Quality of colostrum as estimated by different methods

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Abstract

The main quality marker of colostrum is concentration of immunoglobulins, in the broad sense content of proteins. The standard method for estimation of immunoglobulin IgG1 is radial immunodiffusion (RID), but this method is lengthy and expensive. Thirty samples of spray dried or lyophilized cow colostrum were analysed by several methods, both rapid screening methods and methods that are more precise. Three chromatographic methods were tested: size exclusion chromatography (SEC) and two variants of affinity chromatography (AC) using column with Protein A and Protein G. Good results were obtained from SEC (R2=0.95), but column is very expensive and it has short lifetime. Similar results gave affinity chromatography on Protein G column, its advantage is short analysis time (10 min). Spectrophotometric methods (Bradford and UV spectroscopy) are not demanding for instrumentation, but the sample preparation is quite complex.

Keywords: colostrum, immunoglobulin, radial immunodiffusion, affinity chromatography, size exclusion chromatography, Bradford method.

Introduction

Colostrum is initial secretion produced in mammary glands of mammals following parturition. It is rich in immunoglobulins, lactoferrin, growth factors and many other biologically active substances. Quality of colostrum is important for newborns, which have immature immune system, moreover colostrum is also widely used as food supplement (Godhia and Patel, 2013). The main quality marker of colostrum is concentration of immunoglobulins, in the broad sense content of proteins. Gapper *et al.* (2007) did an overview of methods used for colostrum analysis. The standard method for estimation of immunoglobulin IgG1 is radial immunodiffusion (RID) and many authors used it for comparison with other methods, but this method is lengthy and expensive (Quigley *et al.*, 2013; Stojic *et al.*, 2017). The aim of this work was to prove some alternative methods suitable for estimation of quality of dried colostrum from point of view of IgG content.

Material and methods

Dried colostrum (spray dried or lyophilized) was provided by Ingredia Ltd. (Frydek – Mistek, Czech Republic). IgG from bovine serum (Sigma-Aldrich) was used as standard. Samples for analysis were prepared by dissolving of 1 % (w/v) of colostrum in phosphate buffer pH 8.0. Casein was removed by precipitation at pH 4.6. Immunoglobulins were precipitated by sodium sulphate (Skalka *et al.*, 2017). Radial immunodiffusion (RID)

was used as reference method (Bovine IgG-NL RID Kit RN200.3, Rockland Immunochemicals Inc., USA). Affinity chromatography (AC) was performed as described by Copestake *et al.* (2006) and Abernethy and Otter (2010) using columns HiTrap Protein G HP 1 ml (GE Healthcare, Sweden) and BabyBio (Protein A) 1 ml (Bio-Works, Sweden). Size exclusion chromatography (SEC) was performed on column Superdex Increase 10/300 GL (GE Healthcare, Sweden) using phosphate buffer 0.05 M containing 0.15 M NaCl (pH 7.2). AC and SEC columns were attached to Agilent 1260 Infinity Bio-inert system with DAD (280 nm). Proteins were also estimated according to Bradford method (Bradford, 1976) at 595 nm. Samples after AC were precipitated by acetone and separated on 12.5 % acrylamide gel by SDS-PAGE (Laemmli, 1970). Content of IgG in standard solution was estimated by UV measurement at 280 nm using extinction coefficient ($\varepsilon_{280\ 1\ cm;\ 1\ mg/mL}$) 1.4 (Copestake *et al.*, 2006).

Results and discussion

Immunoglobulins are main part of proteins in acid whey from colostrum (AWC), therefore correlation between RID and Bradford method was examined (Figure 1) and coefficient of determination R^2 was 0,72. AWC was also analysed by size exclusion chromatography (SEC). Proteins were well separated, high R^2 was obtained (0.95), but analysis takes 40 min and high backpressure of column is limiting factor (Figure 2).

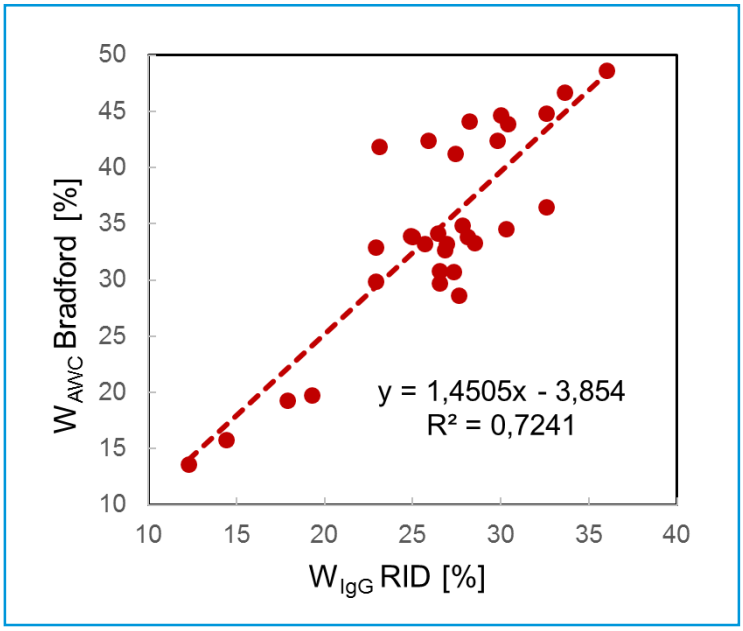

Figure 1. Correlation between RID and spectrophotometric estimation of proteins in acid whey from colostrum.

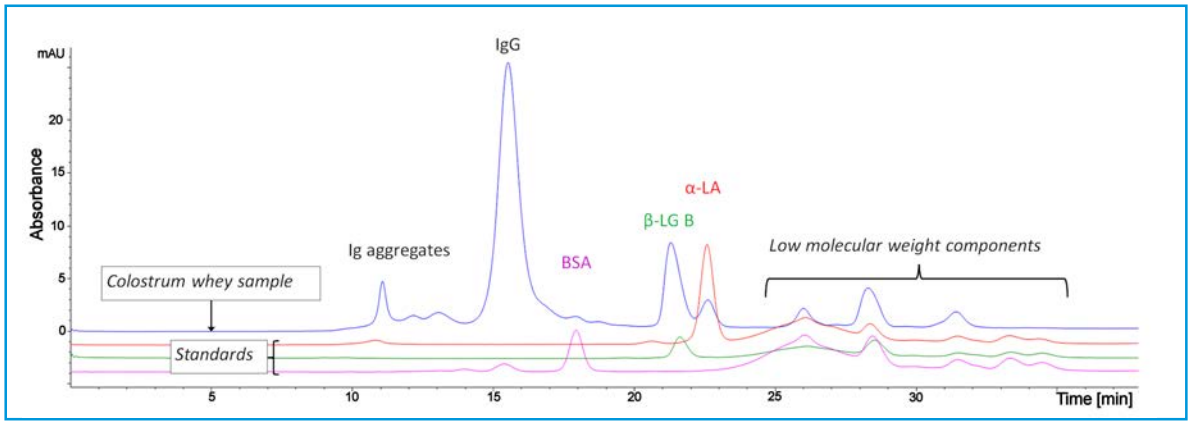

Figure. 2. Separation of acid whey from colostrum and selected standards of whey proteins by SEC chromatography.

Affinity chromatography (AC)

Columns with Protein A and Protein G were examined for AC method. Better response was obtained from Protein G column (Figure 3). Colostrum, acid whey and IgG fraction obtained by precipitation can be analysed by AC in 10 min. Collected peaks were further assessed by SDS-PAGE (Figure 4). IgG isolated by precipitation contained some other whey proteins, particularly β-LG. Peak from colostrum sample is slightly contaminated by casein and results of IgG are distorted. Coefficient of determination between results from analysis of acid whey by AC and RID was 0.88 (Figure 5).

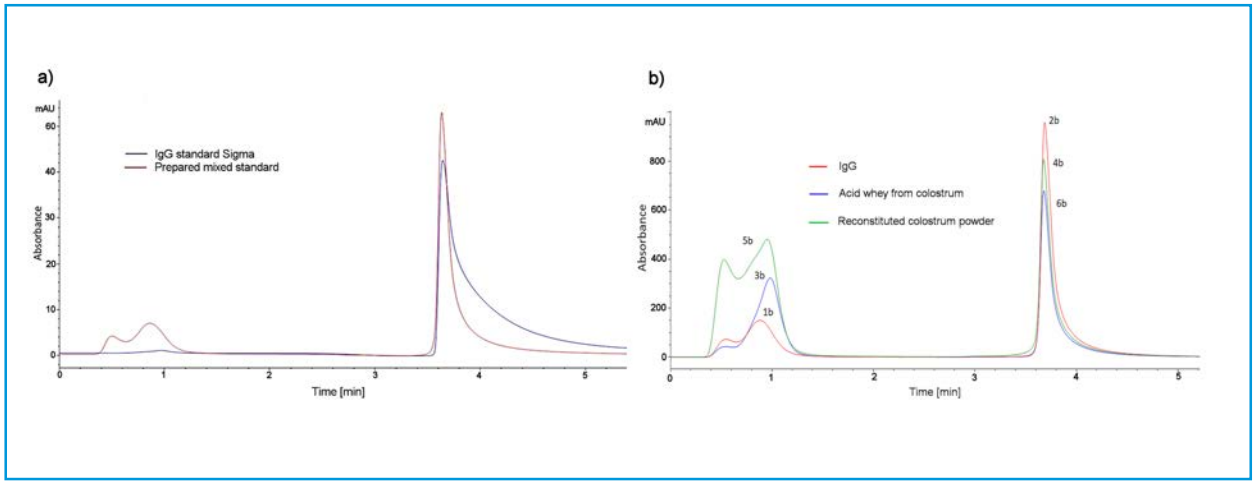

Figure 3. Affinity chromatography on Protein G column: a) chromatograms of standards (sigma and mixed standard obtained by sodium sulphate precipitation from mixture of colostrum samples); b) chromatograms of precipitated IgG (peaks 1 and 2), acid whey from colostrum (peaks 3 and 4) and colostrum sample (peaks 1 and 2).

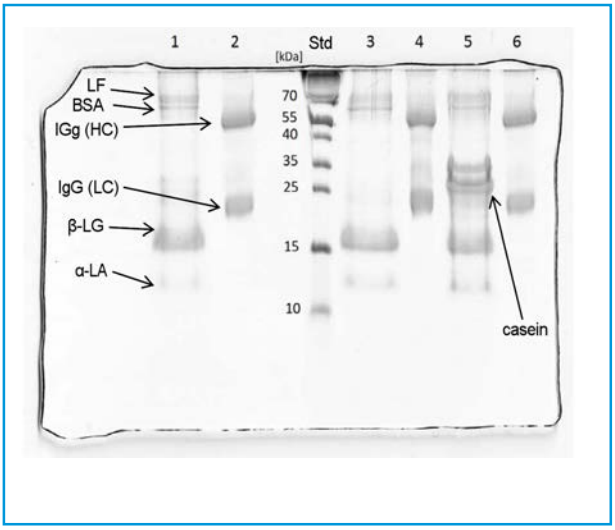

Figure 4. SDS-PAGE of samples from peaks collected from AC Protein G column.

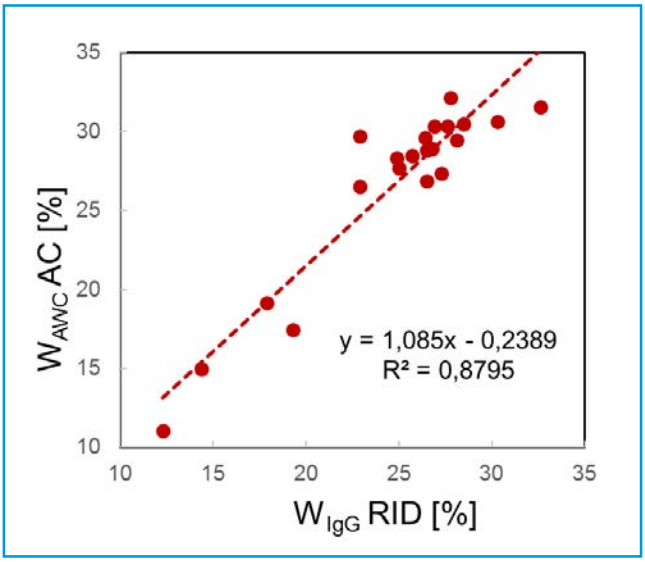

Figure 5. Correlation between RID and affinity chromatography of acid whey from colostrum (column HiTrap Protein G).

Conclusions

Estimation of proteins in acid whey from colostrum by Bradford method is simple and rapid technique for evaluation of colostrum quality. Size exclusion chromatography gave precise results, but the method is lengthy and expensive. Affinity chromatography of acid whey is a rapid method that correlates well to RID.

Acknowledgement

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