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Foam and emulsion properties of potato protein isolate and purified fractions

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ABSTRACT

Spray dried potato protein and specific isolated fractions were used for foaming and emulsification studies. The spray dried protein was separated into a patatin and a protease inhibitor (PI) rich fraction by ion exchange chromatography (IEX), respectively, and these two fractions were purified by hydrophobic interaction chromatography into a low (HIC 1) and a high (HIC 2) hydrophobic fraction. Foam overrun for the spray dried powder and all patatin fractions were highest at pH 3, with gradually lower values at pH 5 and 7, while the PI fractions had highest overrun at pH 5 and equally lower values at pH 3 and 7. Relative foam stability varied from 18 to 78% of the initial foam at pH 3 while lower variation of 67–80% was seen at pH 5 and 7. The HIC fractions did generally perform better than the spray dried powder and IEX fractions, with patatin HIC 1 and HIC 2 having superior performance at pH 3 and PI HIC 2 at pH 5 and 7. Emulsions were characterized by emulsion stability and activity, emulsion droplet size and small scale dynamic rheological measurements. The PI, and especially PI HIC 1 showed poor emulsion properties with low stability, large droplet size and less texture. Interestingly, PI HIC 2 had much better emulsion properties on par with the spray dried powder and patatin, while having a more frequency dependent textural response. Overall, the best emulsion properties were obtained with patatin HIC 2, thus showing the importance of hydrophobicity for protein functionality.

1. Introduction

Potato protein has in recent years drawn more and more attention as a protein source for human consumption and as a unique food ingredient. Industrially potato starch is the main value-added ingredient from potatoes with the proteins being located in a side stream from starch production known as potato fruit juice (PFJ). PFJ contains roughly 2–5% solids of which 35% is N-containing substances i.e. protein, peptides and amino acids (Knorr, Kohler, & Betschart, 1977). The potato proteins have been concentrated from PFJ by acid precipitation, but to preserve the functional properties more gentle techniques needs to be used e.g. expanded bed absorption (Lokra, Helland, Claussen, Straetkvern, & Egelandsdal, 2008), ion exchange chromatography (van Koningsveld et al., 2001) or ultrafiltration (Straetkvern & Schwarz, 2012).

The potato proteins can roughly be divided into three main groups, with the first being the patatins, representing up to 40% of the total protein. The patatins are glycoproteins with a molecular weight of 39–43 kDa (existing as native 80 kDa dimers) with different isoelectric points (pI) from pH 4.45–5.17 and glycosylation patterns (1–3 glycosylations) (Barta, Bartova, Zdrahal, & Sedo, 2012). The second group of potato proteins is the protease inhibitors (PI), which represents up to 50% of the total protein and this group can be further divided into seven sub-groups (Pouvreau et al., 2001). The molecular weight of the PI proteins varies widely, from 4.3 to 20.6 kDa, with pI values of pH 5.1–9.0 (Pouvreau et al., 2001). The last group of proteins is mainly composed of oxidative enzymes, like lipoxygenase, polyphenol oxidase and enzymes associated with starch synthesis (Jorgensen, Stensballe, & Welinder, 2011).

Two important functional properties of food proteins are their ability to form and stabilize foams and emulsions by adsorbing to

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the interface between air and water or oil and water, hence lowering the interfacial tension and providing electrostatic and steric stabilisation of the interface film (Wierenga & Gruppen, 2010). Only limited results have been published on foam and emulsification behavior of potato proteins and even fewer on the purified fractions (Ralet & Gueguen, 2000, 2001; van Koningsveld, Walstra, Gruppen, Wijngaards, van Boekel, & Voragen, 2002; van Koningsveld, Walstra, Voragen, Kuijpers, Van Boekel, & Gruppen, 2006).

The aim of this study was to provide further insight into foam and emulsion properties of potato proteins and purified fractions hereof. It was theorized that purification of a total protein isolate by first ion exchange and then hydrophobic interaction chromatography would lead to unique fractions with more distinct pI and hydrophobicity characteristics that could be tailored for specific food applications or pH regimes e.g. with fractions high in foaming and fractions high in emulsification properties.

2. Materials and methods

2.1. Protein samples

Spray dried potato protein isolate powders from the harvest years of 2014 and 2015 were provided by KMC, Brande, Denmark. The spray dried powder had a water content of 7.2% as determined by heating 5 g of sample at 105 °C in a Mettler Toledo moisture analyser (Greifensee, Switzerland), protein content of 83.4% (Kjeldahl, N × 6.25) and an ash content of 1% as determined by heating 5 g of sample stepwise from 200 °C to 900 °C for a total time of 17 h. Fractionation of resolubilised spray dried protein isolate by ion exchange chromatography results in a patatin fraction (Pat) and a protease inhibitor fraction (PI), further purification by hydrophobic interaction chromatography (HIC) resulted in two patatin fractions (Patatin HIC 1 and Patatin HIC 2) and two protease inhibitor fractions (PI HIC 1 and PI HIC 2). Fractionation was done as described previously (Schmidt et al., 2017). The purified fractions were concentrated and diafiltrated against destilled water on an Ultralab tangential flow system with a 10 kDa Minimate™ membrane (Pall, New York, USA) and freeze-dried before use.

2.2. Preparation of protein dispersions

The powders were suspended in buffer for 1 h before use. The used buffers were 30 mM trisodium citrate dihydrate (target pH 3), 22 mM sodium acetate (target pH 5) and 7.5 mM disoudium hydrogenphosphate dihydrate (target pH 7). All chemicals were of analytical grade and supplied by MERCK (Darmstadt, Germany) or SIGMA (St. Louise, USA). Milli-Q ultrapure water was used throughout the experiments. All buffers were adjusted to an ionic strength of 50 mM with NaCl. For emulsions at pH 3 a 95 mM trisodium citrate dihydrate was used. Prior to foam, zeta potential, or tensiometry measurements suspensions were centrifuged (4700 rpm, 15 min, 4 °C) in order to avoid any insolubilized material in the sample for analysis.

2.3. Protein composition by SDS-PAGE

SDS-PAGE using Criterion™ TGX™ 8–16% precast gels (Bio-Rad, Richmond, CA, USA) was performed essentially as described earlier (Laemmli, 1970). The samples were mixed 1:1 with sample buffer (20 mM Tris, 2% SDS, 20% glycerol, pyronin Y), and reduced with 1/10 vol 0.2 M DTE and boiled for 3 min. A 30 μL sample of concentration (c) 1 g/L were loaded onto the gel. Gels were stained with Coomassie Brilliant blue G-250. Molecular mass was estimated by a prestained broad range molecular weight marker (Thermo Scientific™ Spectra™ Multicolor Broad Range Protein Ladders).

2.4. Protein concentration determination

The bicinchoninic acid assay (BCA, Thermo Scientific™ Pierce™), with bovine serum albumin (2 g/L) as reference protein was used for protein determination. The working solution containing Cu^{2+} and BCA was added to a microtiter plate containing reference or sample protein and incubation was carried out for 30 min at 37 °C following measurement of absorbance at 562 nm measured in a Synergy 2 Microplate reader (BioTek Instruments Inc, USA) and UV absorbance units converted to protein concentration in g/L based on a regression curve of the reference protein. Measurements were conducted in duplicates.

2.5. Zeta-potential

Zeta-potential (mV) of 10 mL protein solutions at a concentration of 1 g/L and at pH 3, 5 and 7 were determined by a Stabino® (Particle Metrix, Meerbusch, Germany) fitted with a 200 μm piston by measuring for 180 s and the endpoint recorded. The Stabino® produces an electric streaming potential by moving a piston in an oscillating motion. Zeta potential is calculated based on the streaming potential following calibrated to the electrophoresis potential of a known zeta potential standard of 0.01 N KCl. A minimum of four measurements per sample were made.

2.6. Turbidity measurement

Spray dried potato protein isolate powder and the purified fractions hereof were dispersed at c = 1 g/L for 1 h in 30 mM trisodium citrate dihydrate, 22 mM sodium acetate or 7.5 mM disoudium hydrogenphosphate dihydrate buffer. Turbidity of unsettled dispersions were measured spectrophotometrically as absorbance units (AU) at λ = 500 nm in a quartz cuvette. Two to three dispersions were made per sample with three measurements per dispersion.

2.7. Foam analysis

Foam was produced by shaking 20 mL of 1 g/L solution in 100 mL closed cylinders at a frequency of 4 Hz for 45 s by hand essentially as described previously (Hammershoj, Peters, & Andersen, 2004). Visual evaluation of the resulting foam and liquid volumes were conducted. Foam overrun (FO) were calculated (equation (1)) as a relative overrun volume based on V_{foam} = volume of foam (L) and V_{liquid} = volume of liquid (L). The total volume of foam and liquid as well as the liquid volume were recorded for 2 h. The foam stability was calculated (equation (2)) as the relative foam volume (FV) (Hammershoj et al., 2004). Minimum three repetitions were made per sample.

$$FO = \frac{V_{foam}}{V_{liquid}}[L/L] \quad (1)$$

$$FV = \frac{V_{foam\ t=2h}}{V_{foam\ t=0h}} * 100\ [\%] \quad (2)$$

2.8. Surface tension

Surface tension was determined using a platinum Wilhelmy plate (19.62 × 10 × 0.1 mm) controlled by a Sigma 700 force