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Influence of unit operations on immunoglobulins and thermal stability of colostrum fractions

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ABSTRACT

Colostrum-based ingredients are gaining significance in the global nutraceutical market as dietary supplements, but compositional variation, bioactive heat sensitivity and lack of appropriate preservation technologies hamper commercial colostrum processing. The effect of commonly employed unit operations on the physicochemical and nutraceutical components of skim colostrum was determined, and effects of membrane processing on its thermal gelation temperature, key to further liquid processing, were monitored. Homogenisation and high-shear mixing did not alter immunoglobulin content. However, commercial thermal pasteurisation (72 °C, 15 s) and batch pasteurisation (63 °C, 30 min) led to 56.4% and 24.6% denaturation of IgG, respectively. Microfiltration eliminated microbial load of diluted skim colostrum to render it practically sterile, while ultrafiltration concentrated the proteins of colostrum fractions. Concentration of protein, particularly immunoglobulins, resulted in lowering of thermal gelation temperature. Skim colostrum and colostrum whey can, therefore, be preserved by employing microfiltration and concentrated with simultaneous purification by ultrafiltration.

1. Introduction

Life expectancy and mortality rates are effective indicators of public health status. The mortality rates for neonates, infants and children under the age of 5 years are considerably higher among the populations of developing and under-developed nations. Mortality and morbidity rates are substantially higher in middle and lower-income groups, probably due to poor feeding and weaning practices. A poor immune system is responsible for mortality during illnesses, including diarrhoea, malaria, pneumonia, measles, prematurity neonatal sepsis conditions, HIV, birth asphyxia and congenital anomalies, that are highly prevalent in developing nations. Immunisation of infants and children may ward off such health problems. Colostrum feeding could be an alternative and possible approach for immunity enhancement (Borad & Singh, 2018).

Colostrum is the first mammary secretion drawn during the initial few days post-partum. It contains high proportion of antibodies (immunoglobulins), antimicrobial components (lactoferrin, lysozyme and lactoperoxidase) and growth factors necessary for the survival and healthy life of offspring. Immediately after birth, calves are fed with colostrum of 10% of their body weight before intestinal permeability for immunoglobulins is reduced. Surplus colostrum is usually available at dairy farms on a daily basis, even after calf feeding. As such, colostrum has applicability for value added product manufacturing, and hence an opportunity exists for utilisation of its nutritional and therapeutic virtues for human nutrition. However, information pertaining to the status of bioactive components upon common commercial processing operations of liquid colostrum is scanty.

To deliver the immunoprophylactic or therapeutic potential of immunoglobulins of colostrum, it must be preserved in the form of a shelf-stable liquid/beverage or dried powder. Colostrum powders of variable compositions are available in the market, most of which contain lower levels of immunoglobulins with substantially higher amount of carbohydrates and minerals as compared with that in colostrum. Therefore, shelf-stable liquid colostrum containing concentrated immunoglobulin would be an attractive option to deliver colostral bioactives as an immunity booster. Colostrum has been proven to confer passive immunity to humans and scientific

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investigations have validated this (Korhonen, Marnila, & Gill, 2000; Weiner, Pan, Hurtig, & Boren, 1999). It also improves the status of gastrointestinal disorders and injuries, as well as athletes' performance (Buckley & Scammell, 2000).

Elfstrand, Lindmark-Mansson, Paulsson, and Nyberg (2002) reported 28% loss of Igs into the lipid fraction upon centrifugal cream separation, due to flocculation of fat globules by the Igs. Rennet coagulation of skimmed colostrum resulted in non-significant changes in the concentrations of IgG2, IgA, IgM and IGF-1 in whey, but resulted in 20% and 30% retention of IgG1 and TGF-β2 in the curd, respectively. High-speed shearing (20,000 ×*g* at 60 °C for 1 min) followed by pasteurisation at 60 °C for 30 min did not alter the IgG1, IgG2, IgA and TGF-β levels but decreased IGF-1 by 33%.

Heat treatment is invariably employed to ensure microbiological safety and check deteriorative reactions of raw materials, but it adversely affects the bioactivity of immunoglobulins (Igs). Singh and Havea (2003) reported onset of unfolding of Igs at 72 °C and complete denaturation at 89 °C, whereas Lindström, Paulsson, Nylander, and Elofsson (1994) observed thermal unfolding of Igs in the range of 62.6–67.6 °C, depending on the pH of colostrum. Igs are vulnerable to heating above 65 °C (Indyk, Williams, & Patel, 2008) and their thermal denaturation causes in an increase in viscosity and gelation depending on the extent of denaturation. The thermo-labile nature of colostrum immunoglobulins and other bioactives is well-established and depends on temperature, treatment time, pH and compositional factors (Borad & Singh, 2018; McGrath, Fox, McSweeney, & Kelly, 2016; Tripathi & Vashishtha, 2006).

Microfiltration (MF) has been reported to remove microorganisms present in liquids including milk, fruit juices and yield a sterile permeate. Ultra-high temperature processed milk supplemented with MF membrane sterilised bovine Igs under aseptic condition was stable up to 5 months at 4, 25 and 35 °C (Fukumoto, Skura, & Nakai, 1994). Piot, Fauquant, Madec, and Maubois (2004) employed microfiltration to eliminate the microbial load of diluted skim colostrum. The microfiltrate contained 9.6–23.7% total solids, 3.8–11.6% proteins and 2.2–5.0% IgG. Microfiltrate was further ultrafiltered using UF membrane of different molecular mass cut-offs to obtain 8.9–10.5% protein, 6.5–8.4% IgG and 9.4–11.2% total solids in retentates.

Therefore, effects of most common dairy processing operations on immunoglobulins, microbial load and viscosity are of utmost importance from product and process development perspective. To the best of our knowledge, long-life liquid colostrum products are not available in market. Therefore, the present investigation was carried out to process colostrum and preserve it in the liquid form.

2. Materials and methods

2.1. Colostrum handling

Colostrum of Indian Zebu cattle (Sahiwal) was procured from the livestock research centre of the ICAR-National Dairy Research Institute, Karnal and frozen (−18 °C) to create a colostrum bank (80 kg, used within 3 months) and ensure the uniformity of the sample. It was thawed to 45 °C using hot water (50 °C) before experimental trials.

2.2. Processing of colostrum

The thawed colostrum was skimmed twice at 45 °C by passing through a pilot scale semi-open type centrifugal cream separator. Skimmed colostrum was subjected to various common dairy processing operations namely two-stage homogenisation (10.34, 13.79 and 17.24 MPa at 50 °C), high shear mixing (5000, 10,000, 15,000 and 20,000 ×*g* for 1 min at 50 °C), thermal treatment (60 °C for 60 min, 63 °C for 30 min, 65 °C for 15 s and 72 °C for 15 s), microfiltration (0.22 μm pore size at 50 °C) and ultrafiltration (UF; 30 kDa at 50 °C). The skim colostrum was diluted in a 1:1 ratio by adding reverse osmosis water before the MF, UF and for the preparation of colostrum whey. The high-shear mixing was carried out using high-shear disperser (T-18 digital Ultra-Turrax; Staufen im Breisgau, Germany).

The thermal treatments of colostrum samples (25 mL sample volume) were applied using a Rapid Visco Analyser (Model RVA-4; Newport Scientific, Australia) at 160 ×*g* at cooling and heating rates of 10 °C min^{-1}. The sample was heated from 40 °C to the treatment temperature and held for specified time before cooling down to 40 °C. Microfiltration was carried out using a lab scale membrane processing unit (Merck; Darmstadt, Germany). The polyethersulfone membrane cassette having 0.22 μm pore size and 0.1 m^2 membrane area was employed for microfiltration of diluted skim colostrum at 50 °C and 0.5 kg cm^{-2} feed pressure. Two-fold volume concentration by ultrafiltration of diluted skim colostrum was carried out using same plant installed with UF membrane having a 30 kDa cut-off polyethersulfone membrane cassette at 50 °C and 1 kg cm^{-2} feed pressure.

Diluted skimmed colostrum was mixed with rennet (5 g per 100 kg diluted skim colostrum) at 30 °C and curd was allowed to set for 30 min. The set curd was then cut using cheese knives to form 1 cm^3 sized curd cubes. The curd was heated from 30 to 40 °C at a heating rate of 2 °C per 5 min before filtration through muslin cloth to obtain colostrum whey. Diluted skim colostrum and colostrum whey were fractionated using UF to examine the possibility of concentration and simultaneous purification of immunoglobulins from the liquid colostrum.

2.3. Physico-chemical analysis

The fat, total protein, ash and total solids of colostrum were estimated using standard AOAC method numbers 920.85, 979.09, 923.03 and 925.10, respectively (AOAC, 1998). Titratable acidity (% lactic acid) was measured by a titration method, whereas pH of the samples at 20 °C was measured using Cyberscan pH meter (pH2100, EUTECH Instruments, Thermo Fisher Scientific, Massachusetts, USA). The IgG, IgA and IgM contents of the samples were determined using the Bovine IgG ELISA Core Kit, pink-ONE (KOMA BIOTECH, Seoul, South Korea) as per the suggested protocol. The samples were diluted to respective immunoglobulin levels in the range of 500.00–15.625 ng L^{-1} using phosphate saline buffer (pH 7.4) for the estimation. The ELISA was performed at 25 °C and absorbance was recorded at 450 nm using Multimode Plate Readers (TECAN Infinite M200 PRO, Switzerland). A ColorFlex (Hunter Associates Laboratory, Inc., Reston, VA, USA) colorimeter equipped with a dual-beam xenon flash lamp was used for measurement of colour in terms of the L*, a* and b* colour coordinates.

The specific gravity of the sample was estimated using a 50-mL specific gravity bottle. The sample was filled in the specific gravity bottle (50 mL) at 20 °C and the weight of sample was measure by deducting the weight of empty bottle from the total weight. The ratio of weight to volume was expressed as specific gravity at 20 °C.

2.4. Rheological analysis

Apparent viscosity (50 s^{-1}, 20 °C) and flow behaviour of samples (2 mL sample volume) were determined using a rheometer (MCR-52, Anton Paar, Germany) equipped with CP-75 (cone-plate, 1° cone angle) assembly having a 0.149 mm gap. The flow behaviour of samples was carried out at 20 °C in the range of 0–1000 s^{-1} shear rate within 200 s of test duration. The temperature sweep test was

performed using the rheometer with a parallel plate, 50 mm diameter (PP-50), to estimate the temperature corresponding to thermal gelation of sample. Two millilitres of sample were poured and probe was lowered down to maintain the gap of 1.00 mm. A temperature range of 50–80 °C was studied with heating rate of 2 °Cmin^{-1} at a constant frequency of 1 Hz using oscillatory rheology. Thermal gelation temperature was recorded at the point where storage modulus (G') rose suddenly or exceeded the loss modulus (G"), whichever occurred earlier.

2.5. Microbiological analysis

The total plate count (TPC), coliform count and yeast and mould count were enumerated as per the methods no. ISO 4833-1:2013 described in ISO (2013). To estimate methylene blue reduction time (MBRT), 10 mL sample was added to 1 mL methylene blue dye (0.005%) at 37 °C in a sterile glass test tube under aseptic conditions and test tube was sealed using sterile rubber cork. The content was mixed by inverting the tubes several times before incubation at 37 °C. A positive control sample was kept without addition of methylene blue dye to judge complete discolouration. Tubes were checked for complete discolouration after every 10 min for first 1 h and then after every 30 min till 5.5 h. Time required to complete discolouration was noted down as MBRT.

2.6. Statistical analysis

The data generated during the study were analysed statistically for F-test using one-way and two-way analysis of variance (ANOVA) by applying Duncan Post Hoc Test using the SAS Enterprise guide (5.1, 2012) supplied by SAS Institute Inc., North Carolina, USA. Data were expressed as means ± standard error.

3. Results and discussion

3.1. Proximate composition and selected properties of whole and skim colostrum

The proximate composition and selected properties of whole and skim colostrum are shown in Table 1. The pooled bovine colostrum contained all the nutritional components at elevated levels, particularly protein content. Out of 28.25% total solid content, 18.68% was contributed by the total protein, with immunoglobulins being the principal protein component of colostrum contributing 13.62% (72.9% of total protein), unlike mature milk, which contains casein as the major milk protein fraction (almost 80% of protein). Lactose content of milk generally varies in the range of 4.0–4.5% for bovine milk. However, whole colostrum contained only 2.05% lactose. Lactose content accounts for 50% of the osmotic pressure of lacteal secretion; therefore, lactose content is inversely proportional to the protein and ash content so as to maintain the milk isotonic with blood (Fox, 2009). Richness of colostrum in terms of proteins and fat result in an exceptionally high specific gravity as well as viscosity, compared with mature milk.

Table 1
Proximate composition and selected properties of whole and skimmed colostrum.[a]

Parameter	Whole colostrum	Skimmed colostrum
Fat (%)	$6.77^{a} \pm 0.07$	$0.70^{b} \pm 0.07$
Total protein (%)	$18.68^{a} \pm 0.11$	$19.90^{b} \pm 0.34$
Lactose (%)	$2.05^{a} \pm 0.07$	$2.15^{a} \pm 0.03$
Ash (%)	$1.04^{a} \pm 0.00$	$1.08^{b} \pm 0.01$
Total solids (%)	$28.25^{a} \pm 0.04$	$23.48^{b} \pm 0.05$
Total IgG (g L^{-1})	$114.73^{a} \pm 1.46$	$109.73^{b} \pm 0.18$
Total IgA (g L^{-1})	$7.71^{a} \pm 0.15$	$5.29^{b} \pm 0.11$
Total IgM (g L^{-1})	$13.79^{a} \pm 0.53$	$11.26^{b} \pm 0.04$
Titratable acidity (% LA)	$0.50^{a} \pm 0.01$	$0.53^{b} \pm 0.01$
Specific gravity	$1.056^{a} \pm 0.001$	$1.059^{b} \pm 0.001$
pH	$6.24^{a} \pm 0.01$	$6.26^{a} \pm 0.00$
Viscosity at 20 °C 50 s^{-1} (cP)	$41.90^{a} \pm 0.21$	$21.50^{b} \pm 0.06$
L* value	$72.41^{a} \pm 0.10$	$65.96^{b} \pm 0.01$
a* value	$1.47^{a} \pm 0.02$	$-2.14^{b} \pm 0.01$
b* value	$24.97^{a} \pm 0.02$	$20.13^{b} \pm 0.02$

[a] Values are means ± SE from triplicate determinations (n = 9); means with different superscript letters in a row differ significantly ($p < 0.05$).

Higher protein and mineral content exhibit exceptionally high buffering capacity and hence acidity (as acidity of milk/colostrum is buffering capacity to change the pH from natural value to pH 8.3) of colostrum. Colostrum exhibited lower pH values, probably because of the dominance of milk proteins, which are acidic in nature. The residual fat content in the skim colostrum was 0.7% as compared with residual fat content of <0.1% in skim milk. This could be due to higher viscosity of whole colostrum. As per Stokes' law, the velocity of fat globule during centrifugal separation is inversely proportional to the viscosity of the serum.

The total protein, lactose and ash content of the skim colostrum were significantly ($p < 0.05$) higher than that in whole colostrum, whereas, unlike other serum solids, the immunoglobulins were reduced significantly ($p < 0.05$) in skim colostrum. However, all the Igs were concentrated in skim colostrum on a dry matter basis. These results were in line with the report of Elfstrand et al. (2002) regarding the loss of Igs in cream due to their flocculation properties on fat globules. Losses of 28% of Igs of whole colostrum in cream obtained upon centrifugal cream separation were attributed to their role in the flocculation of fat globules (Huppertz & Kelly, 2006). Considering the results obtained, whole colostrum sample was diluted to 1:1 with reverse osmosis water before cream separation that yielded diluted skim colostrum having 0.12 ± 0.01% fat. Therefore, whole colostrum should be diluted before the cream separation to improve skimming efficiency.

Skim colostrum had a higher viscosity of 21.50 mPa s (at 20 °C, 50 s^{-1}) as compared with skim milk (~1 mPa s at 20 °C), which could be attributed to a higher protein content at slightly lower pH value of 6.24 for skim colostrum. Factors that affect the stability of protein include pH, ionic environment, protein content and temperature. Colostrum contains high protein and high mineral contents at lower pH, and hence the stability of colostrum proteins is expected to be inferior as compared with normal milk. The colour values were significantly ($p < 0.05$) affected by cream separation. Unlike cow milk wherein skim milk is whiter in colour, the lightness value (as indicated by L*) of colostrum decreased significantly ($p < 0.05$) upon cream separation, which may be due to increase in concentration of serum solids upon cream separation and thereby opacity. This could be correlated well with the significant ($p < 0.05$) increase in L* value of diluted skim colostrum (71.67 ± 0.03) as compared with the undiluted one. A positive a* value of whole colostrum indicated an intense yellow colour owing to fat globule containing higher carotenoids as compared with mature milk. The presence of β-carotene in bovine colostrum contributes to reddish-yellow colour depending on its concentration (Edelsten, 1988). A positive correlation between colour of colostrum and its carotenoid content was reported by Calderón et al. (2007), with β-carotene contributing 65% of variations in its colour characteristics.

3.2. Effects of pre-treatments to skim colostrum on immunoglobulin status

On the basis of dry matter, the IgG content was significantly ($p < 0.05$) higher in skim colostrum (46.73% IgG on DMB) as compared with whole colostrum (40.61% IgG on DMB). Therefore,

Table 2
Effects of physical treatments on the immunoglobulin levels of skimmed colostrum.[a]

Treatment	IgG	IgA	IgM
Whole colostrum	$114.73^{a} \pm 1.46$	$7.71^{ab} \pm 0.15$	$13.79^{a} \pm 0.53$
Skimmed colostrum	$109.73^{ab} \pm 0.18$	$5.29^{ef} \pm 0.11$	$11.25^{b} \pm 0.04$
Homogenization at10.34 MPa	$109.88^{ab} \pm 3.04$	$6.85^{d} \pm 0.60$	$7.52^{de} \pm 0.55$
Homogenization at 13.79 MPa	$111.68^{ab} \pm 2.15$	$8.40^{b} \pm 0.74$	$7.94^{cde} \pm 0.58$
Homogenization at 17.24 MPa	$110.63^{ab} \pm 1.57$	$5.55^{def} \pm 0.49$	$7.70^{de} \pm 0.57$
High shearing at 5000 ×*g*	$108.18^{b} \pm 2.37$	$8.60^{bc} \pm 0.75$	$9.58^{c} \pm 0.70$
High shear mixing at 10,000 ×*g*	$108.94^{b} \pm 1.53$	$6.80^{ade} \pm 0.60$	$6.91^{ef} \pm 0.51$
High shear mixing at 15,000 ×*g*	$109.68^{ab} \pm 2.85$	$9.93^{c} \pm 0.87$	$3.40^{hi} \pm 0.25$
High shear mixing at 20,000 ×*g*	$106.04^{ab} \pm 1.87$	$6.09^{de} \pm 0.53$	$2.00^{i} \pm 0.15$
72 °C for 15 s	$47.80^{hi} \pm 1.32$	$0.63^{jk} \pm 0.06$	$0.00^{j} \pm 0.00$
63 °C for 30 min	$82.74^{d} \pm 2.29$	$0.83^{jk} \pm 0.07$	$0.00^{j} \pm 0.00$
60 °C for 60 min	$110.14^{ab} \pm 1.19$	$1.03^{ijk} \pm 0.09$	$0.00^{j} \pm 0.00$
65 °C for 15 s	$106.89^{b} \pm 1.26$	$0.00^{k} \pm 0.00$	$0.20^{j} \pm 0.01$
Diluted skimmed colostrum	$53.98^{g} \pm 1.05$	$2.64^{h} \pm 0.05$	$5.63^{fg} \pm 0.02$
MF Permeate	$49.12^{gh} \pm 0.96$	$2.48^{hi} \pm 0.09$	$1.85^{i} \pm 0.14$
MF Retentate	$66.38^{f} \pm 1.70$	$4.12^{fg} \pm 1.14$	$12.96^{a} \pm 2.01$
Skim colostrum UF retentate	$73.80^{e} \pm 1.64$	$3.56^{gh} \pm 0.08$	$7.51^{de} \pm 0.17$
Colostrum whey	$42.97^{i} \pm 0.96$	$2.06^{hij} \pm 0.05$	$4.39^{gh} \pm 0.10$
Colostrum whey UF retentate	$88.95^{c} \pm 1.98$	$4.27^{fg} \pm 0.10$	$9.09^{cd} \pm 0.20$

[a] Values, in g L^{-1}, are means ± SE from triplicate determinations (n = 9); means with different superscript letters in a column differ significantly ($p < 0.05$).

dairy processing operations usually employed at industrial scale were applied to skim colostrum to evaluate the stability of immunoglobulins as well as changes in the selected compositional, physico-chemical and rheological properties. The status of immunoglobulin contents in the skim colostrum samples is presented in Table 2. There were no adverse effects of both physical treatments, namely homogenisation and high-shear mixing, on the IgG contents of skim colostrum. Homogenisation had a significant ($p < 0.05$) effect on IgM, but homogenisation pressure did not influence their level. The levels of IgM were reduced with increasing intensity of high-shear mixing treatment. The mechanism of shear-induced breakdown of IgM is unknown, but it is possibly due to physical breakdown of pentameric molecule of IgM.

In contrast to the effects of physical treatment on Igs, thermal processing resulted in denaturation of the Igs of skim colostrum, depending on the temperature and duration of treatment. Commercial HTST pasteurisation (72 °C for 15 s) treatment reduced the IgG to loss of 56.4% of initial levels, whereas LTLT pasteurisation (63 °C for 30 min) decreased IgG to 82.74 g L^{-1}, which corresponded to 24.6% loss of IgG. However, there was insignificant ($p > 0.05$) effects of thermisation treatments (60 °C for 60 min; 65 °C for 15 s) on IgG. Elizondo-Salazar, Jayarao, and Heinrichs (2010) treated bovine colostrum at 57, 60, and 63 °C for 0, 30, 60, or 90 min in a water bath and analysed status of Igs, microbial load and viscosity. Heating at ≥ 60 °C resulted in significant increase in viscosity as a result of denaturation of Igs. Elfstrand et al. (2002) reported that high-speed shearing (20,000 ×*g* at 60 °C for 1 min) followed by pasteurisation at 60 °C for 30 min did not alter the IgG and IgA levels in bovine colostrum. Commercial pasteurisation time–temperature combination (63 °C for 30 min or 72 °C for 15 s) significantly ($p < 0.05$) reduced IgG and IgM levels, and increased viscosity of colostrum as compared with heat treatment of 60 °C for 60 min as well as raw colostrum sample (Elfstrand et al., 2002).

Immunoglobulins are globular proteins and their quaternary structure is stabilised through non-covalent bonds, which are most vulnerable to the thermal treatments. Significant change in the tertiary structure of proteins maintained by hydrophobic bonds and ionic interactions occur during heating. Loss of structural integrity resulted in lower biological activity. The denaturation of Igs resulted in an increase in viscosity of colostrum due to heat induced unfolding of polypeptide chain, exposing the hydrophilic sites for water binding (El-Fattah, Rabo, El-Dieb, & El-Kashef, 2014).

MF permeate contained significantly ($p < 0.05$) less IgG and IgM, without affecting IgA levels; however, it was almost sterile (Table 3). During MF, there was a loss of 9.0% of initial IgG content compared with 56.4 and 24.6% losses upon HTST and LTLT pasteurisation, respectively. Fritsch and Moraru (2008) obtained permeate with unchanged protein composition with reduced the microbial load of skim milk using MF having a pore size of 1.4 μm. Gosch, Apprich, Kneifel, and Novalin (2014) obtained more than 4 log reduction of the total viable count in bovine colostrum using MF having a pore size of 1.4 μm. However, about 10–30% of IgG was lost in the retentate during MF. Upon membrane filtration (MF followed by UF/DF) IgA and IgG2 contents were reduced by 30%, while IgG1 remained unaffected. IgG1 recovery was 82% in freeze-dried colostrum whey prepared using UF retentate of colostrum whey (Elfstrand et al., 2002). The composition of colostrum whey and skim colostrum, in terms of their Igs, were identical. However, the removal of casein upon renneting increased Igs on a dry matter basis and hence whey preparation could be another Ig purification approach. Ultrafiltration concentrated all the Igs of diluted skim colostrum and colostrum whey in their respective retentates, but their concentration was less than 2-fold, which was the volume concentration factor for both samples, probably due to losses in fouling.

Table 3
Effect of pasteurisation or equivalent treatment on microbiological quality of skim colostrum.[a]

Sample/treatment	TPC (cfu mL^{-1})	Coliforms (cfu mL^{-1})	MBRT
diluted skim colostrum	$5.10^{a} \pm 0.04$	$1.06^{a} \pm 0.14$	$3{:}30^{a} \pm 0{:}00$ h
MF Retentate	$5.79^{b} \pm 0.04$	$1.76^{b} \pm 0.13$	$2{:}00^{b} \pm 0{:}00$ h
MF Permeate	nil	nil	$>5.5\ h^{X}$
72 °C for 15 s	$4.67^{c} \pm 0.03$	nil	$>5.5\ h^{Y}$
63 °C for 30 min	$4.26^{d} \pm 0.14$	nil	$>5.5\ h^{Y}$
60 °C for 60 min	$4.36^{d} \pm 0.06$	nil	$>5.5\ h^{Y}$
65 °C for 15 s	$4.76^{c} \pm 0.03$	nil	$>5.5\ h^{Z}$

[a] Abbreviations are: TPC, total plate count; MBRT, methylene blue reduction time. Values are means ± SE from triplicate determinations (n = 9); means with different superscript letters in a column differ significantly ($p < 0.05$). Superscripts X, Y, and Z indicate that the sample exhibited MBRT of 5.5 h at the 21, 5 and 3 days of refrigerated storage, respectively, in the sterile glass test tubes packaged immediately after treatment.

3.3. Effect of heat treatment on immunoglobulins and microbiological load of skim colostrum

Considering the objective of pasteurisation treatment given to colostrum, the thermal treatment is expected to render colostrum free from pathogenic microorganisms to ensure food safety as well as to reducing spoilage-causing microorganisms. However, these treatments have been reported to be detrimental with respect to bioactive components, specifically Igs (Table 2). Furthermore, the pasteurisation time–temperature combination employed for the viscous fluids should be elevated to overcome the viscous protection to microorganism. If this holds true for colostrum, elevated thermal treatment is expected to destroy colostral Igs to even greater extent. Therefore, alternate methods of pasteurisation for colostrum are required to ensure the elimination of pathogens and other spoilage-causing microflora therein without adversely affecting its bioactive proteins. The ability of MF to replace thermal pasteurisation treatment must be judged by evaluating its effects on microbial load of colostrum. Table 3 shows the microbial contents of skim colostrum upon pasteurisation and MF as an alternative treatment to pasteurisation.

It is evident from the Table 3 that thermal treatment reduced total plate counts (TPC) significantly ($p < 0.05$), and the reduction was greater, particularly with thermal treatments for longer duration (60 °C/60 min and 63 °C/30 min) as compared with higher temperatures and shorter times (72 °C/15 s and 65 °C/15 s). Masking effects of higher solid levels and viscosity of skim colostrum required longer thermal treatment time for heat to penetrate and exert bactericidal activity. Vermeer and Norde (2000) reported the protective effects of denatured immunoglobulins over the native protein molecules above thermal denaturation temperature. This phenomenon was observed at high immunoglobulin levels in solution, where larger aggregates may form that physically entrap native immunoglobulins and thereby prevent them from denaturation. During the present investigation, the heat-induced aggregates of immunoglobulins might have exerted protective effects over microorganisms.

Furthermore, all thermal treatments eliminated coliforms from the skim colostrum. Yeast and moulds were absent in all the samples (data not shown). The thermal treatment also increased MBRT significantly ($p < 0.05$) from 3.5 h of initial MBRT to > 5.5 h that remained unchanged up to 5 days of refrigerated storage, except 3 days for the thermised sample treated at 65 °C for 15 s. On the other hand, MF eliminated TPC and coliforms from the MF permeate completely, by retaining them on feed side, as indicated by greater TPC and coliforms in MF retentate.

Retention of microbial load of raw skim colostrum on retentate side resulted in decreased MBRT of MF retentate, whereas the permeate obtained upon MF was practically sterile (as observed and confirmed by microscopy, not shown here) exhibiting MBRT of >5.5 h, which remained unchanged for up to 21 days when packaged in sterile glass tubes directly from the permeate outlet of MF plant. This could be an approach to developing the processing technology for a shelf-stable colostrum beverage. Fritsch and Moraru (2008) obtained permeate with unchanged protein composition with reduced the microbial load of skim milk using MF having a pore size of 1.4 μm. Gosch et al. (2014) employed MF (pore size 1.4 μm) to retain microbial cells on retentate side to achieve a 4-log reduction as well as about 10–30% of IgG. Hence, MF would be the most preferred processing intervention to preserve liquid colostrum for possible utilisation of its therapeutic and nutraceuticals properties to immune-compromised populations. Though MF has been proven to eliminate the microbial load to yield almost sterile permeate, it does not ensure safety against pathogens, like thermal pasteurisation. The combination of MF and thermal treatment may be beneficial with additional advantage of further extension of keeping quality at refrigeration temperature.

3.4. Effects of treatments on thermal gelation temperature of colostrum fractions

To further validate the impact of thermal processing on colostrum Igs, as observed through increase in viscosity, the skim colostrum was treated at 55, 60 and 65 °C for 0, 5, 10, 15, 20 and 30 min. The viscosity of treated samples revealed the gentle nature of thermal treatment below 60 °C, as significantly ($p < 0.05$) increased viscosity was observed with increasing time at 65 °C (Table 4). These data could be best employed to identify the thermal denaturation temperature range of colostral Igs and design the processing conditions for it.

Thermal denaturation phenomenon and temperature are best investigated using differential scanning calorimetry. However, the temperature sweep test is an oscillatory rheological tool, wherein sample is heated at fixed rate at selected frequency and amplitude, and the changes in the rheological behaviour in terms of G' (modulus of elasticity, indicates elastic/solid component) and G" (modulus of viscosity, indicates viscous/liquid component) are monitored. For a sample that undergoes phase changes under the influence of heating, there is a cross over point, where G' and G" curves cross each other, which indicates temperature corresponding to change in phase. Liquid samples (e.g., starch and protein solutions) that tend to form a gel upon heating show higher G" value at lower temperature with low or no G' curve. With temperature-induced phase change phenomenon, there is an increase in the G' value, which overtakes G" value upon gel formation. This rheological tool can be employed to establish the processing time–temperature combinations for heat sensitive food material, as it has advantages like being easy to perform, rapid, cost effective, and suitable for unknown multi-component food matrices as well as ingredients that undergo structural degradation upon heating.

The colostrum fractions were evaluated for their thermal gelation temperature (TGT) using temperature sweep test (Fig. 1, Table 5). The dilution of skim colostrum resulted in significant ($p < 0.05$) increase in pH of diluted skim colostrum and TGT, whereas UF fractionation reduced TGT but increased pH, significantly ($p < 0.05$). Removal of casein by renneting significantly ($p < 0.05$) increased pH, whereas TGT was decreased upon UF fractionation and concentration. This could be due to the fact that

Table 4
Effects of thermal treatment on the apparent viscosity (50 s^{-1}, 20 °C) of skim colostrum.[a]

T (°C)	Time of treatment (min)					
	0	5	10	15	20	30
55	$33.2^{aA} \pm 0.9$	$33.3^{aA} \pm 0.9$	$27.0^{aB} \pm 0.8$	$26.8^{aB} \pm 0.8$	$33.1^{aA} \pm 0.9$	$34.3^{aA} \pm 1.0$
60	$30.5^{abA} \pm 0.9$	$32.3^{aAB} \pm 0.9$	$33.8^{bAB} \pm 1.0$	$36.7^{bB} \pm 1.0$	$43.1^{aC} \pm 1.2$	$37.9^{aBC} \pm 3.7$
65	$29.6^{bA} \pm 0.8$	$33.9^{aA} \pm 1.0$	$51.0^{cAB} \pm 1.4$	$78.9^{cB} \pm 2.2$	$753.6^{bC} \pm 21.2$	$883.7^{bD} \pm 24.9$

[a] Values are means ± SE from triplicate determinations (n = 9); means with different superscript upper case letters in a row and lower case letters in a column differ significantly ($p < 0.05$).

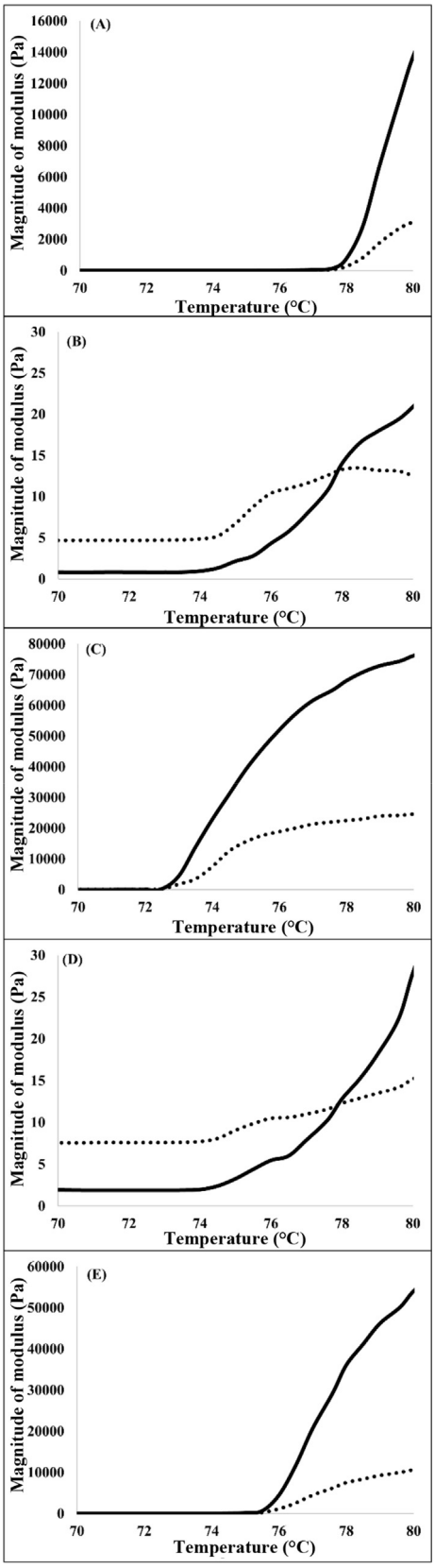

Fig. 1. Rheogram of (A) skimmed colostrum, (B) diluted skimmed colostrum, (C) skimmed colostrum UF retentate, (D) colostrum whey and (E) UF retentate of colostrum whey, showing thermal gelation temperature as crossover temperature of storage modulus (G', ——) and loss modulus (G", ····).

Table 5
The pH values and thermal gelation temperature of colostrum fractions.[a]

Sample	Gelation temperature (°C)	pH
Skim colostrum	$75.83^{a} \pm 0.17$	$6.26^{a} \pm 0.00$
Diluted skim colostrum	$78.00^{b} \pm 0.29$	$6.41^{b} \pm 0.00$
Skim colostrum UF retentate	$72.20^{c} \pm 0.17$	$6.56^{c} \pm 0.00$
Colostrum whey	$77.83^{b} \pm 0.17$	$6.52^{c} \pm 0.00$
Colostrum whey UF retentate	$74.50^{d} \pm 0.17$	$6.54^{c} \pm 0.00$

[a] Values are means ± SE from triplicate determinations (n = 3); means with different superscript letters in a column differ significantly ($p < 0.05$).

the profiles of protein and mineral as well as their concentration play important roles in gelation of proteins. Further, potential of certain thermal protectants to elevate the TGT of Igs to improve processibility of colostrum and its fractions may also be exploited, as the thermisation in conjugation with MF would produce permeate with better shelf-life. Chen and Chang (1998) reported the protective effects of fructose, maltose, sucrose, lactose, glucose, galactose, glutamic acid, glycine, glycerol and sorbitol on bovine milk IgG. The addition of sucrose would also improve the sensory acceptance of the colostrum-based beverage by imparting sweetness and masking characteristic flavour of colostrum.

4. Conclusion

Dilution of colostrum with reverse osmosis water before cream separation assisted in effective fat separation thereby minimising fat losses in skim colostrum. The skimming could the first step for the enrichment of Igs, followed by renneting to remove casein. Physical processing treatments had no detrimental effects on Igs of skim colostrum. The thermal treatments influenced the stability of Igs of skim colostrum. However, the extent of denaturation depends on the time–temperature combinations, and therefore MF could be used as an alternative to thermal pasteurisation to ensure food safety. The microfiltration permeate was practically sterile and exhibited higher shelf-life under refrigerated storage as compared with the pasteurised one. UF fractionation decreased the thermal gelation temperature significantly ($p < 0.05$) due to concentration of proteins and simultaneous removal of minerals and lactose that offer protection to proteins. A temperature sweep test may be employed to standardise processing variables for heat-sensitive food material and optimisation of commercial production thereof. Scaling up of cream separation, microfiltration and ultrafiltration are considered as easy and cost-effective technologies which could be applied to manufacture and commercialise immunoglobulin-rich colostrum for health-conscious individuals, children, and people with poor immune status.

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References

AOAC. (1998). *Official methods of analysis* (16th ed.). Washington, DC, USA: Association of Official Analytical Chemists.
Borad, S. G., & Singh, A. K. (2018). Colostrum immunoglobulins: Processing, preservation and application aspects. *International Dairy Journal, 85*, 201–210.
Buckley, J. D., & Scammell, A. D. (2000). Does a diet of colostrum improve athletic performance? *Asian-Australian Journal of Animal Science, 13*, 312–315.
Calderón, F., Chauveau-Duriot, B., Pradel, P., Martin, B., Graulet, B., Doreau, M., et al. (2007). Variations in carotenoids, vitamins A and E, and color in cow's plasma

and milk following a shift from hay diet to diets containing increasing levels of carotenoids and vitamin E. *Journal of Dairy Science, 90*, 5651–5664.
Chen, C. C., & Chang, H. H. (1998). Effect of thermal protectants on the stability of bovine milk immunoglobulin G. *Journal of Agricultural and Food Chemistry, 46*, 3570–3576.
Edelsten, D. (1988). Composition of milk. In H. R. Cross, & J. A. Overby (Eds.), *Meat science, milk science and technology*. Amsterdam, The Netherlands: Elsevier Science Publishers B.V.
El-Fattah, A. M. A., Rabo, F. H. R. A., El-Dieb, S. M., & El-Kashef, H. A. S. (2014). Preservation methods of buffalo and bovine colostrum as a source of bioactive components. *International Dairy Journal, 39*, 24–27.
Elfstrand, L., Lindmark-Mansson, H., Paulsson, M., & Nyberg, L. (2002). Immunoglobulins growth factors and growth hormone in bovine colostrum and the effects of processing. *International Dairy Journal, 12*, 879–887.
Elizondo-Salazar, J. A., Jayarao, B. M., & Heinrichs, A. J. (2010). Effect of heat treatment of bovine colostrum on bacterial counts, viscosity, and immunoglobulin G concentration. *Journal of Dairy Science, 93*, 961–967.
Fox, P. F. (2009). Lactose: Chemistry and properties. In P. L. H. McSweeney, & P. F. Fox (Eds.), *Advanced dairy chemistry. Vol. 3. Lactose, water, salts and minor constituents*. New York, NY, USA: Springer.
Fritsch, J., & Moraru, C. I. (2008). Development and optimization of a carbon dioxide-aided cold microfiltration process for the physical removal of microorganisms and somatic cells from skim milk. *Journal of Dairy Science, 91*, 3744–3760.
Fukumoto, L. R., Skura, B. J., & Nakai, S. (1994). Stability of membrane-sterilized bovine immunoglobulins aseptically added to UHT milk. *Journal of Food Science, 59*, 757–759.
Gosch, T., Apprich, S., Kneifel, W., & Novalin, S. (2014). A combination of microfiltration and high pressure treatment for the elimination of bacteria in bovine colostrum. *International Dairy Journal, 34*, 41–46.
Huppertz, T., & Kelly, A. L. (2006). Physical chemistry of milk fat globules. In P. L. H. McSweeney, & P. F. Fox (Eds.), *Advanced dairy chemistry. Vol. 2. Lipids* (pp. 184–187). Boston, MA, USA: Springer.
Indyk, H. E., Williams, J. W., & Patel, H. A. (2008). Analysis of denaturation of bovine IgG by heat and high pressure using an optical biosensor. *International Dairy Journal, 18*, 359–366.
ISO. (2013). *Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30 degrees C by the pour plate technique*. Geneva, Switzerland: International Organization for Standardization.
Korhonen, H., Marnila, P., & Gill, H. S. (2000). Bovine milk antibodies for health. *British Journal of Nutrition, 84*, S135–S146.
Lindström, P., Paulsson, M., Nylander, T., & Elofsson, U. (1994). The effect of heat treatment on bovine immunoglobulins. *Milchwissenschaft, 49*, 67–71.
McGrath, B. A., Fox, P. F., McSweeney, P. L., & Kelly, A. L. (2016). Composition and properties of bovine colostrum: A review. *Dairy Science & Technology, 96*, 133–158.
Piot, M., Fauquant, J., Madec, M. N., & Maubois, J. L. (2004). Preparation of serocolostrum by membrane microfiltration. *Le Lait, 84*, 333–341.
Singh, H., & Havea, P. (2003). Thermal denaturation, aggregation and gelation of whey proteins. In P. F. Fox (Ed.), *Advanced dairy chemistry. Vol. 1. Proteins* (p. 1263). London, UK: Elsevier Science Publishers.
Tripathi, V., & Vashishtha, B. (2006). Bioactive compounds of colostrum and its application. *Food Reviews International, 22*, 225–244.
Vermeer, A. W. P., & Norde, W. (2000). The thermal stability of immunoglobulin: Unfolding and aggregation of a multi-domain protein. *Biophysical Journal, 78*, 394–404.
Weiner, C., Pan, Q., Hurtig, M., & Boren, T. (1999). Passive immunity against human pathogens using bovine antibodies. *Clinical and Experimental Immunology, 116*, 193–205.