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A combination of microfiltration and high pressure treatment for the elimination of bacteria in bovine colostrum

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ABSTRACT

Bovine colostrum is a unique source for biomolecules and a valuable raw material for the production of nutraceuticals. The highly variable microbial quality of raw colostrum and its thermo-sensitive nature complicate the manufacture of safe and stable colostrum products for human use. A novel combined process comprising microfiltration (MF) and subsequent high pressure processing (HPP) was investigated. Skimmed colostrum contaminated with various bacteria species was subjected to cross-flow MF at pore sizes of 1.4 and 0.8 μm. The MF permeates contained a residual microbial burden that was reduced to undetectable levels by HPP at 400 and 500 MPa for 10 min. Native IgG was reduced to 36–73% (MF + 400 MPa HPP) and 19–30% (MF + 500 MPa HPP) of the original content. In direct application to contaminated colostrum, HPP was shown to be less detrimental to IgG than heat treatment at conditions giving similar microbial reduction efficacy.

1. Introduction

Colostrum, a secretion similar to milk, is produced in the mammary glands of mammals before and shortly after parturition. In contrast to mature milk, colostrum contains bioactive constituents, such as immunoglobulins, growth factors and antimicrobial factors, in high concentrations (Gauthier, Pouliot, & Maubois, 2006; Pakkanen & Aalto, 1997). Since it provides the first portion of nutrients and passive immunity, it is essential to the newborn.

Bovine colostrum and colostrum from other ruminant species are exceptionally rich in immunoglobulin G (IgG). Surplus colostrum, that is not needed to feed the newborn calf, is a unique source for valuable biomolecules and a raw material for the production of nutraceuticals and food or feed additives. As Uruakpa, Ismond, and Akobundu (2002) showed in their review, many potential health benefits are attributed to colostrum and colostrum products. However, the microbiological quality of raw bovine colostrum may vary considerably. Contamination mostly takes place during milking and storage (Stewart et al., 2005). Reported aerobic mesophilic plate counts often exceed 10^6 colony forming units (cfu) mL^{-1} and the occurrence of potentially pathogenic bacteria is not uncommon (Houser, Donaldson, Kehoe, Heinrichs, & Jayarao, 2008). This fact is of special concern when processing bovine colostrum for human consumption. As one recent study reports, potentially harmful *Bacillus* species can be found in nutraceutical colostrum powders sold in the United States (Hayes, Hughes, & Greene, 2012).

Regarding valuable compounds of bovine colostrum, it is well known that mild heat treatment partially inactivates sensitive proteins. Treatment temperatures above 60 °C are also reported to increase viscosity (Elizondo-Salazar, Jayarao, & Heinrichs, 2010; McMartin et al., 2006).

Microfiltration (MF) is one of the few methods that allows a considerable reduction of the microbial content without affecting the native state of proteins. Ceramic membranes with a pore size of 1.4 μm are usually used in a cross-flow setup to reduce the microbial content of skim milk while leaving the protein composition nearly unchanged. Decimal reduction levels above 3.5 log cycles are reported (Fritsch & Moraru, 2008; Saboyainsta & Maubois, 2000; Walkling-Ribeiro, Rodríguez-González, Jayaram, & Griffiths, 2011). MF membranes with smaller pore sizes (0.1–0.2 μm) are used for concentration of micellar casein from skim milk. The resulting permeate is free from particles and virtually sterile. Despite all efforts of optimisation, a dense fouling layer is usually formed during casein concentration by cross-flow MF. This layer limits the flux performance and the yield of serum proteins in the permeate (Le Berre & Daufin, 1998).

Some results from the application of MF to colostrum whey have been published: Elfstrand, Lindmark-Månsson, Paulsson, Nyberg, and Åkesson, 2002; Ulber et al., 2001; Wu and Xu, 2009. Only one study, however, demonstrates how MF can be directly applied to skimmed colostrum (Piot, Fauquant, Madec, & Maubois, 2004). With the exception of one patent (Mortensen, 2003), which claims

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to provide a 3-log microbial reduction by 1.4-μm MF of defatted bovine colostrum, no information on the feasibility of an MF process, in which microbes are separated but casein remains in the product, could be found.

Although the most valuable colostrum constituents can be found in the serum fraction, avoiding casein separation may prove beneficial. Glycomacropeptide (GMP) associated to bovine colostrum casein is reported to show similar glycosylation to GMP in human milk (Brody, 2000). Moreover, avoiding casein retention would also result in higher serum protein yields and better flux performance by circumventing the limitations caused by casein-fouling. However, considering the high levels of contamination and the possible presence of pathogens, MF at pore sizes around 1.0 μm is probably not sufficient to obtain a safe and stable product as indicated by food legislative regulations for stand-alone MF processing. Therefore, further treatment of the permeate may be necessary.

High pressure processing (HPP) has become a useful process for food preservation in cases where thermal treatment may lead to undesirable alterations of the product. High hydrostatic pressure (100–1000 MPa) is applied to reduce or eliminate viable microorganisms. The susceptibility of common bacterial pathogens and food contaminants to high pressure is well studied (Alpas & Bozoglu, 2000; Alpas et al., 1999; Xu, Hyeon-Yong, & Ahn, 2009). Depending on pressure, temperature and holding time, HPP can also cause irreversible protein denaturation (López-Fandiño, 2006).

A combination of MF and HPP could provide a safe and stable colostrum product by avoiding temperatures above 40 °C. Aside from the separation of fat and a slight loss of casein during MF, the composition of a product manufactured in this way would be very similar to the natural raw material. To the best knowledge of the authors there are no reports on the efficacy of a MF-HPP hurdle technology for any feedstock in current scientific literature.

The objective of this work was to investigate the potential of an MF-HPP combination in the treatment of highly contaminated skimmed colostrum. For that purpose, skimmed colostrum was artificially contaminated with non-pathogenic strains of *Listeria*, *Escherichia coli* and *Bacillus* sp. and then subjected to cross-flow MF at various pore sizes and HPP treatment under various pressures. Additionally, HPP was compared to heat treatment in direct application to contaminated skimmed colostrum. The reduction of total viability as well as the viability of added bacteria species and the loss of bovine IgG were monitored.

2. Material and methods

2.1. Raw material

Raw bovine colostrum in frozen blocks of about 2 kg was provided by OCS Colostrum Vitaplus GmbH, Wörgl, Austria. Colostrum blocks that showed obvious signs of contamination, such as bits of straw and soil, were deliberately included to create a highly contaminated feedstock. Since preliminary experiments showed that thawing, pooling end refreezing results in acidification and even precipitation of protein, raw colostrum was crushed and pooled in frozen state. Batches of 12 kg were then thawed at 30–35 °C and defatted on a cream separator (elecrem 1, Elecrem SA, Fresnes, France) at 35 °C for each run.

2.2. Inocula

For artificial contamination, 24 h cultures of *Listeria innocua* (ATCC 33090), *E. coli* (DSM 30083) and *Bacillus subtilis* (ATCC 6051) were added to skimmed colostrum before treatment by MF and direct heat or HPP treatment. Culture conditions were: brain heart infusion broth at 37 °C, nutrient broth at 37 °C and tryptic soy broth at 30 °C, respectively. Approximate cell densities were determined by optical density measurements at 600 nm (OD600). A calibration curve was obtained for each strain by determining OD600 and plate counts (described in Section 2.6) of decimal dilutions of 24 h cultures. The dilution medium was a sterile sodium chloride peptone solution. Approximate cell densities were 1×10^9 cfu mL^{-1} (OD600 = 0.7) for *L. innocua*, 3×10^8 cfu mL^{-1} (OD600 = 0.4) for *E. coli*, and 2×10^7 cfu mL^{-1} (OD600 = 0.5) for *B. subtilis*, respectively.

For batches A and B 25 mL (inoculation level 1), for batches C and D 250 mL (inoculation level 2) of each culture were added to 10.5 kg of skimmed colostrum. At least 520 g of inoculated skimmed colostrum B and D were spared for direct HPP and heat treatment and stored at −30 °C.

2.3. Microfiltration

The nominal pore sizes of the applied Tubular ceramic ISOFLUX® membrane elements (TAMI Germany, Hermsdorf, Germany) were 1.4 μm (batches A and C) and 0.8 μm (batches B and D). The membrane elements were 1178 mm long and had 8 tubular channels with a hydraulic diameter of 6 mm. The membrane surface of each element was 0.2 m^2. Prior to each filtration run the filtration apparatus including the membrane element was sanitised with a 400 ppm chlorine solution and rinsed with cold tap water. The cross flow velocity was 4.0 m s^{-1}, trans membrane pressure (TMP) was 0.7 bar; the temperature was maintained at 30 ± 2 °C. The observed permeate flux was 75 L m^{-2} h^{-1} in batches A and C and 12.7 and 14.9 L m^{-2} h^{-1} in batches B and D, respectively.

Filtration was stopped when 5.0 kg of permeate were obtained. Samples were taken from permeate and retentate and stored at −30 °C prior to analysis.

2.4. High pressure treatment

Permeate from all four MF runs as well as contaminated skimmed colostrum B and D were submitted to high pressure treatment at 400 and 500 MPa. For each Experiment 200 g of frozen sample was packed in bags made of a polyethylene-polyamide composite and vacuum-sealed to exclude air. After thawing it was vacuum-sealed in a second bag. The pressure unit (FF725, NOVA SWISS SARL, Cesson, France) was tempered to 20 °C before the packed sample was put in the pressure compartment filled with a refrigerant fluid (Friogel®, Dehon, Vincennes, France). The pressure compartment was closed and the pressure pump started. Pressure build-up took 40–42 s for 400 MPa and 50–55 s for 500 MPa. Temperature increased during pressure build-up but did not exceed 22 °C. After 10 min holding time, the pressure pump was deactivated and the pressure was evenly released over 60 s. The packed samples were rinsed with water and stored at −30 °C prior to analysis.

According to literature, the effect of one-time freezing and thawing of colostrum on IgG is negligible (Argüello et al., 2003). Microbial counts can be influenced by freezing and thawing. However, preliminary experiments showed that quick freezing and short storage periods had no appreciable effect on microbial counts. All frozen samples were treated or analysed within two weeks. Controls (skimmed colostrum, inoculated skimmed colostrum) were analysed at the same time as the treated samples.

2.5. Heat treatment

Polypropylene tubes containing 40 mL of contaminated skimmed colostrum B and D were subjected to heat treatment