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ACCEPTED MANUSCRIPT

Isolation of biofunctional bovine immunoglobulin G from milk- and colostral wheywith mixed-mode chromatography at lab and pilot scale

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Highlights

- Serial connection of two mixed mode resins leads to an average purity of 96.1% IgG
- Activity and nativity (>95%) of IgG are retained during isolation and drying
- 130-150 g pure immunoglobulin G are obtained from 3 liters colostral whey in 5 hours
- It becomes feasible to obtain pure, active and stable IgG in therapeutic amounts

Abstract

The aim of the present work was to develop a new scalable and cost-efficient process to isolate bovine immunoglobulin G from colostral whey with high purity and minimal loss of activity. The mixed mode material Mercapto-Ethyl-Pyridine-HypercelTM was identified appropriate for direct capture of immunoglobulin G. The binding mechanism is primarily based on hydrophobic interactions at physiological conditions. As compared to immunoglobulin G, all other low molecular whey proteins such as α -Lactalbumin or β -Lactoglobulin, except lactoperoxidase, are more hydrophilic and were therefore found in the flow-through fraction. In order to remove lactoperoxidase as an impurity the column was combined in series with a second mixed mode material (CaptoTM- with N-benzoyl-homocysteine as ligand) using the same binding conditions. At pH 7.5 the carboxyl group of this ligand is negatively charged and can hence bind the positively charged lactoperoxidase, whose isoelectric point is at pH 9.6. After sample application, the columns were eluted separately. By combining the two columns it

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