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Investigation on solubility, interfacial and emulsifying properties of pumpkin (*Cucurbita pepo*) seed protein isolate

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ABSTRACT

Pumpkin (*Cucurbita pepo*) seed protein isolate (PSPI[1]) with protein content of 94.3 g/100 g was obtained by alkali extraction with isoelectric precipitation. Functional properties of PSPI such as solubility, interfacial and emulsifying properties were tested as a function of pH (3–8), ionic strength ($\mu = 0–1$ mol/dm^3 NaCl) and PSPI concentration. PSPI solubility had a minimum at pH 5 and reached maximal values in alkali region at pH 8. Increase in PSPI suspension concentration led to a decrease in solubility yields at pH 3 and 8, while at pH 5 solubility yield was independent of PSPI suspension concentration. Addition of NaCl caused pronounced salting–out effect at pH 3 and slight salting–in effect at pH 5 and 8. PSPI adsorption at both air/PSPI solution and oil/PSPI solution at all pH and ionic strengths tested was evidenced by an increase in surface and interfacial pressure. Nevertheless, PSPI failed to stabilize 20% oil in water emulsions at pH 5, when phase separation occurred immediately after emulsion preparation. The most stable emulsions, regardless of ionic strength, were obtained at pH 8. All emulsions were susceptible to creaming instability.

1. Introduction

Land plants have always been part of the human diet to provide energy and nutrients for sustainable living. However, indirect consumption, through animal foodstuff, puts huge pressure on the environment. Namely, the inherently inefficient conversion of plant protein into animal protein makes a mere 15% of protein and energy in these crops to ever reach human mouths (indirectly), and 85% are wasted. Therefore, when the supply of animal proteins reaches maximum production capacity better and more efficient utilization of plant proteins will become crucial (Day, 2013).

Cereal grains provide the world with a majority of its food calories and about half of its protein, whereas oilseeds are grown as a source of oil. Nonetheless, oilseeds are considerably higher in protein content than cereal grains (Potter & Hotchkiss, 1995).

Pumpkin (*Cucurbita* sp.) seeds, as one of oilseeds, are gaining attention because they are a very good source of proteins, which content ranges from 24.5 to 36% (Quanhong & Caili, 2005) as well as of oil with content of 31.5%–51% in pumpkin seed kernel (Rezig et al., 2013). The growing interest in the use of pumpkin seed oil is due to its high content of unsaturated fatty acids and other bioactive components which have positive effect on human health in many different ways: they have anti–inflammatory and diuretic properties, alleviate negative symptoms of benign prostatic hyperplasia, help lower cholesterol levels, bind free radicals and decrease the risk of certain forms of cancer (Rabrenović, Dimić, Novaković, Tešević, & Basić, 2014). Owing to increased pumpkin seed oil production, large amount of pumpkin seed cake is being produced as a by–product. Protein content in the pumpkin seed cake can reach up to 60–65% (Vaštag, Popović, Popović, Krimer, & Peričin, 2011). These valuable proteins could be transferred to value–added products such as protein concentrate or isolate and be found as functional ingredients of various products or as a nutritional supplement. However, pumpkin seed oil cake is being used only as low–rated product, livestock feed, so far.

Characterization studies on pumpkin (*Cucurbita* sp.) seed proteins have shown that their major protein fraction is represented by a 12S globulin, called cucurbitin, homologous to those reported in legume seeds (Rezig et al., 2013). Cucurbitin molecule has a

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[1] Pumpkin seed protein isolate.

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molecular weight of 325 kDa and is composed of six similar subunits of molecular weight 54 kDa. These subunits, in turn, contain two disulfide linked polypeptide chains of molecular weights approximately 33 kDa and 22 kDa (Colman, Suzuki, & van Donkelaar, 1980; Marcone, Kakuda, & Yada, 1998). In addition to 12S globulin fraction, Blagrove and Lilley (1980) detected small amounts of 18S globulin fraction which had proved to be dimer of 12S components.

Pumpkin seed globulins are accompanied by albumins and these two protein fractions together make 59% of total crude protein content in pumpkin seed (Fruhwirth & Hermetter, 2007), though albumins are present in considerably smaller amounts (Marcone et al., 1998). 2S albumin is protein with molecular weight around 12.5 kDa composed of two disulfide linked polypeptide chains, a small chain with molecular weight of about 4.8 kDa and a big chain with molecular weight of about 7.9 kDa (Fang, Wong, Lin, & Ng, 2010).

Functional properties of proteins from many plants are being thoroughly investigated. The behavior of proteins during processing, production and storage is influenced by their molecular characteristics (flexibility, conformational stability and distribution of hydrophobic and hydrophilic moieties) which are closely related to external factors such as pH, ionic strength and temperature (Moure, Sineiro, Domínguez, & Parajó, 2006). It was often found that large molecular weight, size and poor water solubility of plant proteins limit utilization of these proteins as functional components of food, cosmetics and pharmaceutical industry products (Day, 2013). Still, proteins are of particular interest in terms of their emulsifying properties, due to their amphiphilic structure, film–forming abilities (Lam & Nickerson, 2013) and the fact that they are natural emulsifiers.

Research on functional properties of pumpkin seed proteins is still in a rather pioneering stage and thus far was mostly related to the solubility studies. Rezig et al. (2013) determined that solubility of pumpkin seed proteins is rather low (<20%) in the acidic pH region (pH < 5), but it drastically increases at pH above pH 6, while it reaches maximal values at pH above 7 (70–80%). As for pumpkin seed globulins maximal solubility of 91% was obtained at pH 7.69 and NaCl concentration of 3.99% (Peričin, Radulović, Trivić, & Dimić, 2008). Denaturing temperatures for pumpkin seed proteins are rather high, and were found to be slightly above 90 °C, what makes them suitable for specific products in which protein native form is needed, because they can resist higher temperature during processing (Rezig et al., 2013).

Information about other functional properties of pumpkin seed proteins is quite scarce. Therefore, the aim of this work was to investigate solubility, interfacial, and emulsifying properties of pumpkin seed protein isolate (PSPI[1]) under different environmental conditions (i.e. pH, ionic strength and PSPI concentration).

2. Materials and methods

2.1. Materials

Pumpkin (*Cucurbita pepo*) seed oil cake was obtained from "Agrojapra", Bosnia and Hercegovina. It was stored at the temperature of 4 °C and ground in a coffee–grinder before use. Sunflower oil was obtained from "Vital" Serbia. Demineralized water was used as a solvent. All other chemicals used were obtained from "Centrohem d.o.o.". Serbia, and were of at least extra pure quality. Buffer solutions were prepared by mixing 0.2 mol/dm^3 di–sodium hydrogenphosphate and 0.1 mol/dm^3 citric acid in proportions defined for each pH.

2.2. Preparation of pumpkin seed protein isolate

Pumpkin seed protein isolate (PSPI) was prepared by alkali extraction with isoelectric precipitation. Firstly, the grounded pumpkin seed oil cake was defatted with hexane (mass ratio 1:5) in two stages and let for air drying at room temperature. The defatted pumpkin cake was suspended in alkali solution at pH 10.00, which was set by 1 mol/dm^3 NaOH, at room temperature to allow protein dissolution. After 30 min of gentle stirring the slurry was filtered. The dissolved proteins in the filtrate were precipitated by adjusting pH to 5.00 with 1 mol/dm^3 HCl. The precipitate was separated from the liquid phase by centrifugation at 4 °C and 10000 rpm for 20 min and dried at temperature of 30 °C for 48 h. In the end, dried protein precipitate was ground in a coffee grinder to obtain PSPI powder.

2.3. Determination of protein content in dry materials and protein recovery

Protein content in pumpkin seed oil cake, c_{OC}, and in PSPI, c_{PSPI}, was determined by Kjeldahl method and it was expressed as g/100 g (AOAC method, 2005). Protein recovery, R, was calculated as

$$R(\%) = (m_{PSPI} * c_{PSPI})/c_{OC}$$

where m_{PSPI} is mass of PSPI recovered from 100 g of pumpkin seed oil cake.

2.4. Determination of PSPI solubility

PSPI suspensions of different concentrations (c_{susp} = 1–10 g/100 cm^3) were prepared by suspending required amount of PSPI in a buffer solution of different pH (3–8) and ionic strength (0–1 mol/dm^3 NaCl). Suspensions were placed in a water bath at 50 °C for 1 h with constant stirring, in order to allow dissolution of PSPI proteins. Soluble proteins were separated from undissolved particles by Sartorius membrane filtration (filter pore size 0.45 μm) to obtain PSPI solution. Concentration of dissolved proteins in PSPI solution, c_{sol}, was determined by Lowry, Rosenbrough, Fair, and Randall (1951) method and was expressed as g/100 cm^3. PSPI solubility, s, was calculated as

$$s(\%) = (c_{sol}/c_{susp}) * 100$$

2.5. Tensiometry

Surface tension (air–water interface) and interfacial tension (oil–water interface) of PSPI solutions were determined at 25 °C according to the *Du Noüy* ring method using a Sigma 703D tensiometer (KSV Instruments Ltd., Finland). The ring was immersed in PSPI solution (20 ml) and the surface was left to equilibrate for 10 min. For interfacial tension measurements, after ring immersing in the solution, 20 ml of sunflower oil was carefully added on top of the solution surface and the interface was left to equilibrate for 10 min. Upon surface/interface equilibration, surface/interfacial tension was measured. The reported values of surface and interfacial tension are the mean values of at least three measurements. Surface tension of buffer solutions of pH 3–8 was 71.7 ± 0.4 mN/m. Interfacial tension of buffer solutions of pH 3–7 was 28.4 ± 0.9 mN/m, whereas at pH = 8 it was 25.7 ± 1 mN/m. Measured surface/interfacial tensions of protein solutions were used to calculate corresponding surface/interfacial pressures: