Food Structure 14 (2017) 104–111

Contents lists available at ScienceDirect

Food Structure

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

Protein nativity explains emulsifying properties of aqueous extracted protein components from yellow pea

Marlies E.J. Geerts[a], Constantinos V. Nikiforidis[a,b], Atze Jan van der Goot[a,*], Albert van der Padt[a,c]

[a] Food Process Engineering Group, Wageningen University, Bornse Weilanden 9, 6708WG Wageningen, P.O. Box 17, 6700AA, Wageningen, The Netherlands
[b] Biobased Chemistry and Technology Group, Wageningen University, Bornse Weilanden 9, 6708WG Wageningen, P.O. Box 17, 6700AA, Wageningen, The Netherlands
[c] FrieslandCampina, Stationsplein 4, 3818 LE, Amersfoort, The Netherlands

ARTICLE INFO

Keywords:
Mild fractionation
Protein functionality
Emulsification
Yellow pea

ABSTRACT

In this paper, the emulsifying properties of a protein-enriched fraction from pea are unravelled. The emulsifying properties of mildly fractionated protein fractions from yellow pea and compared to those of commercial pea protein isolate. The emulsion stability of an oil-in-water emulsions were determined under acidic pH, under acceleration forces and a freeze-thaw treatment. It was found that the emulsions stabilized by the mildly fractionated proteins were less prone to flocculation and coalescence. Those differences were related to the interfacial properties, which indicated that the mildly fractioned proteins were able to form a strong and viscoelastic layer on the interface, providing protection against disruption and high compressive forces. The native state of the mildly fractionated protein was used to explain those results. Denatured protein as obtained after conventional fractionation or after applying an additional heating step resulted in an altered interface characteristics, which could explained increased flocculation and droplet coalescence. Overall, the results indicated the relevance of using mild conditions during fractionation. Mild fractionation, thereby shifting the focus from purity to functionality, could be a route to make novel ingredients, with more natural character in a sustainable manner.

1. Introduction

Many food product are prepared through mixing pure ingredients, such as proteins, oils and starch. Oil-in-water emulsions are made through dispersion small oil droplets into an aqueous phase. Those oil-in-water emulsions must be stable during distribution, retail and preparation at home. Often a combination of proteins and surfactants are used for this purpose (Lam & Nickerson, 2013). Nevertheless, proteins alone should be sufficient to stabilize an oil-water interface. In case of proteins from plant materials, fractionation process is required that maintains the beneficial functionality of proteins to stabilise emulsions.

Commercial yellow pea protein isolates (CPI) are obtained using an alkaline extraction-isoelectric precipitation method (Boye, Zare, & Pletch, 2010; Salome, Verrin, Fache, & Houard, 2007; Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015). In an alkaline extraction-isoelectric precipitation method, protein is fractionated through first solubilising at alkaline condition to remove the insoluble residues. Subsequently, the pH is adjusted to acidic conditions to precipitate protein and to remove soluble impurities. The pH of the CPI dispersion is adjusted to neutral pH and finally the CPI is (spray-)dried. Overall, this protein fractionation method uses chemicals (acids and bases) and high drying temperatures to allow complete disentanglement of the original structures and extract the individual components. However, pH and the heat treatment are linked to a loss in (native) protein functionality (Arntfield & Murray, 1981; Denmat, Anton, & Gandemer, 1999; Taherian et al., 2011; Wang & Corredig, 2011), and negative impact on the environment (Schutyser & van der Goot, 2011; van der Goot et al., 2016).

In literature, alternative methods are described to obtain plant-based protein isolates, like membrane-based extraction methods. Most of these plant-based protein isolates are reported to have improved emulsification functionality when compared to conventional protein fractionation as described above (Alamanou & Doxastakis, 1997; Boye, Aksay et al., 2010; Fuhrmeister & Meuser, 2003; Lam, Can Karaca, Tyler, & Nickerson, 2016; Taherian et al., 2011). Further, Fuhrmeister and Meuser (2003) showed that the emulsification functionality of a wrinkle pea protein isolate obtained using ultra filtration was enhanced compared with the conventional fractionation process. Nevertheless, all

* Corresponding author at: Bornse Weilanden 9, 6708WG Wageningen, PO Box 17, 6700 AA Wageningen, The Netherlands.
E-mail address: atzejan.vandergoot@wur.nl (A.J. van der Goot).

http://dx.doi.org/10.1016/j.foostr.2017.09.001
Received 10 May 2017; Received in revised form 30 August 2017; Accepted 18 September 2017
Available online 19 September 2017

alternative methods proposed still focussed on obtaining highly purified protein isolates.

Berghout, Boom, and Van Der Goot, 2014 proposed that purity might not be essential, since most food products contain more than one ingredient and even owe their attractive properties to the presence or/and interactions between different components. The focus on functional characteristics of the ingredients instead of purity also allows the use of mild conditions during fractionation, which better retain native functional properties of the components. A mild fractionation method was recently proposed for yellow pea yields fractions rich in protein or starch (Geerts, Mienis, Nikiforidis, van der Padt, & van der Goot, 2017; Pelgrom, Boom, & Schutyser, 2015), making use of the weak internal structure of the yellow pea. Fine milling of the yellow pea resulted in detachment of the starch granules from the protein matrix. When the yellow pea flour was suspended in water, it could be fractionated through a layer-by-layer separation using centrifugation forces. Similar fractionation process, based on suspending and subsequently settling, were described for other legumes (Cai, Klamczynska, & Baik, 2001; Czuchajowska & Pomeranz, 1993). The obtained soluble protein fraction (SPF) with modest purity (56 g protein/100 g dry matter) showed potential to be used as emulsifier. The protein content of the mildly fractioned SPF can be increased through the implementation of a dialysing or ultra-filtration membrane step (Pelgrom et al., 2015), thereby removing the additional solutes present. However, the question arises whether this addition purification step is necessary for the eventual emulsification properties.

The aim of this paper is therefore to compare the functional properties of mildly fractionated SPF and CPI. The differences in behaviours were related to state of the protein, the dynamic interfacial tension, interfacial rheology and protein composition at the oil-water interface. The emulsification stability was determined at acidic pH, under acceleration forces and a freeze-thaw treatment. Overall, the outcomes provides insides to what extent purification is essential for the emulsification properties.

2. Materials and methods

2.1. Methods

Pre-dried yellow peas (Pisum sativum) were purchased from Alimex (Sint Kruis, The Netherlands). Commercial pea protein isolate (NUTRALYS® F85 M) was provided by Roquette (Lestrem, France). Sunflower oil was obtained from the local supermarket and used without further purifying, except when determining the interfacial properties. Then, the oil phase was stripped using alumina (MP Alumina N-Super I, MP Biomedicals, Germany) as described by Berton, Genot, and Ropers (2011). Sucrose was obtained from Sigma-Aldrich® (Germany). The pH of the protein solutions was adjusted using 1 M NaOH and HCl (Sigma-Aldrich®, Germany). For all experiments, milliQ-water was used unless stated otherwise.

2.2. Methods

2.2.1. Preparation of pea flour

The pre-dried yellow peas were pre-milled into grits by using a pin mill (LV 15 M, Condux-Werk, Wolfgang bei Hanau, Germany) at room temperature. The pea grits were then milled with a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany) according to the method of (Pelgrom et al., 2015). The impact mill was set at a feed rate of 2 rpm, a speed of 8000, 4000 rpm, an airflow of 52 m^3/h and a classifier wheel speed of 4000 rpm. A thermometer inside the mill assured that the temperature remained between 16 and 34 °C.

2.2.2. Preparation protein fractions

2.2.2.1. Soluble protein fraction (SPF). A 20 wt.% pea flour suspension was prepared and stirred overnight at 4 °C. The suspension was subsequently centrifuged at 10,000*g* for 30 min at 20 °C. The supernatant containing the soluble pea protein fraction (SPF) was collected. The dry matter content was measured using an infrared moisture analyser (MA35, Saltorius AG, Germany). The supernatant was than diluted to obtain a protein solution of 1 wt.%.

2.2.2.2. Dialysed soluble protein fraction (dialysed SPF). The soluble protein fraction (SPF) was prepared as described above and dialysed (cellulose membrane, cut-off 14 kD, Sigma-Aldrich®). After dialysis, the sample was centrifuged at 10,000*g* at 20 °C for 30 min and the supernatant was collected. The dry matter content was measured using an infrared moisture analyser (MA35, Saltorius AG, Germany) and the protein content was adjusted to 1 wt.%.

2.2.2.3. Thermally treated soluble protein fraction (thermally treated SPF). A SPF solution containing 1 wt.% protein was heated till boiling (100 °C). The temperature of the solution was over 90 °C for approximately 5 min, which was indicated to effectively denature legume proteins (Palazolo, Sobral, & Wagner, 2011). Subsequently, the solution was cooled to room temperate before further use.

2.2.3. Compositional analysis

All SPF samples were dried in a freeze dryer (Christ, Germany) prior to determining the protein, starch, ash and oil content. The protein content was determined by using Dumas analysis (Nitrogen analyzer, FlashEA 1112 series, Thermo Scientific, Interscience, Breda, The Netherlands), using a conversion factor of 5.52 (Holt & Sosulski, 1979). The total starch concentration was determined with a Total Starch Amyloglucosidase/a-Amylase Assay Kit (Megazyme International Ireland Ltd, Bray, Ireland). Ash concentration was determined by AACC official method 08-01 (AACC, 1983). The oil concentration was determined with a fully automated Büchi extraction system B-811 LSV (Büchi Labortechnik AG, Flawil, Switzerland). Petroleum ether with a boiling range of 40–60 °C was used in Standard Soxhlet mode with a sample-to-solvent ratio of 1:6 for 7 h.

The protein composition was analysed with a size/exclusion Ultimate 3000 HPLC system (Thermo Scientific, MA, USA) equipped with a TSKgel G2000SWxl column (Tosoh Bioscience LLC, PA, USA). A mixture of 30 wt.% acetonitrile, 70% Milli-Q water with 0.1% Trifluoro Acetic Acid solution was used as running buffer. The flow rate of the running buffer was set at 1.5 mL/min and the UV detector at 214 nm. The molecular weight was standardized and calibrated by using the following purified proteins: ThyroGlobu (670 kD), β −Globulin (158 kD), Ovalbumin (44.3 kD), α-Lactalbumin (14 kD), Aprotinin (6.5 kD), Bacitracin (1.4 kD) and Phenylalanine (0.2 kD). The aqueous serum phase after emulsification was recovered through applying two consecutive centrifugation steps (3500 × *g*, 45 min, 20 °C and subsequently 18,000 × *g*, 45 min, 20 °C). The upper creamed phase was separated from the lower aqueous (serum) phase. The aqueous serum phase was collected and analysed by using HPLC-sec method. Subsequently, the protein content of the serum phase was determined using Dumas method.

2.2.4. Differential scanning colorimetry (DSC)

Differential Scanning Calorimetry (DSC) measurements were performed using a Diamond DSC (PerkinElmer, Shelton, USA) to determine the transition enthalphy and the denaturation temperature. The DSC analyser was calibrated with indium. An empty stainless steel pan was used as reference. Samples were made by suspending protein in water (20 g protein/100 g) and heated from 20 °C to 140 °C at 10 °C/min. Nitrogen was used as carrier gas. Measurements were analysed with Start Pyris Software (PerkinElmer, Shelton, USA). The measurements were performed twice.

2.2.5. Interfacial properties and rheology

The interfacial properties, both the dynamic interfacial tension and