Food Hydrocolloids 44 (2015) 12–22

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Functional analysis of mildly refined fractions from yellow pea

Pascalle J.M. Pelgrom, Remko M. Boom, Maarten A.I. Schutyser*

Food Process Engineering Group, Wageningen University, Bornse Weilanden 9, 6708WG Wageningen, P.O. Box 17, 6700AA Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 4 June 2014
Accepted 2 September 2014
Available online 16 September 2014

Keywords:
Dry fractionation
Pea
Phase separation
Gelatinization
Wet extraction
Protein functionality

ABSTRACT

Dry fractionation offers an attractive route to sustainably produce protein-enriched plant-based ingredients. For example, fine milling of peas followed by air classification separates starch granules from the protein matrix. Unlike conventional wet isolates, dry-enriched pea fractions consist of a mixture of protein, starch and fibre, but have the advantage that protein retains its native state. In this context, dry-enriched pea ingredients were assessed for their functionality in terms of gelatinization and phase behaviour. After suspension in water, starch, protein and fibre separated into distinctive layers. The top layers were concentrated by ultrafiltration into a native protein-rich concentrate with a purity of 67 g protein/100 g dry matter and a protein yield of 63%. Upon heat-induced gelatinization, gel firmness was mainly increased by the presence of starch, while the presence of dispersed components (i.e. protein and/or fibre patches) in the gel weakened its structure. The heating and cooling rates influenced the firmness of the gel prepared from flour. The fine fraction could be gelled by protein crosslinking using transglutaminase. The increased protein gel strength in the presence of dispersed fibre and starch was explained by their water absorption leading to concentration of the protein phase. In conclusion, all pea fractions could be used to prepare firm gels, despite their different compositions, which supports recent insight that development of novel food ingredients should focus on functionality rather than on molecular purity. Finally, the combination of dry and aqueous phase separation is proposed as a more sustainable route compared to conventional wet extraction processes.

1. Introduction

The replacement of animal protein by plant-based alternatives contributes to a more sustainable future food supply (Aiking, 2011). Not only the growing world population, but also the increasing prosperity in large parts of the world have an enormous impact on the global meat consumption. Because of the poor conversion efficiency, i.e. one kilogramme of animal protein can only be obtained by feeding six kilogrammes of plant proteins (Pimentel & Pimentel, 2003), meat production represents a severe burden on the available arable land, water and fossil fuels. Current generation plant-based meat analogues are produced from protein-rich ingredients obtained by wet extraction, which yields a relatively pure protein isolate, but at the expense of high water and energy consumption and loss of native protein functionality (due to dissolution, precipitation and drying). We explore the use of dry fractionation via milling and air classification as a more sustainable extraction route that demands less energy and retains native protein functionality, but with the disadvantage that it produces less pure protein fractions. Schutyser and van der Goot (2011) estimated that dry fractionation requires about 4.2–18 kJ/kg protein, while wet fractionation requires approximately 18 MJ/kg protein.

Dry fractionation of peas involves fine milling during which starch granules are liberated from a protein matrix that breaks in small fragments. During subsequent air classification, the protein fragments are separated from the starch granules on the basis of their size. A pea protein concentrate (fine fraction) is obtained with 50–55 g protein/100 g dry matter and a pea starch concentrate (coarse fraction) is obtained with ~67 g starch/100 g dry matter (Pelgrom, Vissers, Boom, & Schutyser, 2013). Both fine and coarse fractions can be characterised by their specific ratio between three main components: protein, starch and fibre. The application of both fine and coarse fractions may contribute to a more sustainable food production. Moreover, foods themselves usually consist of a mixture of protein, carbohydrate and fibre. Whereas there is a tendency in food industry to compose foods by blending refined ingredient isolates, many traditional foods owe their attractive properties to the presence of and interaction between different constituents in the raw material. For example, the phase separation between gluten and starch in flour determines the texture

* Corresponding author.
E-mail address: maarten.schutyser@wur.nl (M.A.I. Schutyser).

http://dx.doi.org/10.1016/j.foodhyd.2014.09.001
0268-005X/

development in bread to a large degree (Schutyser & van der Goot, 2011). Therefore, dry but not pure fractionated ingredients that retain their native functional properties (Sosulski, Hoover, Tyler, Murray, & Arntfield, 1985; Wright & Boulter, 1980) have large potential for preparation of foods. Here we present the results of our investigations of the interactions between starch, protein and fibre in dry-fractionated peas during phase separation and gelatinization, which are important indicators for practical application into solid, textured protein foods (e.g. meat analogues).

Air-classified native pea protein concentrates are highly soluble in water and are therefore interesting ingredients for preparing liquid high protein foods (Pelgrom, Vissers, et al., 2013). However, for solid foods a firmer texture is required. Wet pea protein isolates have been subject to many investigations to prepare gels (O'Kane, Vereijken, Gruppen, & Boekel, 2005; Shand, Ya, Pietrasik, & Wanasundara, 2007) and to make fibrous structures (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014), but have also been explored for foaming (Aluko, Mofolasayo, & Watts, 2009) or emulsifying (Karaca, Low, & Nickerson, 2011). In contrast, the phase separation or gelling behaviour of air classified pea fractions has received relatively little attention. Pea protein, starch and fibre will phase separate, when suspended in water. Both enthalpic and entropic effects can explain why different biopolymers phase separate when suspended in water (Elgadir et al., 2012). Phase separation between starch and gluten is, for example, observed when wheat flour is suspended in water (Czuchajowska & Pomeranz, 1993; Larsson & Eliasson, 1996). Air classified pea fractions form gels upon heating (Sosulski & Youngs, 1979; Swanson, 1990) and for legumes it is suggested that gelation depends on the type of proteins and on the non-protein components (Sathe & Salunkhe, 1981).

Heat-induced gelatinization has been widely investigated for mixed protein-starch systems. Protein unfolds and aggregates to form a structured matrix. A protein gel may then be formed due to non-covalent crosslinks via hydrophobic interactions, hydrogen bonds and electrostatic interactions (Totosaus, Montejano, Salazar, & Guerrero, 2002). Starch contributes to the gelatinization when starch granules swell upon hydration and leak amylose upon heating. Amylose then forms a network between the starch granules (Morris, 1990).

Heating mixtures of protein and starch isolates may lead to different types of gels. For example, corn starch mixed with whey protein isolate (ratio 50/50) at a dry matter content of 30 g/100 g results in a homogeneous network of leaked amylose and aggregated whey protein isolate in which the collapsed starch granules were tightly packed (Shim & Mulvaney, 2001). A similar gel structure is obtained when lentil protein isolate and lentil starch were mixed in a ratio of starch to protein of less than one, but, at higher starch concentrations, a non-homogeneous amylose network is formed, interrupted by protein-rich domains (Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014). Besides the protein and starch ratio, the heating and the cooling rates also influence gelatinization behaviour. Slow cooling allows protein more time to arrange into a network and fast heating slows down phase separation. These two factors favour the formation of a protein network (Nunes, Raymundo, & Sousa, 2006; Totosaus et al., 2002). Gelatinization can also be enzymatically induced by crosslinking proteins (Sun & Arntfield, 2011).

Air classification can provide fractions of varying protein, fibre and starch composition and with native protein functionality. Our study aimed to explore how pea fractions can be applied for preparation of solid structures, by analysing phase separation and gelatinization behaviour as a function of protein, fibre and starch content. Fractions were first characterised on their composition, rheological properties and phase separation when suspended in water. Subsequently, gelatinization behaviour induced by heating and/or enzymatic treatment was investigated by monitoring the gel strength and by confocal laser scanning microscopy (CLSM).

2. Materials and methods

2.1. Materials

Pre-dried yellow peas, *Pisum sativum*, were purchased from Alimex (Sint Kruis, The Netherlands). The yellow peas were specified by the supplier to contain 10–15 g water/100 g, 23 g protein/100 g, 62 g carbohydrate/100 g (of which 44 g starch/100 g), 2 g oil/100 g, and 3 g ash/100 g. Pea protein isolates (NUTRALYS® F85G) and pea starch isolates (PEA STARCH N—735) were obtained from Roquette (Lestrem, France). Transglutaminase Activa® WM (mTG, Ajinomoto Inc., Tokyo, Japan), with an activity of 100 units/g, was used to crosslink proteins. All experiments were done in duplicate.

2.2. Material preparation

Peas were pre-milled into grits with a pin mill at room temperature (LV 15M, Condux-Werk, Wolfgang bei Hanau, Germany). Subsequently, the grits were milled into pea flour using a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany). The mill contains an internal rotating classifier wheel that allows the passage of fine particles while coarse particles are retained in the chamber. The classifier wheel speed was set at 4000 rpm, the air flow at 52 m^3/h, the impact mill speed at 8000 rpm, and the feed rate at 2 rpm (circa 0.75 kg/h). A thermometer inside the mill indicated that the temperature in the mill was between 16 and 34 °C due to varying ambient air temperatures.

A fine and a coarse fraction were made by air classifying the flour in an ATP50 classifier (Hosokawa-Alpine, Augsburg, Germany). The air flow was fixed at 52 m^3/h, the classifier wheel speed at 6000 rpm, and the feed rate at 20 rpm (circa 1 kg/h) (Pelgrom, Vissers, et al., 2013).

2.3. Phase separation

The pea flour, and the coarse and fine fraction were further fractionated by aqueous phase separation. Solutions of 20 g/100 g were stirred for 30 min at room temperature and were centrifuged at 4500 rpm for 30 min. This set of parameters yielded clear phase separated layers based on visual observation. The layers were separated manually. The upper two layers of the fine fraction, with a dry matter content of 5 g/100 g sample, were concentrated in a stirred Amicon ultrafiltration cell (Millipore Corporation, Billerica, MA, USA) with a 5 kDa regenerated cellulose membrane (Millipore Corporation, Billerica, MA, USA). Batch filtration was carried out at a pressure of 380 kPa until a final solids concentration of 30 g/100 g.

2.4. Compositional analyses

The dry matter content was determined by drying 1 g of sample overnight in an oven at 105 °C (Pelgrom, Schutyser, & Boom, 2013).

The protein content was obtained by Dumas analysis (Nitrogen analyzer, FlashEA 1112 series, Thermo Scientific, Interscience, Breda, The Netherlands). A conversion factor of 5.4 for pea protein was used.

The protein composition was analysed by a non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using a Bio-Rad Mini-Protean 3 cell (BioRad Laboratories, Herculas, California, USA). Samples were prepared by mixing 100 μL of sample solution (1 g protein/100 ml) with 200 μL of sample buffer solution. 15 μL of each sample and a broad range marker