Journal of Biotechnology 168 (2013) 693–700

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Purification of high value proteins from particle containing potato fruit juice via direct capture membrane adsorption chromatography

I. Schoenbeck[a,*], A.M. Graf[b], M. Leuthold[c], A. Pastor[c], S. Beutel[a], T. Scheper[a]

[a] Leibniz Universität Hannover, Institut für Technische Chemie, Callinstr.5, D-30167 Hannover, Germany
[b] Südzucker AG Mannheim/Ochsenfurt, Wormser Str. 11, Obrigheim/Pfalz, D-67283 Obrigheim, Germany
[c] Sartorius Stedim Biotech GmbH, August-Spindler-Str. 11, D-37079 Göttingen, Germany

ARTICLE INFO

Article history:
Received 21 May 2013
Received in revised form
20 September 2013
Accepted 23 September 2013
Available online 30 September 2013

Keywords:
Membrane adsorber
Purification
Protein
Binding capacity
Long-term stability

ABSTRACT

Potato fruit juice (PFJ) is a by-product from industrial starch production. It still contains several valuable components such as amino acids, minerals and proteins. An economic technology for the isolation and purification of different native potato proteins is the ion exchange chromatography, which can be performed either by classical bed chromatography or by membrane adsorption chromatography (MA-IEX). An already published MA-IEX process for the downstreaming of PFJ is based on the following steps: prefiltration/microfiltration, fractionation with MA-IEX, ultra-/diafiltration and finally drying. In order to further minimize process complexity and costs, new MA-IEX-modules were designed and tested in this research project to facilitate the processing of crude, particle-containing solutions using a tangential flow through the membranes. Modules with fleece polymer spacers and extruded polymer spacers, as well as different spacer channel sizes were tested for their binding capacities and their long-term stability. An optimized setup was found for the technical scale. Modules with extruded polymer spacers channel size 250 μm show the highest binding capacities (anion exchanger approx. 0.34 mg/cm^2, cation exchanger approx. 0.16 mg/cm^2), while the modules with extruded polymer spacers channel size 480 μm show the best long-term stability with 23 passes without intermediary cleaning.

1. Introduction

Alongside rice and corn, potatoes are the most important source for industrial starch production. Potato fruit juice (PFJ) is a by-product of this industry. Consisting mostly of water, PFJ also contains approx. 2% (w/w) protein which is subdivided into 50% protease inhibitors (PI), 40% Patatin and 10% high molecular weight proteins such as lectins and phenoloxidases, protein kinases and phosphorylases (Allen et al., 1996; Pots et al., 1999; Ulber, 2002; van Konigsveld, 2001). Currently, potato fruit juice is thermally treated and the denaturated protein is sold as animal feed for 1 €/kg or less. However, potato protein has a high nutritional value due to its high amino acid score of 76% (Graf, 2010). Furthermore, some low molecular weight protein fractions (PI) have been reported to induce a positive effect on the feeling of satiety and may be marketed as an appetite suppressant (Hill et al., 1990). Thus, PI may serve as an interesting ingredient for human nutrition. If isolated natively, PI price is estimated to be 100 €/kg, which is a 100-fold increase in market value. The Patatin fraction show bioactive functions like lipid-acyl-hydrolase- and acyl-transferase-activity. Due to these activities, Patatin is involved in the defence mechanism against plant pests and pathogens (Bohac, 1991; Hirschberg et al., 2001; Pots, 1999; Racusen, 1984; van Konigsveld, 2001). A β-1,3-glucanase-activity is described as a defence mechanism against fungal pathogens as well (Shewry, 2003).

The bioactive potato protein fractions have been isolated from PFJ previously. For example, Menzel et al. have demonstrated that anion exchange membrane adsorbers (functionality: quaternary ammonium ligands) are most suitable for the isolation of the glycoprotein Patatin. They also demonstrated that cation exchange membrane adsorbers (functionality: sulfonic acid ligands) are preferable to isolate the PI (Kokpinar et al., 2006; Menzel et al., 2005; Sartorius Stedim biotech, 2009; Suck et al., 2006).

In order to obtain the proteins in their native form, Steinhoff designed a process comprised of several unit operations (prefiltration/microfiltration, membrane adsorption, ultra-/diafiltration and drying) (Graf et al., 2009; Steinhof, 2007). The used membrane adsorber capsules perform ion exchange chromatography in the dead end filtration mode (MA-IEX-capsules). The capsules contain modified macroporous stabilized reinforced cellulose on which the chromatographic ligands are attached. The adsorptive membrane material is rolled up to form a cylindrical module of a specific chromatographic bed height. The flow through the membrane is redirected from the inlet to the outside channel and then in a radial

* Corresponding author. Tel.: +495117622381.
E-mail address: schoenbeck@iftc.uni-hannover.de (I. Schoenbeck).

0168-1656/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2013.09.018

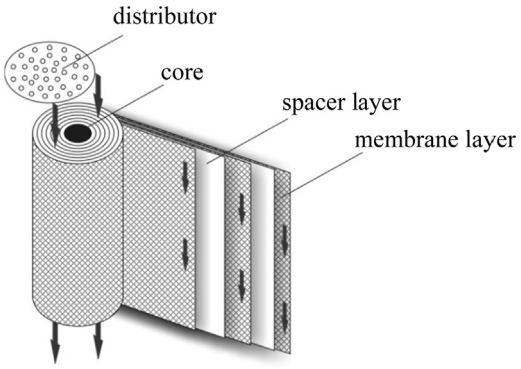

Fig. 1. Setup of the direct capture modules.

path through the centre of the module and the outlet (Sartorius Stedim biotech, 2009). However, MA-IEX-capsules are not suitable for particle-containing liquids. Due to this fact, prefiltration and microfiltration were necessary. Therefore, the overall production process was time-consuming, even if it was faster compared to conventional chromatographic downstreaming. The goal of the following work was to adapt the previously described process for native potato protein isolation to membrane modules which support particle-containing suspensions. In this way, it was possible to decrease the overall number of unit operation steps and to design a more efficient and economic process.

2. Experimental

2.1. Buffer and solutions

The potato fruit juice (PFJ) was provided by Emsland–Stärke GmbH, Emlichheim. Sodium sulphite was added to avoid enzymatic browning (5 g/l). The PFJ was diluted 1:5 with deionized water prior to all experiments. The deionized water was prepared via Arium (Sartorius, Göttingen). The diluted PFJ was centrifugated for 10 min at 40,000× *g* and the supernatant was used for the experiments with the FPLC (BioLogic DuoFlow, BIO-RAD, flow rate 1.5 ml/min). The PFJ used in the experiments was derived from the same batch.

The washing buffer (20 mM sodium phosphate, pH 7) was used for washing and equilibrating the membranes before and after loading, as well as between elution steps.

As elution buffer 20 mM sodium phosphate, pH 7 with 0.5 M NaCl was used to remove the bound proteins of interest.

Regeneration was performed with 20 mM sodium phosphate, pH 7 with 1 M NaCl to detach all proteins still bound on the membranes.

After the experimental setup, all membranes were cleaned with 0.1 M NaOH solution and stored in 20% EtOH in 1 M NaCl.

2.2. Membrane adsorber

The number of membrane adsorber systems available on the market has risen significantly over the past years (Charcosset, 2012; Harkensee et al., 2007). Here, prototype membrane modules, further called “direct capture modules”, were used in the experiment, which contained different membrane adsorber layers and various spacers (fleece and polymer extruded). The advantage of the direct capture modules is tangential flow through the membrane. This allows the processing of particle-containing suspensions with minimal blockage on the membrane surface. The principle setup is shown in Fig. 1.

This setup enables a purification of the potato protein without the unit operation step prefiltration/microfiltration as described in the introduction (Graf, 2010; Graf et al., 2009; Steinhof, 2007).

To simplify the description of these prototype capsules, the different parameters together with their abbreviations are described in the text below.

As membrane ligands, quaternary ammonium was used for the anion exchange membranes (further called “Q”-membrane) and sulfonic acid ligands were used for the cation exchange membranes (further called “S”-membrane). All membranes consist of regenerated cellulose, pore size 3–5 μm, thickness: 275 μm, ligand density 2–5 μeq/cm^2.

The tested modules also vary in their membrane surface area, depending on the spacer material and size. There are three basic types of cases; 2.5 ml cases contain a membrane surface area between 83–116 cm^2 (Q25, S25), 500 ml cases have a membrane surface area between 9040–15,430 cm^2 (Q500, S500) and the 1000 ml cases include a membrane surface area between 34,750–40,900 cm^2 (Q1000, S1000). They also vary in their spacer types; fleece and extruder polymer were tested. The fleece polymer spacers (F) as well as the extruder polymer spacers (E) consist of polypropylene but have differences in their structure. Fig. 2 shows microscope images of the structural differences between fleece and extruded polymer spacers. The spacers differ in the size of their channels, 240/250 μm or 480/500 μm.

An example of the nomenclature used for the direct capture modules tested throughout the paper is Q500 E250. This is an anion exchanger (Q) with a case size of 500 ml (500) and with an extruder polymer as spacer material (E) and a channel size of 250 μm (250).

All modules (with exceptions of the Q25 and S25 module) were loaded in recirculation mode with 15 l PFJ. A complete downstream cycle can be divided into loading (15 min, $V = 5 - 6$ l/min), washing, elution, regeneration and equilibration at $V = 2 - 3$ l/min. The modules were processed five times without any further cleaning step. The Q/S25 modules were loaded in flow through mode with 15 ml PFJ. Afterwards, the modules were washed, eluted, regenerated and equilibrated with a flow rate of: $V = 1.5$ ml/min.

2.3. Binding capacity

The binding capacity of the modules Q/S500 and Q/S1000 was determined with the pilot plant (for description see Section 3.4) by loading PFJ in recirculation mode for 20 min and measuring the total Patatin and PI bound to the membrane after elution. The binding capacity for the Q/S25 Modules was measured with a FPLC instrument after loading PFJ 10 min in a flow through mode. The “Modified Lowry Protein Assay Kit” (product nr. 23240, Thermo Scientific, Rockford USA) was used for the determination of the protein concentration. All measurements were the average of collecting three elution samples from the membranes, determined in triplicate with the Lowry Assay.

3. Results and discussion

All experiments were performed with prototype membrane adsorbers, the direct capture modules described in the experimental section. This new module type allows the downstreaming of particle-containing media. The broth passes a distributor and is tangentially routed through the spacer over the membrane. Impurities and particles pass through, while the target proteins bind on the membrane. Additionally, the speed of binding is increased by the turbulences caused by spacers (Sartorius Stedim biotech, 2009). This allows the adsorption of target molecules in unfiltered suspensions without any prefiltration (Fig. 3). In the experiments described below different spacer materials (described in Section 2.2) as well as different spacer sizes were tested.