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Aqueous fractionation processes of soy protein for fibrous structure formation

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ABSTRACT

Desired properties of ingredients differ for various applications. Here, we use a reverse engineering approach to obtain soy protein fractions targeted for the application of meat analogs. Aqueous fractionation was used to produce these soy protein fractions, which were structured with simple shear flow deformation while heating. The water holding capacity (WHC), nitrogen solubility index (NSI), enthalpy of transition, and viscoelastic properties were determined. We found that a soy protein fraction/full fat flour blend resulted in distinct fibrous structures but only when the soy protein fraction was toasted at 150 °C. At this optimum toasting temperature (150 °C), the protein fractions had a high WHC, intermediate NSI and its viscoelastic property was characterized as G^* between 1 and 10 kPa. These functional properties were shown to be key for fibrous structure formation, whereas, the influence of the state of the proteins was limited.

Industrial relevance: The market for meat analogs is growing. Nowadays, most of the meat analogs are produced with soy protein concentrates and isolates. These concentrates and isolates are obtained with conventional fractionation processes that involve organic solvents to extract the oil first. As a result, the application of these ingredients is limited, *e.g.* the product cannot be classified as organic. In this study, we therefore investigated aqueous fractionation of soy to obtain a soy protein fraction with desired functionality that can be used for the application of meat analogs and satisfy the values of consumers.

1. Introduction

Ingredient production often aims at general applications, which requires defined chemical composition, and a stable product form such as powder. Traditionally, solubility is the targeted functional property for protein ingredients to allow applicability in drinks, emulsions and doughs (Zayas, 1997). Currently, meat analogs is a growing application area. For this application proteins should not solubilize in water, but bind water to allow the creation of a structure. Hence, considering a specific application, one could end up with other or additional requirements for functional properties. Therefore, it can be stated that modern fractionation methods should be designed while keeping a final application in mind. Here, we use fibrous protein structures, which could form the basis for meat analogs as an example to demonstrate a reverse engineering approach to develop ingredients. Soy flour is taken as starting material, because soy-based ingredients are used in many meat analog products currently on the market (Boland et al., 2013; Malav, Talukder, Gokulakrishnan, & Chand, 2015).

Nowadays, meat analogs that are mimicking the fibrous structure of meat are produced with two extrusion processes; low moisture or high moisture. The extruder is used to form the fibrous structure, which is further process into a full meat analogy by freezing and frying (Giezen, Jansen, & Willemsen, 2014). Another innovative technique based on simple shear flow deformation while heating was introduced a decade ago to produce fibrous structures from caseinate (Manski, van der Goot, & Boom, 2007). This concept was later applied to structure soy protein concentrate with a relatively high moisture content (55 wt%) (Grabowska et al., 2016). For fibrous structure formation with high moisture content, it is known that water absorption and gelling are important properties (Asgar, Fazilah, Huda, Bhat, & Karim, 2010; Singh, Kumar, Sabapathy, & Bawa, 2008). Besides protein, other components can contribute to this functionality as well, and might even be required, given the hypothesis that a two phase system is a needed for structuring plant proteins into fibers (Cheftel, Kitagawa, & Queguiner, 1992; Grabowska et al., 2016).

Meat analogs that are currently on the market are consists of

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commercially available protein concentrates and isolates. These concentrates and isolates are mostly prepared with a conventional fractionation process of soy beans, which primary aim was the extraction of oil (Islas-Rubio & Higuera-Ciapara, 2002). Oil is extracted from the soybean meal with organic solvents, but the consumer acceptance of those solvents is decreasing amongst others for environmental reasons (Dunn, Wells, & Williams, 2010). The defatted soy flour is then further processed into protein concentrates or isolates for food applications (Day, 2013; Mulder, can der Peet-Schwering, Hua, & van Ree, 2016). Partial oil extraction can also be achieved using aqueous fractionation, which might be preferred by current consumers of meat analogs, who are caring about the environment (Hartmann & Siegrist, 2017).

Aqueous fractionation is a method in which milled soy flour is mixed with excess water. The application of a centrifugation step yields three phases; *i*) a cream layer, which is rich in oil, *ii*) a liquid phase, which is rich in protein, and *iii*) a pellet, rich in insoluble fibers. The aqueous phase can be used as starting material for protein fractionation by acid precipitation (Campbell et al., 2011; de Almeida, de Moura, & Johnson, 2014; Russin, Boye, Arcand, & Rajamohamed, 2011). Soy beans or soy flour are often toasted to decrease the enzyme activity and anti-nutritional factors (Kakade, Rackis, McGhee, & Puski, 1974), but this toasting step also lowers the solubility (Onimawo & Akpojovwo, 2006; Wu & Inglett, 1974). Clearly, reduced solubility is undesired in the first steps of the fractionation process, because the method is based on solubility (Berk, 1992).

In this study, an aqueous soy protein fractionation process was designed specifically targeted to make fractions that can be structured into fibers for the application of meat analogs. The aim of this study was to reveal the essential functional properties of ingredients required for fibrous structure formation. To reveal these functional properties, a reversed engineering approach was used. Various soy protein fractions were mixed with water, and structured with a high temperature shear cell. We determined the functional properties of fractions in terms of: water holding capacity, nitrogen solubility, and enthalpy of transition (state of the proteins). The viscoelastic properties of the soy protein fractions in water were determined at similar conditions as used during the structuring process. The fractions were compared with commercially available soy protein concentrates (SPC) and isolates (SPI).

2. Materials and methods

2.1. Material

Soy protein isolate (SPI, SUPRO® 500E IP) and soy protein concentrate (SPC, Alpha 6 IP) were both obtained from Solae (Europe S.A.). The manufacturer's specifications indicated that the SPI contained at least 83.4 wt% protein and SPC contained at least 63.1 wt% of protein (N × 5.7). The pH was adjusted using HCl and NaOH, both purchased from Sigma Aldrich (Germany).

2.2. Methods

2.2.1. Preparation of soy flour

Soybeans were pre-milled into grits using a pin mill (LV 15 M, Condux-Werk, Wolfgang bei Hanau, Germany). Subsequently, the soy bean grits were milled using a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany). The impact mill was set at a feed rate of 2–5 rpm, a speed of 8000 rpm, an airflow of 80 m^3/h and a classifier wheel speed of 2500 rpm. A thermocouple inside the mill was used to monitor the temperature, which remained between 16 and 34 °C.

2.2.2. Aqueous fractionation

A soy protein fraction was prepared by suspending soy flour in water (20 wt%) and adjusting the pH between 8 and 9 with 1 mol/L NaOH solution. The suspension was stirred for 1 h and subsequently centrifuged (10,000*g*; 30 min; 20 °C). The aqueous samples were poured through a cheese-cloth to separate the cream layer from the supernatant. The supernatant was collected and the pH was adjusted to 4.8 with 1 mol/L HCl. The solution was stirred for at least 1 h and subsequently centrifuged (10,000*g*; 30 min; 20 °C). The pellet was collected and neutralized (pH 6.5–7) with 1 mol/L NaOH and subsequently freeze dried (Christ, Germany). In this study, the supernatant was discarded.

2.2.3. Toasting

Soy flour and the soy protein fractions were toasted by spreading the powders over an oven tray ensuring an equal distribution of the flour of around 5–10 mm thick. The oven tray was placed in an Heratherm oven (Thermoscientific, USA) at various temperatures (50–200 °C) for 15 min. Subsequently, the samples were cooled till room temperature and stored in a closed container at 4 °C up until further use.

2.2.4. Shear-induced structuring with a high temperature shear cell

A high temperature shear cell was used to structure the soy protein fractions. The soy protein fractions alone and soy protein fractions mixed with soy flour powder in a ratio 70/30 were used for structuring. For the preparation of the sheared samples, salt (1 wt% NaCl in the total blend) was dissolved in demineralized water. The powders were then added to the salt solution and thoroughly mixed to obtain a mixture with 44% dry matter. A hydration time of 30 min was used. The hydrated materials were placed into a preheated high temperature shear cell (HTSC) at 140 °C, and sheared for 15 min at 30 rpm. The HTSC was developed in house (Grabowska et al., 2016; Peighambardoust, Avd, Hamer, & Boom, 2004). It consists of a rotating bottom cone and a stationary cone. An oil bath (JULABO LH46, USA) filled with Thermal H10 oil (JULABO, Germany) was used to heat and cool the cones, while a thermocouple measured the temperature inside the cone. A Haake drive (Haake Polylab QC, Germany) was used to control rotation speed (at 30 rpm). The HTSC was cooled down to room temperature within 5 min, before the samples were taken out. Samples were kept at room temperature for 1 h prior to tensile strength analysis.

2.2.5. Tensile strength analysis

The degree of anisotropy was determined by cutting tensile bars parallel and perpendicular to the shear flow. From each sample, three tensile bars were taken in parallel and perpendicular. A texture analyzer (INSTRON 5564, USA) was used to deform the samples with a constant deformation rate of 1 mm/s. Samples were placed between two sand-coated clamps at a distance of 15.5 mm. From the stress-strain curve we determined the Young's modulus, tensile stress and tensile strain at rupture. The ratio between the measures (Young's modulus, tensile stress, tensile strain) in parallel and perpendicular direction is referred to as the anisotropic index (AI), which is used as an indication of fibrousness of the material.

2.2.6. Composition analysis

The protein content was determined using Dumas analysis (Nitrogen analyzer, FlashEA 1112 series, Thermo Scientific, The Netherlands), using a conversion factor of 5.7. Ash content was determined by AACC official method 08–01 (AACC, 1983). The oil content was determined with a fully automated Büchi extraction system B-811 LSV (Büchi Labortechnik AG, Flawil, Switzerland), using petroleum ether as extraction solvent. The duration of the extraction step was set at 7 h. The carbohydrates were determined by difference.

2.2.7. Water holding capacity & nitrogen solubility

The water holding capacity (WHC) and nitrogen solubility index (NSI) of the soy protein fractions and full fat flour were determined with a 2 wt% dispersion. The dispersions were thoroughly mixed and shaken overnight. Next, the dispersions were centrifuged (10,000*g*, 30 min, 20 °C), and the supernatant and pellet were separated. The