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Estimation of Particle Size Distribution in Bovine Colostrum Whey by Dynamic Light Scattering (DLS) Method

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Abstract. Colostrum whey consist bioactive compounds in considerable concentration. For isolation of these compounds the particle size has crucial importance. The aim of this work was to study possibilities of using dynamic light scattering method – DLS (Malvern Zetasizer Nano ZS) for colostrum whey particle size distribution estimation. The first and second milking colostrum samples were skimmed by centrifugal separation and casein of these was enzymatically coagulated by rennet (chymosin). Obtained whey was diluted (1:200) by distilled water and filtered (cut-off 0.45 μm) to get probes for estimation of particle size. Particle size distribution in colostrum whey probes had maximally three peaks and polydispersity indices from 0.157 to 0.541. Prevailing peak of the distribution was found at size from 144 to 210 nm, which apparently corresponds to hydrodynamic diameter of immunoglobulin IgG1.

Key words: Milk, Colostrum, Whey, Particle size distribution, immunoglobulin.

INTRODUCTION

Colostrum is a complex fluid rich in nutrients and is also characterised by its high level of bioactive (e.g. antimicrobial) components, like immunoglobulins (Ig), especially IgG1, growth factors, especially insulin-like growth factor-1 (IGF-1), transforming growth factor beta-2 (TGF-b2) and growth hormone (GH) as well as lactoferrin, lysozyme and lactoperoxidase (table 1). Colostrum whey contains a significant amount of those bioactive compounds (Pakkanen & Aalto, 1997; Elfstrand et al., 2002; Marnila & Korhonen, 2002; Kehoe et al., 2007), which can be used in livestock husbandry, in food and feed supplements, in medical products, etc. Concentration of proteins and/or bioactive components in bovine colostrum and milk are shown in Table 1.

The pharmaceutical and biotechnological industries have recently shown interest in bovine colostrum as a source of growth factors and other specific bioactive components. Also, a multitude of health products and foods made from various colostrum fractions have been launched on the market. Fractionation of colostrum components (immunoglobulins, lactoferrin, glycomacropeptides, etc.) from colostral whey may result in profitable returns and hence, more research into effects of fractions or individual components compared to whole colostrum is being undertaken. (Tripathi & Vashishtha, 2006)

Table 1. Concentration of proteins and/or bioactive components in bovine colostrum and milk. Data adapted from: Pakkanen & Aalto 1997; Marnila & Korhonen, 2002; Elfstrand et al., 2002; Kehoe et al., 2007

Protein and Growth Factors	Colostrum	Milk
Casein	26 g l^{-1}	29 g l^{-1}
α-lactalbumin	2 g l^{-1}	1.4 g l^{-1}
β-lactoglobulin	8 g l^{-1}	3.3 g l^{-1}
IgG1	48 k–87 g l^{-1}	0.7 g l^{-1}
IgG2	1.6–2.9 g l^{-1}	0.05 g l^{-1}
IgM	3.7–6.1 g l^{-1}	0.05 g l^{-1}
IgA	3.2–6.2 g l^{-1}	0.1 g l^{-1}
Serum Albumin	1.2 g l^{-1}	0.4 g l^{-1}
Lactoferrin	1.0–2.0 g l^{-1}	0.1 g l^{-1}
Lactoperoxydase	30 mg l^{-1}	20 mg l^{-1}
TGF-b2	20–40 mg l^{-1}	1–2 μg l^{-1}
IGF-1	0.1–2 mg l^{-1}	25 μg l^{-1}
Lysozyme	0.1–0.7 mg l^{-1}	0.1–0.3 mg l^{-1}

Values are depending on postpartum timeline and cow´s individuality. Data represent range.

To extract bioactive compounds from colostrum membrane filtration may be used (Elfstrand et al., 2002; Venkiteshwaran et al., 2008). Based on the particle size distribution it is possible to select optimum separation technologies and also evaluate the protein composition of colostrum whey. Although there are some references concerning skimmed milk (Beliciu & Moraru, 2009) and whey (Giroux et al., 2009) particle size distribution (PSD) by dynamic light scattering (DLS) method, there is no such data about PSD in bovine colostrum available. An important factor in use of separation technologies and of DLS is the knowledge about possible polydispersity which is caused by considerable differences in particle size. In application of DLS method for investigation of particle size it is essential to prevent:

1. the presence of large particles in the sample
2. excessively high concentration of nanoparticles in the sample
3. denaturation or aggregation of particles due to pH or some other co-factor as temperature, concentration etc. (Dalgleish & Hallett, 1995; Alexander & Dalgleish, 2006; Beliciu & Moraru, 2009).

The aim of current research was to evaluate the suitability of DLS method for the estimation of particle size distribution and its polydispersity in colostrum whey.

MATERIALS AND METHODS

First and second milking colostrum (1.5 l) was collected and frozen at the EULS Märja experimental cowshed. Five first and seven second milking samples of colostrum were under investigation. To ensure efficient separation of fat frozen colostrum was warmed up to separation temperature 55°C in water bath. Fat separation process was conducted by separator Armfield FT15 (10,000 rpm). Casein was precipitated at 35°C using rennet (Formatase 2200 TL) which is derived from a fermentation process of the fungus *Rhizomucor miehei* and does not affect whey proteins. In order to secure large particles (casein-dust, fat, etc.) free and transparent solution, colostrum whey was diluted (1 : 200) and filtered by syringe filter (cut-off

0.45 μm). Since whey proteins are more stable close to neutral pH, distilled water (pH 6.8–7.2) was used as diluting solution for reduction of particles concentration in current research. Isoelectric points of whey proteins are presented in Table 2 (Pouliot & Gauthier, 2006). Dilution of whey was carried out before filtering to prevent clogging of the filter. The effect of pH and dilution environment on colostrum whey particle size distribution was not studied because this needs a detailed investigation.

Table 2. Isoelectric point of whey proteins and growth factors present in milk (Pouliot & Gauthier, 2006)

Milk protein	IgG1	IgG2	BSA	βLg	αLa	GF	LF	LP
Isoelectrical point, pH	6.5	8	4.7	5	4.7	4.7–9.5	9	10

BSA – serumalbumin, βLg – β-lactoglobulin, αLa – α-lactalbumin, GF – Growth factors, LF – Lactoferrin, LP – Lactoperoxydase.

For estimation of colostrum whey particle size distribution Malvern Zetasizer Nano-ZS analyser which is based on dynamic light scattering (DLS) method was used. This device measures the time dependent fluctuations in the scattering intensity of light to determine the translational diffusion coefficient, and subsequently the hydrodynamic diameter by the Stokes-Einstein equation. Each measurement consisted of 3 subsequent individual runs of which the average result was calculated. Measurements were conducted at 22.0ºC and the light scattering was detected at 173 degrees. The detection range of device is from 0.1 nm to 10 μm. The data obtained by the Malvern Zetasizer Nano-ZS analyser was exported into Microsoft Excel for further analyses.

RESULTS AND DISCUSSION

The particles size distribution (PSD) of diluted and filtered colostrum whey (DFCW) was found to be in wide range and can therefore be described as multimodal dispersion (indicates polydispersity). Polydispersity in PSD is reflected clearly by existence of three peaks (Fig. 1).

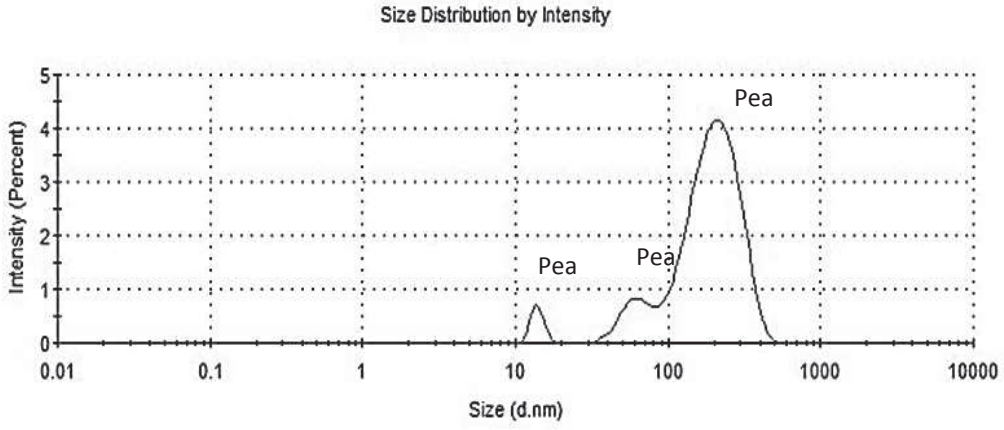

Figure 1. Example of particles size distribution (PSD) graph with three peaks of diluted and filtered first milking colostrum whey (cow No. 550).

PSD is usually described by overall average or cumulant average (z-average) of particle diameter (size). In case of polydisperse solutions interpretation of PSD results using z-average might be insufficient. To describe the polydispersity of the PSD in DFCW z-average, mean intensity size and area intensity percentage of each peak and polydispersity index (PDI) were calculated (Table 3).

Table 3. Results of particle size distribution in colostrum whey estimated by DSL method. (PDI – polydispersity index, MI – mean intensity which corresponds to mode of the peak, AI – area intensity which corresponds to partial area under the peak of the total distribution)

Cow No/ milking	z-average, nm	PDI	Peak 1 MI, nm	Peak 2 MI, nm	Peak 3 MI, nm	Peak 1 AI, %	Peak 1 AI, %	Peak 1 AI, %
528/ I	94.5	0.541	177.7	45.1	11.6	80.1	9.1	7.5
624/ I	106.5	0.533	209.6	51.8	12.6	80.3	13.5	6.3
550/ I	122.8	0.458	211.0	61.1	14.0	96.1	3.9	0.0
70/ I	132.2	0.226	167.7	30.9	0.0	99.2	0.8	0.0
439/ I	113.5	0.310	173.9	39.8	16.7	92.8	3.7	3.5
Average of 1st milking	113.9	0.414	188.0	45.7	11.0	89.7	6.20	3.46
St.dev.	13.0	0.13	18.5	10.3	5.75	8.0	4.53	3.11
550/ II	148.3	0.290	200.5	63.4	0.0	97.5	2.5	0.0
92/ II	132.3	0.192	155.6	0.0	0.0	100.0	0.0	0.0
44/ II	105.1	0.311	144.1	13.7	0.0	96.1	3,9	0.0
60/ II	185.5	0.157	199.8	0.0	0.0	100.0	0.0	0.0
17/ II	129.7	0.239	168.9	0.0	0.0	100.0	0.0	0.0
70/ II	138.0	0.271	184.1	50.48	0.0	97.4	2.6	0.0
76/ II	135.0	0.328	196.9	43.86	0.0	90.1	9.9	0.0
Average of 2nd milking	139.1	0.255	178.6	24.50	0.0	97.3	2.7	0.0
St.dev.	22.51	0.06	21.07	25.29	0.0	3.28	3.28	0.0
Average of 1st and 2nd milking	126.5	0.335	183.3	35.1	5.50	93.5	4.5	1.7
St.dev.	22.82	0.12	20.57	22.95	6.56	6.86	4.22	2.63

PSD of first milking probes had 2–3 peaks with average mean intensity sizes 188.0, 45.7 and 11.0 nm for peaks 1, 2 and 3 (may also be absent) respectively. Mean intensity and area intensity varied between 173.9–209.6 nm and 80.1–99.2% for peaks 1 and between 30.9–61.1 nm and 0.8–13.5% for peak 2. PSD of second milking colostrum probes had 1–2 peaks with average mean intensity sizes of 178.6 nm for peak 1 and 24.5 nm for peak 2. Mean intensity and area intensity of the dominating peak 1 varied between 144.1–200.5 nm and 90.1–100.0% respectively.

Polydispersity index (PDI) indicates how homogenous the probes appeared to be, at least from a light scattering perspective. PDI larger than 0.2 indicates that the simple cumulant fitting is not a complete representation and that more than a single species are present (Nobbmann, 2007). It is acknowledged that polydispersity affects the DLS measurement results. Although it is also clear that the mean intensity results of the dominating peak are less affected and the role of smallest particles to it may be neglected. The second milking colostrum probes showed clearly more homogenous PSD results having only two peaks maximally and lower mean PDI (0.255) compared

to first milking colostrum probes (mean PDI = 0.414). This can be explained by rapid postpartum changes in colostrum composition (Elfstrand et al., 2002). According to published investigations about the content of colostrum and normal milk protein compounds (Table 1), and the fact that casein and fat fractions were removed from probes, it can be assumed that dominating peak of PSD in our experiments represented major colloidal compound of colostrum whey – immunoglobulin IgG1.

Because of wide variance in polydispesity index values (PDI = 0.157–0.541) and considerably large mean size of dominating particles certain aggregation of those in DFCW probes can be assumed also. There are several studies about the influence of various factors including storage conditions and processing variables such as heat, pH and pressure on stability of bovine IgG (Elfstrand et al., 2002; Godden et al., 2006; Mcmartin et al., 2006; Indyk et al., 2007). Still, from the point of view of PSD estimation by DLS-method the question about possible aggregation of IgG remains to be answered.

Further on more profound investigations about sample treatment impact (dilution media, pH, heat treatment, refrigerated preservation, etc.) towards PSD of bovine colostrum whey proteins are required. The certain aggregation degree of whey proteins (especially IgG1) by regulation of temperature and pH could even enhance efficiency of filtration or other extraction technology (bigger particles ensure more effective separation). However, it is essential to preserve the bioactivity of protein during this kind of treatment.

CONCLUSIONS

This study showed that DLS method is applicable for evaluation of particle size distribution and polydispersity of the colostrum whey proteins. Based on the cited knowledge about content of colostrum protein compounds and the fact that casein and fat fractions were removed, it can be assumed that highest mean intensity of PSD in our study represents major colloidal compound of colostrum whey – immunoglobulin IgG1. Further research about the effect of pH and dilution environment in the phase of sample preparation and possible aggregation of IgG on colostrum whey particle size estimation by DSL-method is needed.

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