Whey processed by Pressurized Gas eXpanded (PGX) liquid Technology

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1. Introduction

Polysaccharides and proteins have been successfully processed with the patented Pressurized Gas eXpanded (PGX) Technology.[1] The tuneable PGX solvent consisting of carbon dioxide (CO_2) expanded ethanol (EtOH) precipitates, purifies, micronizes, and dries biopolymer from solutions forming highly porous materials with large specific surface areas and low bulk densities. Whey is the liquid effluent of cheese and yogurt production and was previously considered to be of low value. It is composed mainly of lactose, proteins, salts and small quantities of fat. Research on the valorization of whey, particularly the protein (WP) fraction, has revealed a wide range of applications[2-4]. Conventional processing of whey liquid (bovine cheese production effluent) requires consecutive filtration steps before spray drying or freeze drying.[5] The objective of this study was to investigate the PGX processing of whey liquid and the characterization of the resultant dried protein-rich powder.

2. Materials and Methods

Sweet whey feedstock (6.94% total solids) was purchased from The Cheese Factory (Edmonton, AB, Canada). PGX processing involves the co-injection of whey liquid together with the PGX solvent (CO_2 + EtOH) into a pressurized chamber at 100 bar and 40°C, equipped with 5 µm felt filters at the bottom of the basket. After injection of whey, water removal proceeds using CO_2 and EtOH and finally, SC-CO_2 to remove EtOH. Whey feedstock was lyophilized (FD-WP) using a LABCONCO Freeze Zone Plus 12 at <-80°C and <40 x 10^{-3} mbar. Composition of the feedstock and dried materials was determined (Table 1). The protein profile was analyzed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Protein aggregations due to disulfide interactions were disintegrated by heating the samples with 0.05% wt. 2-mercaptoethanol (100°C, 5 min). Acrylamide gels (4-20%) were run at 80V, stained with 0.01% wt/vol. Coomassie Blue solution and destained in an acetic acid/methanol solution (10% v/v methanol). The Zeiss Orion NanoFab Helium Ion Microscope equipped with an electron flood gun to neutralize positive charges allowed for direct imaging of PGX-WP. Intrinsic fluorescence intensities (IFI) of dried WP were determined at 0.05 mg protein/mL phosphate buffer (pH 6.8-7.8) using a SpectraMax M3. Protein surface hydrophobicity was determined using a fluorometric method with 1-anilinonaphthalene-8-sulfonate (ANS).[6] The hydrophobicity of the protein was reported as the slope of the plot of relative fluorescence intensities (RFI) vs protein concentration (mg/mL).

3. Results and discussion

SDS-PAGE patterns of FD-WP confirmed the presence of the major whey proteins α-lactalbumin (α-LA) and β-lactoglobulin (β-LG), as well as minor fractions, immunoglobulin (IgG), bovine serum albumin (BSA), and Lactoferrin (Lf). The PGX process successfully fractionated and concentrated the proteins (~13% to 46% w/w) of the whey liquid (Table 1). The protein profile of PGX-WP is composed of mainly α-LA and β-LG. The absence of other proteins in the dried powder may be attributed to the processing conditions favouring the retention of α-LA and β-LG in the PGX system. The PGX effluent contained 1.33 ± 0. 21% total solids, 12.77 ± 4.86% crude protein, and 11.17 ± 0.43% lactose. The PGX-effluent contained the remaining proteins fractions, IgG (heavy and light chains), Lf and BSA, in addition to α-LA and β-LG. Other scCO_2 fractionation methods for WP[7-8] utilize direct acidification and heat denaturation of whey, as well as post-processing steps to obtain separate, dry fractions of concentrated α-LA and β-LG. The compositional analysis of PGX-WP and effluent revealed both proteins and lactose in both fractions with more protein in the PGX-WP and more lactose in the effluent. Alternative CO_2: EtOH: H_2O ratios and filter setups may be considered to evaluate the change in recovery, composition (i.e. relative ratios of protein to lactose) and consequently, the functional properties of the dried powder obtained.

Table 1. Composition analysis of whey samples, percentage on a dry weight basis.

Sample	Ash (%)	Crude Protein (%)	Total Fat (%)	Total Carbohydrate (%)	Lactose (%)
Feedstock	6.63	12.97[1]	5.04[3]	71.6[4]	*
FD-WP	*	10.58 ± 0.18[2]	*	*	5.03 ± 0.49[5]
PGX-WP	*	46.00 ± 0.97[2]	*	*	2.99 ± 0.14[5]

[1] Kjeldahl Block Digester Method; [2] Organic Elemental Analysis (Thermo Fisher Flash 2000); [3] Mojonnier Method; [4] Calculation; [5] Megazyme Lactose Assay Kit - Sequential/High Sensitivity (K-LOLAC), as is basis; * *Awaiting results.*

The average particle sizes of PGX-WP and FD-WP were 10.06 ± 8.63 µm and 92.44 ± 41.68 µm, respectively. The untapped bulk densities of PGX-WP and FD-WP were 0.05 ± 0.06 g/mL and 0.22 ± 0.09 g/mL, respectively. HiM images of PGX-WP are characterized by nano-sized ball-and-stick morphologies (highlighted in the blue circle), with 100 nm – 2 µm spherical particles embedded (highlighted in the red circle, Fig. 1). Previously, PGX-processed gum arabic (a mixture of glycoprotein and polysaccharides) was reported to have similar nano-sized ball-and-stick features thus it is suggestive that these features of PGX-WP are glycoprotein and lactose. The ζ-potential of the reconstituted PGX-WP and FD-WP solutions in deionized water (pH 6.89) were -11.5 mV and -12.8 mV respectively.

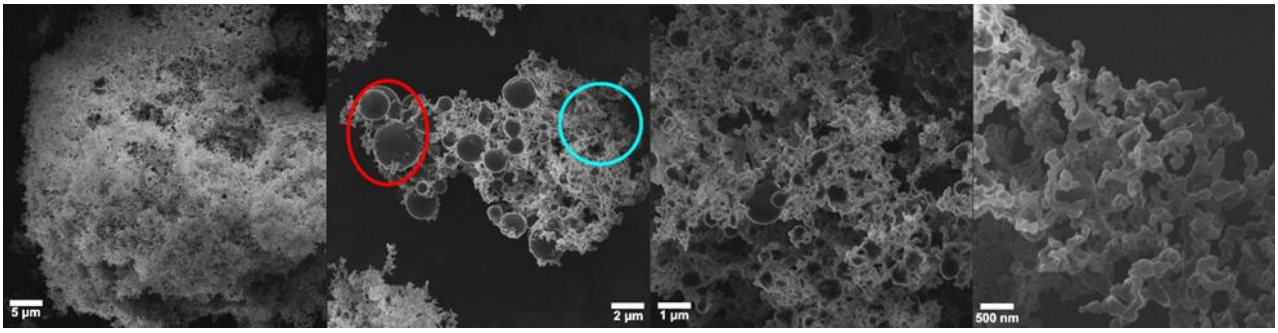

Figure 1. HiM images of PGX-WP.

The surface hydrophobicity of PGX-WP is affected by pH, suggesting a change in the protein-solvent interactions with varying pH. At pH 6.8, the hydrophobicity of PGX-WP is like that of FD-WP, however, at pH 7.8, PGX-WP is more hydrophilic compared to its FD counterpart. The interaction with the PGX solvent may cause a change in the protein folding patterns thereby resulting in the shielding of hydrophobic amino acids such as Trp and Tyr.

4. Conclusions

Whey was successfully processed with the PGX Technology, producing a low bulk density, high protein powder with nano-features. WP was fractionated, purified and concentrated (by 33%) using the PGX process. When compared to FD-WP, the PGX-WP had a 9-times smaller average particle size, 2% less lactose and 35% more protein consisting mainly of α-LA and β-LG. The PGX-WP was also more hydrophilic than FD-WP at pH 7.8. Future experiments should consider operating at different fluid ratios to explore the extent of fractionation and purification by the PGX process.

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