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Clarification of aqueous corn extracts by tangential flow microfiltration

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ABSTRACT

The effectiveness of tangential flow microfiltration for the solid/liquid clarification of aqueous corn endosperm and germ extracts was examined using recombinant type I human collagen (rCollagen) and green fluorescent protein (GFP) as model proteins. We identified the effects of transmembrane pressure (TMP), crossflow rate, protein molecular weight, and membrane chemistry on permeate flux, protein rejection, and internal membrane fouling in microfiltration. In dead-end filtrations, both endosperm and germ extracts formed highly compressible cakes that provided the dominant hydraulic resistance. For tangential flow filtration using a ceramic membrane, increasing the crossflow rate had a significant beneficial effect on the permeate flux for all TMPs examined. High fouling of the ceramic membrane occurred during the filtration and this was likely due to the presence of soluble corn starch in the feed. Filtration with a ceramic membrane resulted in low rejection (<10%) of both the host cell proteins (HCP) and GFP and very high rejection (~90%) of rCollagen. In contrast to the ceramic membrane, tangential flow filtration using a poly(vinylidene fluoride) membrane resulted in much less internal fouling and no measurable rejection of HCP, GFP, or rCollagen. Microfiltration was an effective method for the solid/liquid clarification of corn protein extracts, except in cases where high internal membrane fouling resulted in increased rejection of large molecular weight proteins.

1. Introduction

Production of high-volume, lower value recombinant proteins in transgenic corn seed offers an economic alternative to protein production from cell culture and animal sources. Plant-based production avoids the risk of contamination from mammalian-associated infectious agents (i.e. prions and viruses) and retains considerable capability for post-translational modifications similar to those occurring in mammalian cell cultures [1]. Plant hosts also have the ability to target the expression of recombinant proteins to specific tissues or organs, including those which allow for prolonged storage (seeds). Extraction of only the targeted tissue reduces the extraction volume and impurity level [1].

Among plant-based recombinant-protein production systems, corn seed offers the advantages of low water-soluble protein content and well-established methods for genetic manipulation, transformation, cultivation, and processing by bio-refining into multiple industrial products. Corn has been explored as an expression host for several recombinant proteins including dog gastric lipase [2], monoclonal antibodies [3], vaccines [4], proinsulin [5], and, more recently, collagen-related proteins [6,7], which have also been expressed in tobacco [8,9] and barley [10]. Corn seed consists largely of endosperm (83% dry weight), which is comprised mainly of starch and water-insoluble proteins, along with germ (11% dry weight), which contains most of the seed's oil and water-soluble protein. Combining endosperm-targeted expression with dry milling to isolate an endosperm-rich fraction provides an extract with the lowest host cell protein (HCP) burden [1]. While issues of contamination of non-GMO corn with transgenes has delayed large-scale use of corn for recombinant-protein production, methods of risk reduction that would enable future adoption are being developed [11].

The low cost that is associated with the production of recombinant proteins in plants and the potential for coupling protein production to current crop-based biorefineries makes the plant host system ideal for the production of low-value/high-volume proteins (e.g. protein-based materials, industrial enzymes) [7]. However, the purification process becomes the major cost, comprising up to 90% of the overall production cost of recombinant proteins in plants [12]. Thus, development of efficient and inexpensive methods for the clarification of plant extracts and purification of recombinant proteins from the host plants is crucial.

A major step that is required in the purification process is the solid/liquid clarification of the protein extracts. Currently, most of the work regarding the clarification of recombinant proteins from corn extracts has used centrifugation [2,13], rotary drum vacuum filtration (RVDF), or expanded bed chromatography [14] as the solid/liquid clarification step. Impediments to the alterna-

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tive of microfiltration include membrane fouling by soluble feed components and the buildup of a compressible layer of rejected particles on the membrane surface (i.e. cake layer), both of which can decrease permeate flux and increase protein rejection [15,16]. However, microfiltration offers the advantages of low cost operation, the elimination of filter aids [17], the ability to handle the high solids content (10–25%, w/w) and large particle size range (sub-micron to millimeter) associated with corn extracts [14], and relatively mild operating conditions, which minimize protein denaturation.

Formation of a cake layer often provides the greatest hydraulic resistance to permeate flux in microfiltration. The cake resistance, R_c, influences permeate flux J, as seen in Darcy's law,

$$J = \frac{dV_{perm}}{Adt} = \frac{\Delta p}{\mu_0(R_m + R_c)} \quad (1)$$

where V_{perm} is the permeate volume, μ_0 is the viscosity of the permeate, R_m is the membrane resistance, Δp is the transmembrane pressure (TMP), and A is the filtration area. In tangential flow microfiltration, the formation of the cake layer is affected by the filtration operating conditions (crossflow rate and TMP) as well as the feed conditions (particle size, pH, and ionic strength). Several methods exist to reduce the resistance and formation of the cake layer. Increasing the crossflow rate generally increases the permeate flux by reducing the mass of the deposited cake layer due to increased sweeping of the particles from the membrane surface [18–22]. Also, larger feed particle size can help form a more porous cake layer, thus, increasing the permeate flux [15,23,24]. Reduction of cake layer formation and solute rejection by the cake layer can also be achieved by operating the filtration below the critical TMP (i.e. in the pressure-dependent permeate flux regime) [25–27].

In this study, the suitability of tangential flow microfiltration for the solid/liquid clarification of corn protein extracts from endosperm- and germ-rich dry milling fractions was examined. We used triple helical type I recombinant human collagen (rCollagen, 265 kDa) and green fluorescent protein (GFP, 27 kDa) as model proteins to assess the effect of protein molecular weight on rejection during microfiltration of corn protein extracts. The effect of crossflow rate, TMP, and membrane chemistry on the permeate flux, product transmission, and membrane fouling during tangential flow microfiltration of corn extracts was examined. In addition, the compressibility of the filter cake that formed during the filtration of corn extracts was determined in dead-end microfiltration.

2. Materials and methods

2.1. Materials

All chemicals were reagent grade and purchased from Fisher Scientific (Itasca, IL) unless noted otherwise. Preparation of rCollagen, expression of GFP in transgenic corn, and methods for rCollagen spiking and protein extraction are described by Aspelund and Glatz [28].

2.2. Dry milling

Kernels were dry milled into germ- and endosperm-rich fractions as described by Aspelund and Glatz [28]. Kernels were first tempered in a sealed bag for 2.5 h with deionized water to achieve a moisture content of 21%, then ground using a laboratory Beal-type drum degermer and a series of corrugated Witt mills (Witt Corrugating Inc., Wichita, KS) using the method and mill settings optimized for fractionation of recombinant dog gastric lipase-containing corn [29], and finally separated into fractions by screening.

2.3. Corn defatting

GFP-expressing germ- and endosperm-rich fractions were milled a burr-type coffee mill (KitchenAid model KPCG100) with 400 g of fractionated grain in each batch. The resultant flours had particle sizes ranging from 0.01 to >1 mm for both the endosperm- and germ-rich fraction, as determined by dry sieving. Oil was extracted from the milled endosperm- and germ-rich fractions as described in Aspelund and Glatz [28].

2.4. Solids settling and clarification

All dead-end and tangential flow filtrations described in this work were performed using either the suspension that remained after settling or clarified extracts. To prevent clogging of the filtration modules used in these experiments, a solids settling step was performed as follows. Subsequent to extraction, the corn slurry was allowed to settle for 30 s, from a maximum settling distance of 15 cm, and the supernatant was decanted to separate the rapidly settling solids from the solids that remained in suspension. Alternatively, the extracts were clarified by centrifugation at $3000 \times g$ and 4 °C for 15 min, followed by filtration through a 0.2 μm syringe filter (Sigma–Aldrich; St. Louis, MO).

2.5. Unstirred dead-end filtration

Dead-end filtrations were performed using a 0.2 μm pore size flat-sheet polyethersulfone (PES) membrane (Koch Membrane Systems, Wilmington, MA) in a dead-end filtration cell (Model 8050, Millipore Corp., Billerica, MA) with an effective membrane area of 13.4 cm^2. Circular (4.45 cm diameter) membranes were cut from a larger sheet. Fifty milliliter of endosperm or germ extract was added to the filtration cell, which was then pressurized to the desired filtration pressure using compressed nitrogen. Dead-end filtrations were performed at several TMPs between 0.34 and 3.4 bar. Permeate mass was measured using an electronic balance (Mettler-Toledo PG6002-S, Columbus, OH). The specific cake resistance, α, for unstirred dead-end filtrations can be obtained from the slope of a plot of tA/V_{perm} versus V_{perm}/A using the following equation:

$$\frac{tA}{V_{perm}} = \frac{\rho_c \alpha \mu_o}{2\Delta p}\left(\frac{V_{perm}}{A}\right) + \frac{\mu_o R_m}{\Delta p} \quad (2)$$

where, V_{perm} is the permeate volume, μ_0 is the dynamic viscosity of the permeate, R_m is the membrane resistance, Δp is the transmembrane pressure, A is the filtration area, m is the mass of the cake layer per membrane area, and ρ_c is the mass of dry filter cake per unit volume of permeate [30]. For compressible cakes, the specific cake resistance varies with position in the cake [31]; therefore, Eq. (2) estimates a specific cake resistance averaged over the entire thickness of the cake layer. The compressibility index, n, of the filter cake was calculated using Eq. (3)

$$\alpha = \alpha_o \Delta p^n \quad (3)$$

where, α_0 is the zero-pressure specific cake resistance. A value of zero for n represents an incompressible cake and an increasing value of n represents increasing cake compressibility. Flux data were collected during the linear range of the plot of tA/V_{perm} versus V_{perm}/A, determined experimentally, for both endosperm and germ extracts. Samples of the feed and final permeate were collected and assayed for HCP, GFP, and rCollagen concentration. The filter cake was collected and allowed to air dry for 24 h to determine the dry cake mass. Each filtration was performed in duplicate.

The protein rejection coefficient was calculated as,

$$R = 1 - \frac{C_{perm}}{C_{feed}} \quad (4)$$