$ value which ranges from +60 to −60. Yellow and blue colors are depicted by $b^*$ values which ranges from +60 to −60. The $L^*$ values of skimmed bovine colostrum powder from different milkings were found to be in the range of 93.21–91.80. For bovine colostrum whey powder prepared using colostrum from different milkings, $L^*$ values were found to be in the range of 95.06–92.52. The $a^*$ values of skimmed bovine colostrum powder from different milkings were found to be in the range of 5.10–1.80. For bovine colostrum whey powder prepared using colostrum from different milkings, $a^*$ values were found to be in the range of −3.80 to −7.32. The $b^*$ values of skimmed bovine colostrum powder from different milkings were found to be in the range of 21.39–25.64. For bovine colostrum whey powder prepared using colostrum from different milkings, $b^*$ values were found to be in the range of 22.68–16.35. The color of powder samples was slightly yellowish as bovine secretions contain more carotene as physiological system of cow does not allow hundred percent conversion of carotene into vitamin A. Wettability (in sec) values of skimmed bovine colostrum powder prepared from different milkings were found to be in the range of 64.87–150.10, and those of bovine colostrum whey powder prepared from different milkings were found to be in the range of 18.20–35.80. From the observations of wettability, it can be inferred that bovine colostrum whey powder gets wet readily as compared to bovine skimmed colostrum powder which might be due to the presence of colloidal casein particles which have fewer pores with less pore size as evidenced by the microstructure of bovine skimmed colostrum powder.




(a) (b) (c)

Figure 4: Internal microstructure of freeze-dried BCWP prepared from (a) 1st milking, (b) 2nd milking, and (c) 3rd milking.




(a) (b) (c)

Figure 5: Internal microstructure of freeze-dried BSCP prepared from (a) 1st milking, (b) 2nd milking, and (c) 3rd milking.

*3.4. Microstructure of the Bovine Colostrum Whey Powder and Bovine Skimmed Colostrum Powder.* The microstructure of the bovine colostrum whey powder was investigated, and the images of SEM (Figure 4) show that the freeze-dried powder particles showed a sponge-like rough internal microstructure with numerous pores observed. The microstructure of the bovine skimmed colostrum powder was investigated, and the image of SEM (Figure 5) shows that the microstructure has rough surfaces and had comparatively less pore size. Bhatta et al. [44] reported that the ice sublimation process in freeze drying influences the shape and volume of powders because of the formation of large pores. From the figures, it can be observed that freeze-dried colostrum powder samples showed rough or flat shaped surface and amorphous or uneven form rather than individual particles. Sublimation of ice crystals has resulted in numerous pores which might increase water penetration and this might increase the reconstitutability of the products.

*3.5. In Vitro Immunomodulatory Activity of the Dried Colostrum Products.* The observations of *in vitro* immunomodulatory activity of freeze-dried bovine skimmed colostrum powder and colostrum whey powder are presented in Table 5. Lymphocyte proliferation index of bovine skimmed colostrum powder was found to be 1.79, 1.64, and 1.41 for powders prepared from colostrum samples collected from 1st milking, 2nd milking, and 3rd milking, respectively, after parturition whereas for bovine colostrum whey powder, it was observed to be 1.26, 1.13, and 0.96, respectively, for colostrum whey powders prepared from samples collected from 1st milking, 2nd milking, and 3rd milking after parturition. Skimmed bovine colostrum powder prepared from colostrum samples collected from 1st milking, 2nd milking, and 3rd milking after parturition showed 35.47%, 31.87%, and 29% phagocytic activity, respectively, whereas bovine colostrum whey powder prepared from 1st milking, 2nd milking, and 3rd milking showed 20.27%, 18%, and 14.67% phagocytic activity, respectively. There is a significant ($P < 0.05$) difference in phagocytic activity as well as lymphocyte proliferation index between all the samples of freeze-dried BSCP and BCWP. Bovine colostrum whey powder exhibited significantly lower phagocytic activity as well as a lower lymphocyte proliferation index as compared to skimmed bovine colostrum powder.

Table 5: The observations of immunomodulatory activity (*in vitro*) of freeze-dried bovine skimmed colostrum powder and colostrum whey powder.

| Type of powder | Bovine skimmed colostrum powder | | | Bovine colostrum whey powder | | |
|---|---|---|---|---|---|---|
| Immunomodulatory parameters | 1st milking | 2nd milking | 3rd milking | 1st milking | 2nd milking | 3rd milking |
| Lymphocyte proliferation index | $1.79^{a} \pm 0.037$ | $1.64^{b} \pm 0.045$ | $1.41^{c} \pm 0.018$ | $1.26^{d} \pm 0.05$ | $1.13^{e} \pm 0.04$ | $0.96^{f} \pm 0.073$ |
| Phagocytic activity (%) | $35.47^{a} \pm 0.38$ | $31.89^{b} \pm 0.31$ | $29.03^{c} \pm 0.47$ | $20.27^{d} \pm 0.25$ | $18.08^{e} \pm 0.36$ | $14.67^{f} \pm 0.18$ |

$n = 15$, mean ± SE; values followed by different superscripts coloumnwise are significantly different ($P < 0.05$).

## 4. Conclusion

The unique biomolecules of colostrum have aroused interest among consumers in the post-COVID period, wherein many industries are looking forward for manufacturing innovative functional food using colostrum-based ingredients. Bovine colostrum can be subjected to heat treatment at 60°C/45 minutes with a minimal decrease in immune factors and growth factors. At the same time, there is considerable reduction in microbial count which renders it safe for human consumption. Bovine skimmed colostrum powder as well as bovine colostrum whey powder can be prepared by the freeze drying method, wherein there is a minimal loss of biomolecules, and such powders can be used as a constituent or as a bioactive ingredient in beverages, infant formulas, or protein-rich supplements. The functional attributes of such powders become an essential property when it is to be incorporated into protein-rich supplements or when it is to be used as bioactive ingredient for other food products or for development of a functional food product using immune factors as the basic constituents. The major challenges that were being faced with the utilization of this ingredient was the huge variation in composition, uncertain availability, and inadequate processing technologies. This issue can be resolved by converting colostrum into a powdered form which can be easily made available, easily transported, and stored throughout the year with assured bioactivity of its components.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Anamika Das proposed the methodology, validated the study, investigated the study, and wrote the original draft. Raman Seth conceptualized the study, proposed the methodology, supervised the study, and performed project administration. Ayon Tarafdar reviewed the article, provided interpretation of results, and edited the draft. Swarnima Dey, Yogesh Kumar Saini, and Ranjna Sirohi wrote and edited different sections of the draft.

## Acknowledgments

The authors acknowledge the Director, National Dairy Research Institute, Karnal (Haryana, India), for providing necessary funding and facilities to conduct the experiments.

## References

[1] G. Di Matteo, M. Spano, M. Grosso et al., "Food and COVID-19: preventive/Co-therapeutic strategies explored by current clinical trials and in silico studies," *Foods*, vol. 9, p. 1036, 2020.

[2] V. Tripathi and B. Vashishtha, "Bioactive compounds of colostrum and its application," *Food Reviews International*, vol. 22, no. 3, pp. 225–244, 2006.

[3] S. G. Borad and A. K. Singh, "Colostrum immunoglobulins: processing, preservation and application aspects," *International Dairy Journal*, vol. 85, pp. 201–210, 2018.

[4] R. J. Playford, M. Cattell, and T. Marchbank, "Marked variability in bioactivity between commercially available bovine colostrum for human use; implications for clinical trials," *PLoS One*, vol. 15, no. 6, Article ID e0234719, 2020.

[5] A. J. Lopez and A. J. Heinrichs, "Invited review: the importance of colostrum in the newborn dairy calf," *Journal of Dairy Science*, vol. 105, no. 4, pp. 2733–2749, 2022.

[6] A. W. Scammell and J. Billakanti, "Colostrum," in *Encyclopedia of Dairy Sciences*, P. L. H. McSweeney and J. P. McNamara, Eds., pp. 18–30, Academic, Oxford, UK, 3rd edition, 2022.

[7] H. Singh and P. Havea, "Thermal denaturation, aggregation and gelation of whey proteins," in *Advanced Dairy Chemistry Volume 1: Proteins*, P. F. Fox, Ed., p. 1263, Elsevier Science Publishers, London, UK, 2003.

[8] A. C. Stoy, P. T. Sangild, K. Skovgaard et al., "Spray dried, pasteurised bovine colostrum protects against gut dysfunction and inflammation in preterm pigs," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 63, pp. 280–287, 2016.

[9] L. Elfstrand, H. Lindmark-Mansson, M. Paulsson, L. Nyberg, and B. Akesson, "Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing," *International Dairy Journal*, vol. 12, no. 11, pp. 879–887, 2002.

[10] B. J. Chelack, P. S. Morley, and D. M. Haines, "Evaluation of methods for dehydration of bovine colostrum for total replacement of normal colostrum in calves," *Canadian Veterinary Journal*, vol. 34, no. 7, pp. 407–412, 1993.

[11] K. Stelwagen, E. Carpenter, B. Haigh, A. Hodgkinson, and T. T. Wheeler, "Immune components of bovine colostrum and milk," *Journal of Animal Science*, vol. 87, no. suppl_13, pp. 3–9, 2009.

[12] S. Bagwe, L. J. P. Tharappel, G. Kaur, and H. S. Buttar, "Bovine colostrum: an emerging nutraceutical," *Journal of*

*Complementary and Integrative Medicine*, vol. 12, no. 3, pp. 175–185, 2015.

[13] A. Lee, M. C. F. Pontin, E. Kosmerl, R. Jimenez-Flores, D. B. Moretti, and O. Ziouzenkova, "Assessment of adipogenic, antioxidant, and anti-inflammatory properties of whole and whey bovine colostrum," *Journal of Dairy Science*, vol. 102, no. 10, pp. 8614–8621, 2019.

[14] J. Antonio, M. S. Sanders, and D. van Gammeren, "The effects of bovine colostrum supplementation on body composition and exercise performance in active men and women," *Nutrition*, vol. 17, no. 3, pp. 243–247, 2001.

[15] E. Mizelman, W. Duff, S. Kontulainen, and P. D. Chilibeck, "Chapter 4: "The health benefits of bovine colostrum," in *Nutrients in Dairy and Their Implications on Health and Disease*, R. Watson, R. J. Collier, and V. R. Preedy, Eds., , pp. 51–60, Elsevier, 2017.

[16] D. S. O. A. Rangel, L. Mürmam, M. Bezerra, and J. Oliveira, "Bovine colostrum: benefits of its use in human food," *Food Science and Technology*, vol. 39, no. suppl 2, pp. 355–362, 2019.

[17] A. W. Scammell, "Production and uses of colostrum," *Australian Journal of Dairy Technology*, vol. 56, pp. 74–82, 2001.

[18] M. L. Godhia and N. Patel, "Colostrum—its composition, benefits as a nutraceutical: a review," *Current Research in Nutrition and Food Science Journal*, vol. 1, pp. 37–47, 2013.

[19] Z. P. Rona, "Bovine colostrum emerges as immunity modulator," *American Journal of Nature Medicine*, vol. 5, no. 2, pp. 19–23, 1998.

[20] A. B. Sabin and A. H. Fieldsteel, "Antipoliomyelitic activity of human and bovine colostrum and milk," *Pediatrics*, vol. 29, pp. 105–115, 1962.

[21] R. J. Playford, D. N. Floyd, C. E. Macdonald et al., "Bovine colostrum is a health food supplement which prevents NSAID induced gut damage," *Gut*, vol. 44, no. 5, pp. 653–658, 1999.

[22] R. Mehra, R. Singh, V. Nayan et al., "Nutritional attributes of bovine colostrum components in human health and disease: a comprehensive review," *Food Bioscience*, vol. 40, Article ID 100907, 2021.

[23] K. Sarabandi, S. Peighambardoust, A. R. Sadeghi Mahoonak, and S. Samaei, "Effect of different carriers on microstructure and physical characteristics of spray dried apple juice concentrate," *Journal of Food Science and Technology*, vol. 55, no. 8, pp. 3098–3109, 2018.

[24] S. Borad, A. Singh, G. Meena, and H. Raghu, "Storage related changes in spray dried colostrum preparations," *Lebensmittel-Wissenschaft and-Technologie*, vol. 118, Article ID 108719, 2020.

[25] A. Tsioulpas, A. S. Grandison, and M. J. Lewis, "Changes in physical properties of bovine milk from the colostrum period to early lactation," *Journal of Dairy Science*, vol. 90, no. 11, pp. 5012–5017, 2007.

[26] Sp, *Handbook of Food Analysis. Part XI. Dairy Products*, Bureau of Indian Standards, New Delhi, India, 1981.

[27] Aoac, "Official methods of analysis," *The Association of Official Analytical Chemist*, AOAC, Maryland, MD, USA, 15th edition, 2000.

[28] A. G. Houghtby, L. J. Maturin, and K. E. Koenig, "Microbiological count methods," in *Standard Methods for the Examination of Dairy Products*, R. T. Marshall, Ed., American Public Health Association, Washington, DC, USA, 16th edition, 1993.

[29] A. M. E. Sulieman, O. M. Elamin, E. A. Elkhalifa, and L. Laleye, "Comparison of physicochemical properties of spray-dried camel's milk and cow's milk powder," *International Journal of Food Science and Nutrition Engineering*, vol. 4, pp. 15–19, 2014.

[30] M. Caric and Ibrahim, "Effect of drying techniques on milk powders quality and micros tructure: a review," *Food Microstructure*, vol. 6, pp. 171–180, 1987.

[31] A. Naik, S. N. Raghavendra, and K. S. M. S. Raghavarao, "Production of coconut protein powder from coconut wet processing waste and its characterization," *Applied Biochemistry and Biotechnology*, vol. 167, no. 5, pp. 1290–1302, 2012.

[32] F. C. Hay and O. M. R. Westwood, *Practical Immunology*, Blackwell Science, Oxford, UK, 4th edition, 2002.

[33] D. J. Saldana, S. L. Gelsinger, C. M. Jones, and A. J. Heinrichs, "Effect of different heating times of high-medium-and low-quality colostrum on immunoglobulin G absorption in dairy calves," *Journal of Dairy Science*, vol. 102, no. 3, pp. 2068–2074, 2019.

[34] S. Godden, S. McMartin, J. Feirtag et al., "Heat treatment of bovine colostrum. II: effects of heating duration on pathogen viability and immunoglobulin G," *Journal of Dairy Science*, vol. 89, no. 9, pp. 3476–3483, 2006.

[35] K. Puppel, M. Gołębiewski, G. Grodkowski et al., "Composition and factors affecting quality of bovine colostrum: a review," *Animals*, vol. 9, no. 12, p. 1070, 2019.

[36] B. A. McGrath, P. F. Fox, P. L. H. McSweeney, and A. L. Kelly, "Composition and properties of bovine colostrum: a review," *Dairy Science and Technology*, vol. 96, no. 2, pp. 133–158, 2016.

[37] Y. R. R. S. Rezende, J. P. Nogueira, and N. Narain, "Microencapsulation of extracts of bioactive compounds obtained from acerola (Malpighia emarginata DC) pulp and residue by spray and freeze drying: chemical, morphological and chemometric characterization," *Food Chemistry*, vol. 254, pp. 281–291, 2018.

[38] K. G. Upadhyay, "Physico-chemical and functional properties of milk powders," *Lecture Compendium on Advances in spray Drying*, NDRI, Karnal, Indiapp. 59–81, 1999.

[39] C. Turchiuli, Z. Eloualia, N. El Mansouri, and E. Dumoulin, "Fluidised bed agglomeration: agglomerates shape and end-use properties," *Powder Technology*, vol. 157, no. 1-3, pp. 168–175, 2005.

[40] J. W. Carson and B. H. Pittenger, "Bulk properties of powders," in *Powder Metal Technologies and Applications*, P. W. Lee, Y. Trudel, R. Iacocca, R. M. German, B. L. Ferguson, and W. B. Eisen, Eds., vol. 7, pp. 287–301, ASM International, Russel Township, OH, USA, 1998.

[41] M. N. Febriyenti, N. Mohamed, M. R. Hamdan, S. N. M. Salleh, and S. B. B. Baie, "Comparison of freeze drying and spray drying methods of haruan extract," *International Journal of Drug Delivery*, vol. 6, no. 3, pp. 286–291, 2014.

[42] G. Hols and P. J. J. M. V. Mil, "An alternative process for the manufacture of whole milk powder," *International Journal of Dairy Technology*, vol. 44, no. 2, pp. 49–52, 1991.

[43] L. J. Jallo, C. Ghoroi, L. Gurumurthy, U. Patel, and R. N. Davé, "Improvement of flow and bulk density of pharmaceutical powders using surface modification," *International Journal of Pharmaceutics*, vol. 423, no. 2, pp. 213–225, 2012.

[44] S. Bhatta, T. Stevanovic, and C. Ratti, "Freeze-drying of maple syrup: efficient protocol formulation and evaluation of powder physicochemical properties," *Drying Technology*, vol. 38, no. 9, pp. 1138–1150, 2020.