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Application of a membrane technology to remove bacteriophages from whey

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ABSTRACT

Whey from former cheese batches can be recycled either to increase the yield or to improve texture properties of fat reduced cheeses. However, in the case of the presence of bacteriophages, pasteurization may not be sufficient to eliminate phages in whey. Therefore, in this work, a cross-flow membrane filtration process was designed to separate whey proteins from whey-derived phages. Filtration experiments were carried out using native whey as model filtration medium, three polyethersulfone membranes (100, 300 and 500 kDa) that were studied in detail, and lactococcal phage P008. Filtration performance was characterized by phage retention, total whey protein permeation, and permeation of the major whey proteins α-lactalbumin and β-lactoglobulin. Filtration experiments showed that it is possible to reduce the number of phages in whey by filtration to a level at which subsequent phage multiplication is minimized and, concomitantly, high protein permeation through the membrane is ensured.

1. Introduction

In traditional cheese manufacturing, only 6 to 30% of the milk constituents used are obtained as cheese curd depending on the type of cheese, whereas the major residual part accrues as cheese whey (Hinrichs, 2001). This whey has been frequently used for animal feed, biogas plants or disposed with the wastewater (Jelen, 2002; Rektor & Vatai, 2004); however, it has been recognized that further use of the by-products to optimize processes and to develop new products in the dairy industry has economic and environmental advantages.

Whey can be transformed into diverse native whey protein supplements or can be directly used as additives in various fermented products to achieve a significant increase in product yield or to improve product texture and nutritional value (Atamer, Samtlebe, Neve, Heller, & Hinrichs, 2013; Atra, Vatai, Bekassy-Molnar, & Balint, 2005; Schenkel, Samudrala, & Hinrichs, 2013; Toro-Sierra, Tolkach, & Kulozik, 2013; de Wit, 1998). Whey can be incorporated in cheese in its native state by standardizing the cheese milk prior manufacturing, or in its denatured form as micro-particulated whey protein or heat treated whey cream (Brown & Ernstrom, 1982; Hinrichs, 2001; Lawrence, Creamer, & Gilles, 1987; Lo & Bastian, 1998; Schenkel, Samudrala, & Hinrichs, 2011).

However, cheese whey may contain high numbers of bacteriophages, which limit these applications. Phages are still inducing most fermentation failures in the dairy industry (Deveau, Labrie, Chopin, & Moineau, 2006; Garneau & Moineau, 2011). In raw milk, phages of lactic acid bacteria are present with phage titres, varying from 10^1 to 10^4 plaque-forming units (pfu) mL^{-1} (McIntyre, Heap, Davey, & Limsowtin, 1991). In a recent study on phage dissemination in whey powders, it was shown that 94% of the samples contained lytic phages up to titres of 10^7 pfu g^{-1} (Wagner, 2012). In previous studies, heat inactivation has been studied in detail, and it was documented that most phages can withstand pasteurization processes of 72–75 °C for 15–30 s (Atamer et al., 2009; Atamer & Hinrichs, 2010; Müller-Merbach, Neve, & Hinrichs, 2005; Quiberoni, Guglielmotti, & Reinheimer, 2003; Suárez & Reinheimer, 2002). Consequently, thermo-resistant

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phages are capable of propagating during cheese processing, resulting in phage titres up to 10^9 phages per mL in drained cheese whey (Neve, 1996; Neve, Berger, & Heller, 1995). According to Atamer and Hinrichs (2010), a reliable thermal inactivation of heat resistant phages in whey and whey cream is possible, but such harsh heat treatments also cause a significant (approx. 60%) denaturation of whey proteins (Kessler, 2002) and hence, either a loss of or a change in functionality. Thus, to minimize the risk of fermentation failure due to re-utilization of cheese whey, whey treatments that ensure an efficient phage reduction as well as a high yield of native whey proteins are required.

Membrane separation is used for various applications in the dairy industry. The current main applications in the dairy industry aim at using membrane filtration as an alternative to existing, well established, technological processes (e.g., centrifugation, heating), enhancing functional properties of dairy products, and using various milk components effectively (Rosenberg, 1995). Filtration processes with membranes having a cut-off of approximately 1.4 μm can increase the microbiological quality by removing microorganisms (Maubois, 2002; Pouliot, 2008; Saboya & Maubois, 2000; Schmidt, Kaufmann, Kulozik, Scherer, & Wenning, 2012). The separation of milk proteins into casein micelles (average diameter of 200 nm) and whey protein (average diameter <20 nm) is possible with membrane pore size diameters ranging from 0.05 to 0.2 μm (Heino, Uusi-Rauva, Rantamäki, & Tossavainen, 2007; Jimenez-Lopez et al., 2008; Kersten, 2000; Punidadas & Rizvi, 1998).

However, only a few publications have so far addressed filtration of milk or whey containing bacteriophages. In an early paper it was reported that not only milk proteins but also bacteriophages were retained by ultrafiltration (20 kDa) of skim milk (Mistry & Kosikowski, 1986). More subsequent work suggested that phage retention of 99.86% in raw milk by a 0.1 μm membrane is due to the formation of a deposit layer by casein micelles and fat globules (Gautier, Rouault, Méjean, Fauquant, & Maubois, 1994). There are many publications available dealing with the reduction of bacteriophages, used as indicator for pathogenic viruses, in water treatment facilities by means of filtration (Grabow, 2001; Langlet et al., 2009; Madaeni, Fane, & Grohmann, 1995; Marti et al., 2011). However, to the best of our knowledge, transmission of whey protein and removal of phages at the same time from a milk system by a membrane-based filtration process has not been studied in detail yet.

The objective of the present study was to establish a non-thermal process to eliminate bacteriophages from whey by means of membrane technology. Generally, phage particle dimensions are similar to whey protein sizes (<20 nm), while the length of phage tail ranges from 120 nm to 450 nm and the head varies from approximately 50 to 75 nm (Atamer et al., 2013). Hence, the aim of this study was to characterize membranes that retain bacteriophages and allow whey proteins to permeate. Both filtration fractions, the phage reduced permeate containing the smaller major whey proteins β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) as well as the retentate containing (in addition to phages) all whey protein fractions, could be used for further individual processing to prevent a loss of mass flow. Filtration studies were conducted with the *Lactococcus lactis* phage P008 and membranes having different molecular mass cut-offs. To assess the filtration efficiency, the phage retention, the total protein permeation as well as the permeation of the major whey proteins, and the mass flux were determined.

2. Materials and methods

2.1. Native whey used for cross-flow phage filtration experiments

To avoid a fouling layer by aggregated whey components such as dust, fat or bacterial starter cells, native whey separated from milk by cross-flow microfiltration was used as feed solution for the phage filtration experiments. Raw milk obtained from the Dairy Research Station Meiereihof, Universität Hohenheim, Germany, was first skimmed (<0.1%, w/w, fat) and pasteurized (74 °C for 30 s). The skim milk was micro-filtered with a ceramic gradient of permeability (GP) membrane (Membralox, type 7P19-40 GP; Pall Exekia, Bazet, France) having a nominal pore diameter of 0.1 μm and a total membrane area of 1.69 m^2 in a pilot plant scale filtration device (model TFF; Pall GmbH, Dreieich, Germany). The microfiltration was carried out at constant transmembrane pressure (TMP), filtration temperature and tangential velocity of 0.1 MPa, 50 °C and 7 m s^{-1}, respectively. The pressure drop over the membrane was 0.2 MPa and the resulting wall shear stress was calculated as 196 Pa.

The composition of the native whey, measured with the LactoScope™ FTIR Advanced (Delta Instruments B.V., Drachten, the Netherlands), was: 6.29 ± 0.01% (w/w) dry matter, 0.68 ± 0.01% (w/w) protein, 0.01 ± 0.002% (w/w) fat, and 5.19 ± 0.02% (w/w) lactose.

2.2. Bacteriophage and host strain

The lactococcal host strain F7/2 was used to prepare a 1 L lysate of phage P008 in M17 broth (Merck Millipore, Schwalbach, Germany; Terzaghi & Sandine, 1975) supplemented with 0.5% (w/v) lactose (i.e., L-M17) and 10 mM $CaCl_2$. The incubation temperature was 30 °C. Further purification and concentration of the phages by CsCl step gradient ultracentrifugation was essentially performed according to Sambrook and Russel (2001). The final titre of phage P008 collected after centrifugation and suspended in SM-buffer without gelatin (modified from Sambrook & Russel, 2001) was 5×10^{12} pfu mL^{-1}. Phage P008 (Fig. 1) is the type phage of the widespread lactococcal 936 phage species and has been characterized in detail in earlier studies (Braun, Hertwig, Neve, Geis, & Teuber, 1989; Loof, Lembke, & Teuber, 1983; Mahony et al., 2006; Müller-Merbach et al., 2005). Phage titres were determined using the double agar layer method (plaque assay) of Adams (1959).

2.3. Filtration rig and experimental design for cross-flow phage filtration experiments

The cross-flow membrane phage filtration experiments were conducted using a SEPA CF II membrane cell system (Sterlitech Corporation, Kent, WA, USA). The experimental rig was built by the mechanical workshop of the Universität Hohenheim. It consisted of a 3.5 L feed tank with a double jacket connected to a water bath, a diaphragm pump (Hydracell M-03S, Wanner Engineering Inc., Minneapolis, MN, USA) to recirculate the feed, and a hose pump to recirculate the permeate with a flow rate of 5 L h^{-1}. In this study, three omega flat sheet membranes made of modified hydrophilic polyethersulfone (PES) (Pall Life Sciences, Port Washington, NY, USA) were investigated for their performance. The specifications provided by the supplier with respect to molecular mass cut-off for the membranes were 100 kDa, 300 kDa and 500 kDa and the effective filtration area was 1.39×10^{-2} m^2. A feed spacer (Sepa CF high foulant spacer 65 Mil (1.651 mm), Sterlitech Coporation) and a permeate carrier (Sepa CF permeate carrier membrane, Sterlitech Coporation) were inserted in the membrane cell body.

Filtration experiments were performed in batch mode. After the filtration system was set to 20 °C, 2 L of native whey (pre-tempered at 20 °C) were spiked with 100 μL of high-titre P008 phage stock and added to the feed tank. The TMP was set to 0.2 MPa and the wall shear stress was calculated as 64 Pa. Retentate and permeate were recirculated for 15 min. After each filtration experiment, samples of the retentate and permeate were taken to determine the