Functional properties of Chinese yam (*Dioscorea opposita* Thunb. cv. Baiyu) soluble protein

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Abstract In this study, yam soluble protein (YSP) was extracted from Chinese yam (*Dioscorea opposita* Thunb. cv. Baiyu) and the functional properties were investigated under the influence of pH and ionic strength. As results, YSP was highly soluble and had better emulsifying activity over a wide range of pH. The solubility of YSP decreased in 0.5 and 1.0 M NaCl solution. An increment in NaCl concentration reduced the emulsion activity index and emulsion stability index of YSP. The oil absorption capacity of YSP was 3.2 ml/g protein. With the increase of pH, the foam capacity (FC) and stability (FS) decreased and then increased. FS of YSP increased as the salt concentration increased from 0 to 0.5 M, and then decreased. The minimal gelation concentration of YSP was 8% (w/v) and 10% YSP gel (w/v) had maximum gel strength in 0.1 M NaCl. These results suggested that YSP produced by acid precipitation may be used as a protein source with remarkable functional properties.

Keywords Yam soluble protein · Composition · Functional properties · pH · NaCl

Introduction

Crop plants including yam are indispensable part of human nutrition used for ages. Different parts of crop plants have been used not only for nutrition purposes but also to meet personal and social needs such as curing diseases, beautifying the planet etc. (Bozokalfa et al. 2016; Zorenc et al. 2016). Yams (*Dioscorea* spp.) are an important tuber crop in Africa, Asia, and Middle and South America. General compositions (dry-weight basis) of 75–84% starch, 6–8% crude protein, and 1.2–1.8% crude fiber had been reported (Liu and Lin 2009). Yam tubers contain functional components such as dioscin, diosgenin, allantoin, choline, and proteins (Hooker 2004). Dioscorins, the major storage proteins in yam tubers, contribute to approximately 85% of the total soluble protein content of the tubers. In the past two decades, many studies showed that dioscorin exhibited biological activities both in vitro and in vivo, including the enzymatic, antioxidant, antihypertensive, immunomodulatory, lectin and airway epithelial cell protecting activities (Xue et al. 2012).

With the increase of protein requirement, novel proteins have been extracted from various sources. It is ideal to obtain proteins with both outstanding functionality and bioactivity, such as rapeseed protein isolate, with free radical-scavenging activity (Yumiko et al. 2008), and sweet potato protein, with trypsin inhibitor activity (Sun et al. 2012). Important protein-based functional properties include protein solubility, water and oil absorption, emulsion capacity, foaming capacity and gelation capacity. Functional properties of proteins, in general, are affected

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by various intrinsic and extrinsic factors. Protein molecular structure, size, net charge and surface hydrophobicity are important intrinsic factors, whereas extrinsic factors include the method of protein extraction, pH, ionic strength, and the components in the food system and processing conditions (Moure et al. 2006).

Although the content of protein is not high in yam, the resource of yam is rich in Asia. However, little information about the functionality of yam soluble protein (YSP) has been reported, which might primarily affect its utility in food systems. This study aimed to evaluate YSP's potential as food ingredients by studying its chemical composition and functional properties, such as soluble, water- and oil-holding, emulsifying, foaming and gelation properties.

Materials and methods

Materials

Rhizoma of yams (*Dioscorea opposita* Thunb.) were bought from a local market (Shenyang, China). When arriving to the laboratory, the yams were sorted at room temperature until use. Unless otherwise stated, all reagents used in this study were of reagent grade.

Extraction of crude protein from yam

YSP was prepared by alkali-extraction and acid-precipitation. Yams were washed, peeled, cut, mixed with distilled water (containing 1% sodium bisulfite, 3 L/kg of fresh yam) and adjusted to pH 9 using 1 M NaOH. After ground and stirred with a magnetic stirrer at 4 °C for 20 min, the slurry was centrifuged at 5000g for 30 min. After filtering the supernatant through a double-layer cheese cloth, pH of the filtrate was adjusted to approximately 3.5 (the pH where, based on our preliminary study, highest amount of the YSP was precipitated) using 2 M HCl, and magnetically stirred for 1 h. The slurry was then centrifuged at 5000g for 30 min at room temperature. YSP was prepared by resolubilizing the pellet in distilled water and then adjusted to pH 7 using 1.0 M NaOH, ultrafiltrated and lyophilized. The protein powder was kept in a desiccator until use (Mu et al. 2009).

Proximate composition

The crude protein, total sugar, crude lipid, crude fiber and ash contents of the YSP were determined according to the standard method of analysis (AOAC 2005).

SDS-PAGE analysis

SDS-PAGE analysis was carried out using a modified version of the method of Shiu et al. (2013). The sample was prepared by dissolving the YSP (1 mg/mL) in 2 × sample buffer with or without β-mercaptoethanol (β-ME), and heated in boiling water for 5 min. SDS-PAGE electrophoresis was performed on a 12% acrylamide resolving gel and a 5% acrylamide stacking gel. Approximately 15 μL of the sample solution was loaded onto the gel. The electrophoresis was conducted at 130 kV for 110 min. After the gel was soaked in methanol-based coomassie blue, protein bands were visualized by destaining the background color of the gel using acetic acid in methanol.

Intrinsic fluorescence intensity

Intrinsic fluorescence intensity was determined as described by Agboola and Aluko (2009) with some modifications. FluoroMax-4 spectrofluorometer (Horiba Jobin–Yvon Inc., Edison, N.J., USA) was used to determine the intrinsic fluorescence for 0.05% (w/w) protein solutions at a constant excitation wavelength of 275 nm using a slit width of 2.5 nm as a function of emission wavelength between 285 and 450 nm using a 5.0 nm slit width at increments of 0.5 nm. This technique measures the fluorescence of the aromatic amino acids tyrosine, tryptophan and phenylalanine. The maximum fluorescence intensity (F_{max}) was determined for each spectra captured.

Protein solubility

Protein solubility of the YSP was measured according to the method of Casella and Whitaker (1990). The YSP was solubilized in distilled water and NaCl solutions (0.1 M, 0.5 M, 1.0 M) (1% YSP, w/v) by mixing with a vortex. After adjusting the pH from 1 to 10 using 0.5 M HCl or 0.5 M NaOH, the YSP solutions were magnetically stirred for 1 h and centrifuged at 8000 g for 20 min. Protein concentrations of the supernatants were measured according to the method of Markwell et al. (1978) with bovine serum albumin as the standard. Protein solubility was calculated as (protein content in the supernatant)/(protein content in the YSP solution) × 100% (Mu et al. 2009).

Water- and oil-holding capacity

Water and oil absorption capacities were measured by the method of Beuchat (1977). 1 g of sample was mixed with 10 mL distilled water or oil (Peanut oil) for 30 s. The samples were then allowed to stand at room temperature (30 ± 2 °C) for 30 min before centrifuging at 5000g for

30 min. The volume of supernatant was noted in a 10 mL graduated cylinder.

Emulsifying properties

YSP solutions (1%, w/v) in distilled water and NaCl solutions (0.1, 0.5, 1.0 M) of different pH were prepared as described above before forming emulsion with peanut oil.

Emulsifying activity index (EAI) of the YSP was determined according to the method of Pearce and Kinsella (1978).

The emulsion stability index (ESI) of the YSP was estimated by measuring the turbidity of the emulsion at 500 nm immediately after emulsion formation and after heating at 100 °C for 30 min (Aluko and Yada 1995). ESI was calculated as the turbidity ratio (%) between heated emulsion and newly-formed emulsion.

Foaming properties

The tests on foaming capacity (FC) and stability (FS) were based on the method of Sze-Tao and Sathe (2000). YSP (500 mg) was mixed with water (25 mL). The mixture was blended for 2 min, and was immediately transferred into a 100 mL graduated cylinder. FC and FS were then calculated according to the following formulae:

Foam capacity (%) = (volume after whipping-volume before whipping) mL/(volume before whipping) mL.

Foam stability (%) = (volume after standing for 30 min-volume before whipping) mL/(volume after whipping-volume before whipping) mL.

FC and FS of the YSP were measured at different pH values (pH = 1–10) and NaCl concentrations (0.1, 0.5, 1.0 M).

Gelation properties

Gelation properties were investigated using the method of Coffman and Garcia (1977). 10 mL of sample suspensions of 2–14% (w/v) were prepared in distilled water in a test tube. The dispersion was thoroughly mixed on a vortex mixer for 5 min and then heated in a boiling water bath for 1 h, followed by rapid cooling in a bath of cold water. The test tubes were cooled further at 4 °C for 2 h. The least gelation concentration was taken as the concentration when the sample from the inverted tube did not fall or slip. YSP suspensions of 10% (w/v) were prepared in distilled water and NaCl solutions (0.1, 0.5, 1.0 M). The gel strength was determined by a CT3 texture analyzer (Brookfield Company, USA).

Gel microstructure was studied on freeze-dried gel samples by using scanning electron microscope (SEM)

(Hitachi High Technologies Corp., Tokyo, Japan). 10%, w/v Gel samples were prepared as described above, and freeze-dried. Freeze-dried gels were fractured to expose the cross-section area and attached to the aluminum stub of the SEM with the help of a double-sided adhesive tape. After gold sputtering, the gel samples were viewed under SEM at an accelerating potential of 10 kV.

Statistical analysis

All experiments were triplicated, and the data are presented as mean $\pm$ standard deviation. Data were subjected to analysis of variances, and Duncan's multiple range test was used to separate the means.

Results and discussion

Proximate composition

YSP was composed of 65.31% protein, 2.30% fat, 1.62% crude fiber, 3.28% ash and 18.52% total carbohydrate. Proximate composition of the sweet potato protein (SPP) was composed of 87.0% protein, 0.6% fat, 0.16% crude fiber, 2.19% ash and 1.56% total carbohydrate (Mu et al. 2009). The total carbohydrate content of YSP was considerably higher than that of SPP, which might be caused by the fact that YSP is a glycoprotein and non-specific binding might have occurred between YSP and polysaccharide (Nagai and Nagashima 2006).

SDS-PAGE

As shown in Fig. 1a, without β -ME, there were 2 protein bands, while in the presence of 2-ME, there was only one single protein band with molecular weight of 29 kDa. This result was consistent with previous reports (Liu and Lin 2009; Harvey and Boulter 1983) suggesting that dioscorin contained an intramolecular disulfide bond.

Intrinsic fluorescence intensity

As shown in Fig. 1b, Fmax of YSP was the lowest at pH 4 (34.2) but increased significantly as the pH increased from 5 to 10 (61.6–109.5). The Fmax of YSP within an acidic pH range indicates a closed protein conformation and aggregation resulting from the neutralization of electrostatic charges as pH decreased towards its isoelectric point at pH 3.5. The Fmax of YSP within an alkaline pH range indicated more open structures thus the internal hydrophobic groups were exposed, while the aromatic amino acids may have produced more fluorescence signals (Nakai et al. 1986).

Fig. 1 Coomassie-stained SDS-PAGE gel (12% acrylamide) containing YSP (a). Left-right: Molecular weight (MW), Marker (M), YSP with β -ME (Lane 1), YSP without β -ME (Lane 2). Maximum

fluorescence intensity (Fmax) of YSP at pH 1–10 (b), and aromatic amino acids distribution in dioscorin (c): green (Phe), blue (Trp) and yellow (Tyr). (The image was generated by “Pymol 1.3” software)

The crystal structure of dioscorin (PDB code: 4TWM) was reported earlier by us (Xue et al. 2015), and the aromatic amino acids distribution of dioscorin is shown in Fig. 1c. As we can see, Phe46, Phe146, Phe182, Phe230, Phe234, Phe236 and Tyr235 are on the surface of the protein which can produce fluorescence signals directly. While residues Phe109, Phe133, Trp25, Tyr89 and Tyr176 were located in the deeper position of the protein which may be involved in the generation of fluorescence signals with the change of pH.

Protein solubility

Protein solubility of the YSP in distilled water, NaCl solutions (0.1, 0.5, 1.0 M) as a function of pH is shown in Fig. 2. In distilled water, YSP had minimum solubility (32.74%) at pH 3, as pH shifted away from the pI (3.5), solubility of the YSP in distilled water increased drastically ($P < 0.05$). At pH 1 and 10, 56.45 and 86.99% protein were soluble, respectively. Proteins have net positive charges at low pH and have net negative charges at high pH, which leads to an electrostatic repulsive force. The electrostatic repulsive force helps maintain protein molecules apart, destroy the native protein structure, transfer the equilibrium into the unfolded form and then reveal the buried functional groups in protein molecules, thus lead to an increase in protein solubility. In contrast, near the pI, proteins aggregate due to strong intermolecular interactions, resulting in less interaction with water and thus reducing protein solubility (Yuliana et al. 2014; Mu et al. 2009). At pHs above 8, solubility did not increase significantly with the increase of pH, because extreme alkali pH is detrimental to protein quality as it destroys Lysine (Sathe

Fig. 2 Protein solubility determined in YSP solubilized in distilled water (filled rectangle), 0.1 M NaCl (filled circle), 0.5 M NaCl (filled triangle) and 1 M NaCl (filled diamond) (1% YSP, w/v)

1994). Additionally, YSP contains a number of polysaccharide (18.52%), which also contains hydrophilic parts, such as polar or charged side chains, so that YSP has excellent solubility (Jitngarmkusol et al. 2008).

The presence of NaCl exerted ‘salting-out’ effect, i.e. reduction in protein solubility, on the YSP (Fig. 2). The solubility of the YSP in 0.5 and 1.0 M were lower than those in water and 0.1 M NaCl. The addition of a small amount of NaCl increased protein solubility due to salting-in phenomenon. However, further increases in NaCl concentration resulted in the interaction of negatively charged chloride ions with positively charged protein molecules, leading to a decrease in electrostatic repulsion, thus enhancing hydrophobic interactions (Yuliana et al. 2014).

Water- and oil-holding capacity

The oil-holding capacity of YSP was found to be 3.20 mL oil/g of protein which is higher than ginkgo seed protein isolate (2.95 mL/g) (Deng et al. 2011), sesame protein isolate (1.5 mL/g) (Khalida et al. 2003). According to Kinsella (1979), the ability of proteins to bind fat is very important for applications such as meat replacement or meat extenders, mainly because it enhances flavor retention and improves mouth feel. The water holding capacity of YSP have not been detected, which is caused by its excellent solubility.

Emulsifying properties

EAI of YSP in distilled water, NaCl solutions (0.1, 0.5, 1.0 M) as a function of pH are shown in Fig. 3a. As pH shifted away from the pI, the EAI of YSP in distilled water increased drastically ($P < 0.05$). The highest EAI of YSP (81.7%) was found at pH 10. The results showed that the EAI could increase with the enhancement of pH values in distilled water. At extremes of alkalinity, protein molecules unfolding exposed most of the buried lipophilic functional groups on the protein molecules and limited interfacial energy between oil phase and aqueous phase, which resulted in improved EAI (Olayide 2004). The distribution of hydrophobic residues on the surface of dioscorin at neutral pH is shown in Fig. S1. As we can see, the surface hydrophobic residues of dioscorin constituted by Ala (59, 136, 174), Ile (8, 45, 219), Leu (208, 209, 239), Phe (46, 146, 182, 230, 234, 236), Val (87, 162, 199), Pro (12, 23, 99, 138, 204, 224, 227), Gly (10, 77, 106, 134, 161, 190) and Tyr (235), which most likely involved in the emulsifying reaction at neutral pH. With the increase of pH, the

internal hydrophobic groups exposed, resulting in an increased EAI.

At pH 1–3, increasing the NaCl concentration from 0.1 to 1.0 M led to an increase in EAI of YSP ($P < 0.05$). Changes in EAI of YSP in NaCl solutions became stagnant or lower at pH 3–5, then the EAI values increased again until pH 7 beyond which the values fell again ($P < 0.05$). Lam and Nickerson (2015) postulated that aggregation was the highest due to reduction in electrostatic repulsion between proteins near isoelectric point and the larger aggregates would require greater time to migrate, and less capable to reconfigure and align with the oil–water interface which would reduce EAI.

ESI of the YSP in distilled water, NaCl solutions (0.1, 0.5, 1.0 M) as a function of pH is shown in Fig. 3b. ESI in the distilled water increased on increasing pH from 4 (54.7%) to 9 (77.2%). When the pH was increased toward alkaline values, the coulombic repulsion increased between neighbouring droplets, coupled with increased hydration of the charged protein molecules. These factors resulted in reduction of interface energy and combination of emulsion droplet, which might account for the higher ESI obtained (Chavan et al. 2001).

An increase in NaCl concentration caused a decline of the ESI of YSP. The ESI of YSP in the presence of 0.5 and 1.0 M NaCl were lower than those in distilled water and 0.1 M NaCl. This can be attributed to the electrostatic screening of charges of proteins adsorbed in droplets by salt ions. The electrostatic repulsive force is helpful for proteins to overcome various attractive forces, for example, van Der Waals, hydrophobic force (McClements 2005). The increase in ESI resulting from a low sodium chloride concentration might have been achieved through formation of charged layers around the fat globules, resulting in mutual repulsion and/or

Fig. 3 EAI (a) and ESI (b) determined in YSP solubilized in distilled water (filled rectangle), 0.1 M NaCl (filled circle), 0.5 M NaCl (filled triangle) and 1 M NaCl (filled diamond) (1% YSP, w/v)

formation of a hydrated layer around the interfacial material, which lowers interfacial energy and retard droplet coalescence (Aluko and Yada 1995). However, at NaCl concentrations above 0.1 M, the salting-out effect resulted in a sharp decrease in ESI as NaCl concentration increases.

Foaming properties

The effects of pH and salt concentration on FC and FS of YSP are shown in Fig. 4. In distilled water, the minimum FC occurs at pH = 3 (10.4%) because of the behavior of proteins close to pI, and FC increases with pH above the pI, with FC values ranging from 22.4% (pH 4) to 57.2% (pH 10). Pearson (1983) confirmed that the lowest foam properties of proteins coincided with the lowest solubility at their isoelectric point. The increase in FC with pH above the pI is likely due to the increased net charges on the proteins, which weakened the hydrophobic interactions and increased the flexibility of protein (Benelhadj et al. 2016). This likely allowed the protein to diffuse more rapidly to the air–water interface to encapsulate air bubbles and to enhance the foam formation (Aluko and Yada 1995). A similar trend was observed in the dependence of FS on pH. FS was the lowest (34.62%) at pH = 3, while increasing the pH to 10 resulted in the highest FS. The increase in FS at higher pH may have been due to a decreased tendency for foam particles to coalesce as a result of the higher net negative charge of the protein (Yuliana et al. 2014).

The results showed that FC and FS of YSP increased as the salt concentration increased from 0 to 0.5 M, and then decreased. Initial increase in foaming properties might results from increase in protein solubility at these concentrations. Increased protein solubility facilitated

formation of stable and viscous films surrounding the vacuole and enhanced protein concentration at the air–water interface. However, the ion-screening effect of high salt concentration promoted the hydrophobic interactions of proteins and disrupted the protein film, which encouraged flocculation, aggregation and precipitation of proteins (Lawal et al. 2005).

Gelation properties

Gelation is an important function in food formulation and the least gelation concentration (LGC) is regarded as the index of gelation capacity. The LGC of YSP was 8% (w/v) in distilled water, which is lower than lupine protein concentrate (10%) (Lqari et al. 2002), pea protein isolate (14.5%) (Sun and Arntfield 2010) and defatted cashew nut shell protein (12%) (Yuliana et al. 2014).

Low concentration of NaCl (0.1 M) increased the gel strength from 302.9 ± 2.3 g (0 M) to 415 ± 1.3 g (0.1 M). Followed by the increase of concentration (0.5 and 1.0 M), the gel strength decreased to 171.1 ± 3.6 and 163.5 ± 0.42 g, respectively. Initial increase in ionic strength, up to 0.1 M, facilitated a shielding effect on the surface charges; this development reduced the repulsive forces acting among the protein molecules, creating an identical situation to the isoelectric region, whereas further increase in ionic strength influenced the gel forming process negatively (Olayide 2004).

Figure 5, the morphology of 0.1 M NaCl gel appear to have finer and better developed gel network compared to the control. In addition, in the presence of 0.1 M NaCl and with increased solubility, the gel has smaller pores and dense gel compared to the control which also provided higher gel firmness. However, 0.5 and 1.0 M NaCl protein gels appeared uneven porous and are discontinuous which

Fig. 4 Foam Capacity (a) and Foam Stability (b) determined in YSP solubilized in distilled water (filled rectangle), 0.1 M NaCl (filled circle), 0.5 M NaCl (filled triangle) and 1 M NaCl (filled diamond) (2% YSP, w/v)

Fig. 5 SEM photomicrographs of freeze dried gels of (a) distilled water; (b) 0.1 M NaCl; (c) 0.5 M NaCl; (d) 1 M NaCl (10% YSP, w/v)

provided lower gel firmness due to the decreased solubility of protein (Joshi et al. 2014).

Conclusion

The results showed that YSP had desired physicochemical properties. Compared with other vegetable-derived proteins, YSP presented better solubility, oil-holding capacity, emulsifying properties and foam stability, but less water-holding and foaming capacity. The adjustment of pH or the addition of sodium chloride modified the functionalities, e.g. solubility, emulsifying properties, foaming properties and gel strength of YSP.

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