

Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing

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Received 12 December 2001; accepted 17 July 2002

Abstract

In colostrum collected 0–80 h postpartum the contents of immunoglobulins (Igs), transforming growth factor beta-2 (TGF- β 2), insulin-like growth factor-1 (IGF-1) and growth hormone (GH) were analysed. Colostrum initially contained 90 mg mL⁻¹ IgG1, 2.8 mg mL⁻¹ IgG2, 1.6 mg mL⁻¹ IgA, 4.5 mg mL⁻¹ IgM, and these concentrations declined by 92%, 87%, 93% and 84%, respectively, in the samples collected later. Of the growth factors, colostrum initially contained 289–310 ng mL⁻¹ TGF- β 2 and the concentration diminished to 66 ng mL⁻¹. The content of IGF-1 and GH postpartum decreased from 870 to 150 ng mL⁻¹, and from 0.17 to <0.03 ng mL⁻¹, respectively. Heat treatment and freeze-drying of colostrum whey decreased the content of Igs to 75%, while the contents of IGF-1 and TGF- β 2 were unaffected. A similar processing, including filtration steps reduced also the IGF-1 and TGF- β 2 by 25%. IgM seems to be the most sensitive of the Igs to processing.

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Keywords: Bovine colostrum; Immunoglobulins; Growth factors; Growth hormone; Processing effects; Immunological techniques

1. Introduction

Many components in bovine colostrum and milk exhibit specific biological activity in addition to their established nutritional values. During the past two decades, interest in these beneficial physiological effects and the possibility to utilise the components from milk have increased. Colostrum is a complex fluid rich in nutrients and is also characterised by its high level of bioactive components, e.g. immunoglobulins (Igs), especially IgG1, growth factors, especially insulin-like growth factor-1 (IGF-1), transforming growth factor beta-2 (TGF- β 2) and growth hormone (GH) as well as lactoferrin, lysozyme and lactoperoxidase (Butler, 1994; Pakkanen, 1998; Register, Smithers, Mitchell,

McIntosh, & Dionysius, 1997; Reiter, 1985). Colostral antibodies provide passive immunity to the new-born calf and growth factors control some fundamental life processes such as cell division, cell differentiation or apoptosis, and stimulate the growth and development of the gastrointestinal tract of new-born animals (Kurokawa, Lynch, & Podolsky, 1987; Xu, 1996). The effect on human health after consuming some of these components has not been thoroughly examined. Moreover, heat treatment during processing and isolation of bioactive components may affect their bioactive state and functionality, which implies that a good understanding of the relation between thermal stability and bioactivity is important. Knowledge concerning the influence of processing and isolation procedures on bioactive compounds in milk and milk-based products is, however, limited.

The major Ig present in ruminant milk is IgG, with IgG1 representing more than 90%, but also IgA and IgM are found (Larson, 1992). The predominant Ig in most other mammalian milks, including human milk is

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IgA, IgG1 and IgG2 are monomers (MW approx. 150 kDa), IgA occurs as a monomer or a dimer (approx. 385 kDa), while IgM consists of five monomers (MW approx. 900 kDa). The concentrations of Ig classes in colostrum have been investigated using immunological techniques (Benheng & Chengxiang, 1996; Kulkarni & Pimpale, 1989; Larson, 1992; Levieux & Ollier, 1999; Quigley, Martin, Dowlen, Wallis, & Lamar, 1994; Vacher & Blum, 1993). The most significant factors affecting the Ig concentrations in colostrum are breed, age of the cow, lactation number and volume of the first colostrum milking (Melchor, Grohn, McDowell, & van Saun, 1992; Muller & Ellinger, 1981; Pritchett, Gay, Besser, & Hancock, 1991; Vacher & Blum, 1993). Thermal treatment of Igs influences their unfolding behaviour and biological activity (Dominguez, Perez, & Calvo, 1997; Li-Chan, Kummer, Losso, Kitts, & Nakai, 1995; Lindström, Paulsson, Nylander, Elofsson, & Lindmark-Månsson, 1994; Mainer, Dominguez, Randrup, Sanchez, & Calvo, 1999). For instance, heat treatment at 72°C for 15 s resulted in a 10–30% loss of Ig activity, whereas UHT treatment (138°C, 4 s) and evaporation processing destroyed most of the activity of Igs in milk (Kummer et al., 1992; Li-Chan et al., 1995). However, virus-neutralising activity of individual immunoglobulins in colostrum against rotavirus were analysed after a similar heat treatment and no reduction of the activity was observed (Mainer et al., 1999).

The major growth factors in bovine colostrum are TGF and IGF (Pakkanen & Aalto, 1997). It has been reported that specific growth factors and growth factor extracts can improve wound healing (Scammell, 2001). Most forms of TGF- β are homodimeric proteins with a molecular weight of approximately 25 kDa (Jin, Cox, Knecht, Raschdorf, & Cerletti, 1991), although heterodimers have also been found. The major TGF- β form in bovine milk and colostrum is TGF- β 2 (85–95%), the remainder being TGF- β 1 (Jin et al., 1991; Tokuyama & Tokuyama, 1993). More than 90% of the total TGF- β 2 in colostrum is in an inactive, latent form (Pakkanen, 1998), which can be activated by changes in ionic strength, acidification or by proteolytic enzymes. The amino acid sequence of bovine TGF- β 2 and the N-terminal end (29 residues) of bovine TGF- β 1 are identical to those of their corresponding human counterparts (Jin et al., 1991). The concentration of TGF- β 2 in activated bovine colostrum as determined by enzyme-linked immunosorbent assay (ELISA) was 150–1150 ng mL⁻¹ (Pakkanen, 1998). To our knowledge the effect of heat treatment on these growth factors has not been studied previously.

The major form of IGF in bovine colostrum is IGF-1 and its amino acid sequence is identical to that of the human form (Francis, Upton, Ballard, McNell, & Wallace, 1988; Marcotty, Frankenne, van Beeumen, Maghuin-Rogister, & Hennen, 1991). IGF-1 and IGF-2

are single-chain polypeptides of approximately 7.6 kDa (Pakkanen & Aalto, 1997). IGF-1 is usually bound to its binding proteins (IGFBP) in biological fluids, which has also been detected in the case of bovine milk. Some of the binding proteins inactivate IGF-1, others enhance the activity, and some binding proteins have biological activity of their own (Tucker, 2000). IGF-1 appears in mature milk mainly in the bound form (85–90%), but in the first milkings postpartum the free form of IGF-1 predominates (73%). The slightly acidic pH (6.3) of the colostrum secretion is correlated with an increased proportion of free IGF-1 (Einspanier & Schams, 1991). The IGF-1 content of colostrum has been determined and it varied from 50 to 2000 μ g mL⁻¹ (Pakkanen & Aalto, 1997). Pasteurisation of bovine milk (79°C, 45 s) did not alter the concentration of IGF-1, but treatment at 121°C for 5 min destroyed this hormone (Collier et al., 1991). GH, secreted by the anterior pituitary is the single most important hormone for postnatal growth (Vander, Sherman, & Luciano, 1998). GH exerts its stimulating effect on cell division indirectly through the mediation of a chemical messenger, IGF-1, whose synthesis and release are induced by GH.

The aim of this study was to examine the content of Igs (IgG1, IgG2, IgA and IgM), growth factors (TGF- β 2 and IGF-1), growth hormone (GH), total protein, total fat and fatty acid composition, lactose and urea in colostrum collected at different times after calving. The concentration of Igs, growth factors and GH was analysed by immunological techniques. Furthermore, the impact of dairy processes, such as heat treatment, ultrafiltration and freeze-drying, on the recovery of these bioactive components was investigated.

2. Materials and methods

2.1. Collection of bovine colostrum

Colostrum samples from the first milkings (3 or 4 milkings) postpartum within 0–80 h were collected from Swedish Friesian cows. The cows were in different lactation number: 6 cows in the first lactation, 8 cows in the second lactation and 6 cows in lactation \geq 3. The cows were milked manually twice a day after suckling by the calf. The colostrum samples were immediately frozen and stored at -20°C. After thawing the lipid fraction was removed by centrifugation at 10,000g for 10 min at 2°C (Sorvall RC-5B, DuPont, Wilmington, DE, USA). The skimmed colostrum samples were again immediately frozen and stored at -20°C. Colostrum samples were divided into two different pools before further analysis. Pool A included colostrum collected from three time intervals postpartum: 0–10 h (14 samples), 11–30 h (22 samples) and 31–50 h (34 samples).

Pool B included samples from seven time intervals: 0–6 h (6 samples), 7–10 h (8 samples), 11–20 h (11 samples), 21–30 h (11 samples), 31–40 h (15 samples), 41–50 h (19 samples) and 51–80 h (7 samples) postpartum.

2.2. Processing of colostrum

Colostrum whey was prepared from pool B by renneting at 32°C for 30 min before cutting (rennet strength 1:15000, chymosin/pepsin ratio of 75/25, Kemikalia, Lund, Sweden). The colostrum whey was further directly heat-treated or microfiltered before heat treatment, followed by freeze-drying (Fig. 1). These processing steps resulted in two different preparations called colostrum whey powder and colostrum concentrate powder, respectively.

The colostrum concentrate was prepared from the colostrum whey by microfiltration (30°C, ceramic membrane, pore size 0.8 µm, Alfa Laval, Lund, Sweden), ultrafiltration (20°C, cut-off of 100 kDa, Pellicon System, Millipore Corporation, Bedford, MA) and diafiltration (20°C, cut-off of 100 kDa, Pellicon System, Millipore Corporation, Bedford, MA) (Lindström et al., 1994). Colostrum whey and colostrum concentrate were pasteurised batch-wise at 60°C for 45 min and then freeze-dried (Leybold-Heraeus GT20, Leybold-Heraeus GMBH, Cologne, Germany). The samples obtained in the different processing steps were analysed for Igs, growth factors and GH.

In one experiment, the homogenisation of colostrum was simulated at 60°C for 1 min using an Ultra Turrax

(TP 18–10, 20,000 rpm, Janke & Kunkel KG, IKA Werk, Staufen i. Breisgau, Germany) and the samples were further heated at 60°C for 30 min to resemble pasteurisation (72°C, 15 s).

2.3. Analyses

2.3.1. Total protein, total fat, lactose, urea and fatty acid composition

Total protein, total fat, lactose and urea were measured in all individual colostrum samples by the IR technique (CombiFoss 5000, Foss Electric A/S, Hilleröd, Denmark) before the fat had been removed. The composition of fatty acids in colostrum samples of pool A was determined by gas chromatography (Varian 3400, Varian AB, Solna, Sweden) (DeMan, 1964). A silica capillary column was used (Supelco 2380, Supelco Pak, Bellefonte, PA).

2.3.2. Immunoglobulins

The native forms of IgG1, IgG2, IgA and IgM were analysed in colostrum samples from pool B by the radial immunodiffusion (RID) technique after fat removal. RID plates containing antibodies to the different bovine Igs were used (Code RN201.3, RN 202.3, RN 206 and RN203.2, The Binding Site Ltd., Birmingham, UK). In wells in the agarose layer, 5 µL of the samples or standards containing different Igs were applied. After 96 h at 20°C the diameter of the precipitation ring around each well was measured and the concentration

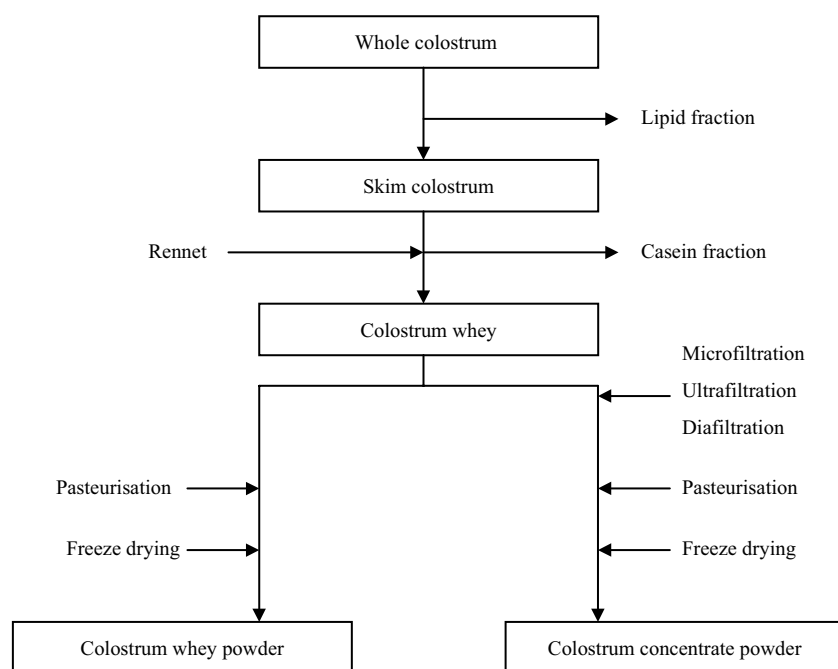


Fig. 1. Processing scheme of colostrum concentrate and colostrum whey powder. The filtration steps involve microfiltration, ultrafiltration and diafiltration.

of the specific Ig present in the sample was calculated. The analyses were performed in duplicate.

2.3.3. TGF- β 2

TGF- β 2 was analysed in colostrum samples from pool B by ELISA after fat removal (Pakkanen, 1998). An ELISA for human TGF- β 2 was obtained from R&D Systems Europe Ltd. (Abingdon, UK). Before analysis, the samples (125 μ L) were activated to the immunoreactive form by adding 25 μ L 1 N HCl. After 10 min of incubation the samples were neutralised by adding 25 μ L 1.2 N NaOH/0.5 M HEPES buffer. The samples were diluted up to 1:500 before analysis. All colostrum samples were analysed in duplicate.

2.3.4. IGF-1 and GH

IGF-1 and GH were analysed in colostrum samples from pool B by the radioimmunoassay (RIA) technique (Medilab, Stockholm, Sweden) after fat removal. The samples (100 μ L) were treated with ethanol (900 μ L 87.5%), centrifuged and the supernatant was analysed. The reagents for analysis of human plasma and serum were obtained from Nichols Institute Diagnostics, San Juan Capistrano, CA, (No. 40-2100) and were used for the quantitative determination of IGF-1 and GH in bovine colostrum. All colostrum samples were analysed in duplicate.

2.4. Statistical methods

Pearson correlations and statistical significances according to Bonferroni were calculated using Systat 5.02 for Windows (SYSTAT Inc., Evanstone, IL).

3. Results and discussion

3.1. Composition of colostrum

3.1.1. Total protein, total fat, lactose, urea and fatty acid composition

The concentration of total protein, total fat, lactose and urea in all colostrum samples (pool B), collected during the first 80 h after calving, is presented in Table 1. The protein content decreased with time, while the concentration of lactose increased. No effect on the fat content was observed. These observations of the general composition of colostrum are in line with previous reports (Foley & Otterby, 1978; Walstra, Geurts, Noomen, Jellema, & van Boekel, 1999). In our study the urea level increased slightly, while Foley and Otterby (1978) reported a decrease in non-protein nitrogen content. The mean value of the freezing point of the first milkings (0–6 h postpartum) was -0.47°C and decreased gradually with time to that of normal milk (-0.53°C). The pH of the pooled colostrum was 6.3–6.4,

Table 1

Mean values (standard deviation) of total fat, total protein, lactose and urea in pooled colostrum samples (pool B) at various time intervals postpartum

Time interval postpartum (h)	Fat (%)	Protein (%)	Lactose (%)	Urea (mM)
0–6	4.6 (1.7)	12.4 (2.3)	3.0 (0.6)	8.8 (4.8)
7–10	3.4 (1.1)	11.0 (1.8)	2.9 (0.5)	11.3 (5.0)
11–20	3.7 (2.0)	8.6 (2.4)	3.5 (0.7)	11.6 (5.0)
21–30	4.3 (2.4)	6.9 (1.0)	3.2 (1.0)	13.3 (4.4)
31–40	3.9 (1.4)	5.6 (1.3)	3.5 (0.8)	12.9 (4.6)
41–50	4.9 (2.9)	5.2 (1.6)	3.5 (0.6)	13.0 (4.7)
51–80	4.3 (2.3)	5.1 (1.5)	3.8 (0.7)	14.1 (3.6)

which was in agreement with Foley and Otterby (1978) and this is lower than that in normal milk (pH 6.7).

During the first two days after parturition the fatty acid composition of colostrum fat (pool A) altered gradually. The proportion of saturated and polyunsaturated fatty acids decreased, while the content of mono-unsaturated acids increased with time postpartum. The fatty acid composition of colostrum fat was compared with that of mature milk fat (Lindmark Månsson, 1999). The proportions of saturated short-chain fatty acids (C_4 – C_{12}) and stearic acid (C_{18}) were considerably higher in mature milk fat, but the content of palmitic fatty acid (C_{16}) was lower. The amount of *trans* fatty acids was lower in colostrum fat. Our findings are in agreement with previously published studies, in which the concentration of unsaturated fatty acids tended to be lower and the concentration of saturated fatty acids tended to be higher in mature milk than in colostrum (Benheng & Chengxiang, 1996; Bitman & Wood, 1990; Parodi, 1974). The lower amount of *trans* fatty acids and the higher amount of unsaturated fatty acids compared to mature milk may be explained by an alteration of the microflora in the rumen of the cow in close connection with calving, for instance, due to a changed feeding regime and mobilisation of body fat.

3.1.2. Immunoglobulins

The concentration of all Ig subclasses decreased with the number of milkings, but at different rates over time (Table 2). The IgA concentration was highest (1.6 mg mL^{-1}) during the first 10 h postpartum and decreased by 50% during the next 10 h. The concentration of IgG1 was at almost the same level during the first 10 h postpartum and then diminished to 20% after 2 days. IgG2 diminished by 30% during the first 10 h, but remained at that level for the next 10 h. IgM concentration was reduced by 50% 11–20 h postpartum, by another 10% in the next 10 h and then reached a level of 17% 31–80 h after calving. In total, IgA, IgG1, IgG2

Table 2

Mean concentration (standard deviation) of IgA, IgG1, IgG2 and IgM in colostrum at various time intervals postpartum. The results are compared with the Ig content in mature milk^a

Time interval postpartum (h)	IgG1 (mg mL ⁻¹)	IgG2 (mg mL ⁻¹)	IgA (mg mL ⁻¹)	IgM (mg mL ⁻¹)
0–6	90 (7.1)	2.8 (0.85)	1.6 (0.12)	4.5 (0.20)
7–10	79 (28)	1.9 (0.08)	1.7 (0.01)	4.0 (0.50)
11–20	65 (12)	1.8 (0.50)	0.9 (0.06)	2.3 (0.08)
21–30	24 (1.2)	1.1 (0.14)	0.7 (0.02)	1.8 (0.01)
31–40	31 (4.2)	0.5 (0.03)	0.3 (0.02)	1.0 (0.01)
41–50	17 (0.9)	0.4 (0.03)	0.2 (0.01)	0.8 (0.12)
51–80	12 (0.2)	0.2 (0.01)	0.1 (0.01)	0.7 (0.08)
Mature milk	0.51 (0.2)	0.03 (0.02)	0.02 (0.02)	0.10 (0.07)

^aLindmark-Månsson et al. (2000).

and IgM declined by 92%, 87%, 93% and 84%, respectively, from the first to the last time of the sampling. Thus, the concentration of individual Ig classes in colostrum decreased at every consecutive milking for individual cows, but at different rates over the time interval. The large variations in Ig concentrations among cows observed at the same lactation stage are due to different factors, such as lactation number and breed (Muller & Ellinger, 1981; Pritchett et al., 1991; Vacher & Blum, 1993). The IgA concentration in bovine colostrum reported by Stott, Fleenor, and Kleese (1981) and Benheng and Chengxiang (1996), were almost 10-fold higher compared to our results. By comparison, the content of total IgG was somewhat higher in our study, while the content of IgM was in the same range (Stott et al., 1981). Also, in other investigations of the amount of Igs large variations have been reported (Muller & Ellinger, 1981; Quigley et al., 1994). This can be explained by differences in analytical techniques, or in variation between individuals and breed. Compared to mature milk (Lindmark-Månsson et al., 2000), the concentration of different Ig classes in colostrum milk after 80 h postpartum was 24, 7, 5 and 7 times higher for IgG1, IgG2, IgA and IgM, respectively (Table 2). This shows that the concentrations of the individual Igs decline at different rates over the time it takes to reach the concentration in mature milk.

Analyses of colostrum samples from individual cows showed considerable variations in the concentrations of Igs, with minimal and maximal values of IgG1 in the first milking of 48 and 120 mg mL⁻¹, in the second milking of 26 and 42 mg mL⁻¹, and in the third milking of 11 and 40 mg mL⁻¹. The oldest cow, with lactation number 5, had the highest concentration of IgG1, namely, 120 mg mL⁻¹, but the lowest concentration of IgG2, 1.5 mg mL⁻¹, and IgA, 1.4 mg mL⁻¹. This is in accordance with the findings of Muller et al. (1981) and Stott et al. (1981).

Table 3

Mean concentration (standard deviation) of TGF- β 2, IGF-I and GH in colostrum at various time intervals postpartum

Time interval postpartum (h)	TGF- β 2 (ng mL ⁻¹)	IGF-1 (ng mL ⁻¹)	GH (ng mL ⁻¹)
0–6	289 (10)	870; 590 ^a	0.17; 0.04 ^a
7–10	310 (1)	330 ^a	<0.03 ^a
11–20	154 (5)	490	0.05
21–30	113 (35)	290 ^a	0.04 ^a
31–40	72 (15)	140	<0.03
41–50	85 (0)	110 ^a	<0.03 ^a
51–80	66 (13)	150	<0.03

^aAnalysed 2 months later after storage at -20°C .

3.1.3. TGF- β 2, IGF-1 and growth hormone

TGF- β 2 analysis in the pooled colostrum samples (pool B) using the ELISA technique demonstrated that TGF- β 2 was at a similar level (300 ng mL⁻¹) during the first 10 h after parturition (Table 3). Its concentration was reduced by 50% during the next 10 h, by a further 14% during 21–30 h and then remained at the same level 30–80 h after parturition. In the present study an ELISA kit for the analysis of human TGF- β 2 was used, which has been validated for bovine TGF- β 2 analysis (Pakkanen, 1998). Our results are in line with those reported by Pakkanen (1998), who found TGF- β 2 concentrations of the first and the fifth milking of individual cows to be 150–1150 and 12–71 ng mL⁻¹, respectively.

IGF-1 and GH were analysed in pool B using the RIA technique (Table 3). The results demonstrated that the IGF-1 concentration of pooled colostrum was at the highest level (870 ng mL⁻¹) in the colostrum samples collected during the first 6 h postpartum. Its concentration was reduced by 44% already after 11–20 h. It then remained at the same level (150 ng mL⁻¹), which is approximately 16% of the original concentration, in the time interval 31–80 h postpartum. The concentrations of IGF-1 reported by Ginjala and Pakkanen (1998) were 248–1850 and 27–101 ng mL⁻¹ for the first and the fifth milking of pooled colostrum, respectively, which is in agreement with our results. In the present study, also GH was at the highest level in the colostrum samples from the first 6 h postpartum, 0.17 ng mL⁻¹, and then decreased to below the detection level (<0.03 ng mL⁻¹) 31–80 h postpartum. To our knowledge, this decrease in concentration has not previously been reported. In milk, however, less than 1 ng mL⁻¹ GH has been found (Campana & Baumrucker, 1995).

The concentration of IGF-1 and GH was also analysed in colostrum samples after storage for 2 months at -20°C and a decrease in the content of IGF-1 by 32% and of GH by 76% was observed in the samples collected within the first 6 h (Table 3). These components seem to be less resistant to storage than Igs under these conditions (Lindmark Månsson, 2000).

3.1.4. Correlations between Ig subclasses, total protein, total fat and TGF- β 2

In the samples of pooled bovine colostrum, significant correlations between TGF- β 2 and IgA ($r = 0.98$, $p = 0.001$), IgG1 ($r = 0.93$, $p = 0.04$) and IgM ($r = 0.98$, $p = 0.002$), respectively, were found. The concentrations of growth factors TGF- β 2 and TGF- β 1 have earlier been shown to have a high correlation ($r = 0.97$) in colostrum samples (Ginjala & Pakkanen, 1998). In our study we measured the content of TGF- β 2, the major form in bovine milk and colostrum. Kim and Kagnoff (1990) reported that TGF- β 1 could interact with other lymphokines and selectively modulate the IgA response and increase IgA secretion 10-fold. Tokuyama and Tokuyama (1993) suggested that TGF- β 2 might be a positive regulator for IgA production in the mammary gland. The high correlations between

TGF- β 2 and IgA, IgG1 and IgM, respectively, found in the present study, may indicate that TGF- β 2 has a role as stimulator for the secretion of these Igs in the mammary gland. The correlation between total protein and total Igs of pooled colostrum was 0.95, between total protein and TGF- β 2 it was 0.94 and between total protein and IGF-1 it was 0.75.

3.2. Effects of processing

3.2.1. Preparation of colostrum whey

The impact of different processing steps on the native form of Igs and growth factors is shown in Fig. 1 and Table 4. The lipid fraction of the colostrum was removed and after this treatment the total Ig content was approximately 28% lower than in the original colostrum (40 mg mL^{-1}). The reduction of Igs in the

Table 4

Concentrations (standard deviation) of immunoglobulin subclasses, growth factors TGF- β 2 and IGF-1, and growth hormone (GH) after different processing treatments of colostrum

Colostrum fraction	GH (ng mL^{-1})	IGF-1 (ng mL^{-1})	TGF- β 2 (ng mL^{-1})	IgG1 (mg mL^{-1})	IgG2 (mg mL^{-1})	IgA (mg mL^{-1})	IgM (mg mL^{-1})	Ig total (mg mL^{-1})
Fat-free colostrum	<0.03	190	187 (8)	22.5 (1.1)	0.8	0.6	1.7	25.6
Colostrum whey (Yield based on fat-free colostrum)	<0.03	190	125 (8)	18 (0.2)	0.8	0.6	1.6	21.2
		100	67	80	100	100	94	83
Colostrum concentrate after filtration (Yield based on colostrum whey)	n.d.	n.d.	n.d.	57 (3)	n.d.	n.d.	n.d.	n.d.
				100				
Colostrum permeate after filtration	n.d.	n.d.	0	0.1	0.02	0	0	0.12
Colostrum concentrate after pasteurisation (Yield based on colostrum concentrate after filtration)	n.d.	n.d.	n.d.	47 (0.2)	1.6	1.1	0	49.7
				82				
Colostrum concentrate powder after freeze-drying (Yield based on colostrum whey)	0.3 ^a	5000 ^a	3248 ^a (30)	468 ^b (8)	16.0 ^b	10.8 ^b	0 ^b	495 ^b
	100^c	74	73	73	56	51	0	66
Colostrum whey after pasteurisation (Yield based on colostrum whey)	n.d.	n.d.	113 (5)	18 (0.1)	n.d.	n.d.	n.d.	n.d.
			90	100				
Colostrum whey powder after freeze-drying (Yield based on colostrum whey)	0.7 ^a	2300 ^a	1682 ^a (36)	186 ^b (12)	6.1 ^b	4.8 ^b	0.4 ^b	197 ^b
	100^c	97	100	83	61	65	2	75

^aThese entries have units ng g^{-1} .

^bThese entries have units mg g^{-1} .

^cApproximate value.

n.d. not determined.

Calculated yield (%), based on various colostrum fractions after the processing steps, is indicated by value in bold.

fat-free colostrum could be caused by flocculation of the fat globules by the immunoglobulins (Walstra et al., 1999). On the other hand, fat removal had a concentrating effect on IGF-1, which had a 50% higher concentration in fat-free colostrum than in original colostrum. This may be due to the interference of fat in the analytical method. In contrast, the concentration of TGF- β 2 and GH was not altered by fat removal.

The casein fraction was removed by adding rennet to the fat-free colostrum resulting in approximately the same concentration of IgG2, IgA, IgM and IGF-1 in the colostrum whey fraction, while IgG1 and TGF- β 2 were reduced (by 20% and 33%, respectively). A lower reduction has been reported in acid whey of colostrum in which 94% of the TGF- β 2 remained (Pakkanen, 1998). The overall loss of Igs and growth factors could be an effect of interaction with fat globules and the high concentration of Igs in colostrum entrapped in the casein matrix or cleaved by proteolytic enzymes. Colostrum whey was further freeze-dried directly after heat treatment (colostrum whey powder) or separated by filtration techniques, followed by heat treatment prior to freeze-drying (colostrum concentrate powder).

3.2.2. Filtration and pasteurisation of colostrum whey

Colostrum whey was treated by microfiltration, ultrafiltration and diafiltration, followed by heat treatment (60°C, 45 min) and the concentration of Igs in the retentate and permeate was analysed (Table 4). The reduction of IgG2 and IgA in the colostrum concentrate after these filtration steps and pasteurisation (calculated on the concentration and the total volume of the colostrum fraction) was approximately 30%, while the amount of native IgG1 was not effected. In contrast to other Ig subclasses, IgM could not be detected after the filtration process in the concentrate. In the permeate, only traces of IgG1 and IgG2 could be detected. The loss of IgM in the colostrum concentrate after heat treatment might be caused by the filtration procedures, heat treatment or a combination of these processes. Mechanical treatment can effect the protein conformation and activity. It has been reported that high pressure (10 MPa) can inactivate IgM (Walstra et al., 1999), but the pressure used in the filtration steps in our study was much lower (0.1–1 MPa). After filtration the IGF-1 and TGF- β 2 would be expected to be found in the permeate depending on their molecular weight and conformation. However, no detectable amount of native TGF- β 2 was observed in this fraction. A possible explanation is that these peptides are bound to carrier proteins with the result that they have a higher concentration in the retentate. An experiment to verify these results was performed, in which the effect of homogenisation and pasteurisation was studied in whole colostrum. The homogenisation was simulated at 60°C for 1 min, followed by heat treatment at 60°C for 30 min. The

pentameric molecule IgM and GH could not be detected after these treatments, while the concentrations of IgG1, IgG2, IgA and TGF- β were not effected. Furthermore, the IGF-1 concentration decreased by 33%. This indicates that different mechanical and thermal treatments have an effect on these components.

Colostrum whey was also heated (60°C, 45 min) without the filtration steps and this heating procedure did not alter the concentration of IgG1 and TGF- β 2 (Table 4). As a comparison, whole colostrum was heat treated (60°C, 30 min) and likewise no effect on the concentration of the Igs was observed. These experiments show that Igs in whole colostrum and colostrum whey were more resistant to heat treatment than in colostrum concentrate after removal of fat, casein, salts and lactose. These components may protect the immunoglobulins during heat treatment and filtration.

Differential scanning calorimetry (DSC) measurements of colostrum concentrate showed a higher thermal unfolding temperature (80.9°C) compared with other whey proteins, e.g. β -lactoglobulin (77°C), α -lactalbumin (67°C), lactoferrin (65–93°C depending on the iron content) and bovine serum albumin (64°C) (Lindström et al., 1994; Paulsson & Dejmeck, 1990; Paulsson, Svensson, Kishore, & Naidu, 1993). Among the individual Ig classes, IgG1 unfolded at 79.4°C, IgG2 at 76.7°C and IgM at 80.3°C (Lindström et al., 1994). They also found that the unfolding of the individual Igs was irreversible and almost independent of pH. The combination of temperature and time used in processing can affect the structure of the proteins and involve unfolding and aggregation. Heat sensitivity can also be measured by other techniques, such as HPLC and electrophoresis. The heat treatment used in this study was 60°C for 45 min, which is below the unfolding temperature of Igs. However, the long heating time could cause aggregation. Further, the filtration used in our study might also affect the conformation, making the proteins more susceptible to the following heat treatment. This supports the finding that the yield of IgG1 after pasteurisation of colostrum whey was 100%, while the yield after filtration and pasteurisation of colostrum concentrate was 82% (Table 4). No DSC data have been published on TGF- β 2 or IGF-1 and, therefore, the unfolding temperature of these milk components could not be compared with those of immunoglobulins. Heat treatment at 56°C for 30 min had no effect on the amount of native IGF-1 in human milk as reported by Donovan, Hintz, and Rosenfeld (1991). In our study the yield of TGF- β 2 after pasteurisation of colostrum whey was 90% (Table 4).

3.2.3. Freeze-drying

Colostrum concentrate and colostrum whey were freeze-dried after pasteurisation (Fig. 1). In the freeze-dried colostrum concentrate the amount of native IgG1,

IgG2 and IgA decreased by 25% compared to colostrum whey. In freeze-dried colostrum whey the decrease in IgG2 and IgA was 35–40%, while IgG1 was affected to a less extent (17%). The freeze-drying step in combination with pasteurisation had a more pronounced reducing effect on IgM with only 2% remaining after this treatment of colostrum whey. A similar result was obtained when filtration steps were included in the process. Thus, IgM seems to be the most sensitive of the Igs, resulting in a loss during processing.

Freeze-drying also reduced the amount of native TGF- β 2 and IGF-1 in colostrum concentrate by 30%, and a minor reduction could also be observed in the freeze-dried colostrum whey. Our results imply that salts and lactose play a protective role both for Igs and TGF- β 2 in colostrum whey during freeze-drying. The original concentration of GH in fat-free colostrum was below the detection limit of the assay, $<0.03 \text{ ng mL}^{-1}$, but the GH concentration in the freeze-dried colostrum concentrate powder and colostrum whey powder were 0.3 and 0.7 ng g^{-1} , respectively. These results indicate that the filtration procedures reduced the content of GH.

Overall, the yield after heat treatment and freeze-drying of colostrum whey resulting in a colostrum whey powder was approximately 100% for IGF-1 and TGF- β 2, while the total yield of Igs was 75% (Table 4). However, the yield of IgM was only 2%. The yield in colostrum concentrate powder after various filtration steps was approximately 74% for IGF-1 and TGF- β 2 and the total yield of Igs was 66%. The recovery of IgG1 was 73%, while IgM could not be detected after these processing steps.

4. Conclusions

The content of bioactive substances in colostrum and colostrum fractions after filtration procedures, heat treatment and freeze-drying decreased gradually after removal of fat, casein, lactose and salts. These compounds seem to have a protective function on Igs, growth factors and GH during processing. The greatest influence of the processing steps was found on the content of IgM. This implies that it is important to optimise processes to retain the activity of the bioactive components in milk.

Acknowledgements

We would like to thank Gun Aldén, Swedish Dairy Association, Lund, Sweden, for analytical advice and Håkan Andersson and Benny Wraae, Kemikalia, Lund, Sweden, for their contributions to the processing part of this work.

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