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Physicochemical and functional properties of protein extracts from *Torreya grandis* seeds

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ABSTRACT

Proteins extracted from *Torreya grandis* seeds were investigated for their physicochemical and functional properties. The results showed protein extracts from two cultivars of *T. grandis*, Shengzhou I (SZPI) and Dazinaiyou (DNPI), had similar protein contents and appropriate amino acid balances with about 41% of the essential amino acid. The molecular weights of seed protein fractions were mostly about 31–37 kDa and 20–21 kDa. SZPI and DNPI had similar denaturation temperature of around 93.7 °C while free sulfhydryl group and disulfide bond contents were found to differ slightly. The surface hydrophobicity of DNPI was 982, significantly ($p < 0.05$) greater than that of SZPI (649). Both proteins exhibited high solubilities and favourable emulsifying abilities, foaming and fat absorption capacities, although their *in-vitro* digestibilities were rather low. Therefore, *T. grandis* seed proteins have potential as valuable nutrition sources and functional ingredients in food industry.

1. Introduction

Torreya grandis (*Taxaceae*) is a large evergreen tree mainly cultivated in hilly subtropical areas in China, Florida in the United States, and some parts of Japan and Korea (Kang & Tang, 1995). It has dioecious flowers and drupe-like fruits with nut seeds, which normally take 2–3 years to mature (Ni & Shi, 2014). The seeds are quite popular and valuable dried fruits consumed worldwide for their high nutritional value, unique flavour, special texture and medicinal functions (Li, Luo, Cheng, Feng, & Yu, 2005). Moreover, they are rich in oil, fatty acids, protein, vitamins (i.e., nicotinic and folic acid) and mineral elements (i.e., Mg, Ca, Fe, Zn and Se) (Li et al., 2005; Ni & Shi, 2014). In recent years, *T. grandis* seeds have been found to contain bioactive constituents such as tocopherols, sterols, and phenolic compounds (He et al., 2016; Ni et al., 2015) and to exhibit pharmacological properties such as anti-proliferative and apoptosis-inducible, anti-oxidative and acute anti-inflammatory activities (Chen et al., 2006, 2010).

The protein content of *T. grandis* seeds ranges from 10.34% to 16.43% depending on the cultivar (He et al., 2016; Li et al., 2005), with high amino acid contents (up to 118.1 g/kg) and essential amino acids accounting for about 38% of the total (Li et al., 2005). Some investigations have focused on identifying different cultivars of multi-variety *T. grandis* by analyzing the protein patterns of different *T. grandis* varieties (Chen, Chen, Hou, Xu, & Zheng, 2000). In addition, there is only one research known to us that has reported *T. grandis* seed proteins primarily containing peptides with molecular weights of 33 kDa, 31 kDa and 20 kDa and were similar to Amentotaxus argotaenia proteins (Hu, Wang, Liu, & Qian, 1986). In recent years, a vast number of studies have been conducted on the physicochemical and functional properties of seed proteins aiming at utilizing them in food systems. Some of them concentrated on pumpkin seed proteins working as the functional ingredients, for they hold high solubility, appropriate amino acid balances and high denaturation temperatures (Rezig et al., 2013). There are also some investigations that have focused on *Bilphia sapida* seed proteins and its pulp to produce soups, sausages and cakes with improved flavour-retaining capacities and mouth-feel, as a result of high emulsion and oil absorption capacities of the proteins (Akintayo, Adebayo, & Arogundade, 2002). To be relatively more, some researches have focused on soybean proteins and hemp proteins. The results generally tended to show that although the functional properties of hemp proteins were poorer than those of soybean proteins, the former can be used as valuable nutrition sources for infants and children due to their high essential amino acid contents (Tang, Ten, Wang, & Yang, 2006). Others

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also have concentrated on kidney bean, field pea and fenugreek and other seed proteins for they hold unique properties in terms of their foaming capacities and foam and emulsion stabilities (Feyzi, Varidi, Zare, & Varidi, 2015; Shevkani, Singh, Kaur, & Rana, 2015). However, to the best of our knowledge, the characteristics of *T. grandis* seed proteins remain fairly unknown. Therefore, to utilize *T. grandis* seed proteins effectively, it is necessary to understand their physicochemical and functional properties.

Therefore, the main objectives of this study were to characterize the physicochemical properties (molecular weights, denaturation temperature [T_d], sulfhydryl group [SH] contents and surface hydrophobicities [H_0]) and functional properties (emulsifying and foaming capacities, fat absorption capacities [FACs] and *in-vitro* digestibilities [IVDs]) of protein extracts from the seeds of two *T. grandis* cultivars (Shengzhou I [SZPI] and Dazinaiyou [DNPI]) and to better assess their potentials as nutritional and functional ingredients in the food industry.

2. Materials and methods

2.1. Materials

Dry Shengzhou I and Dazinaiyou *T. grandis* seeds (with moisture contents of about 6%) were obtained from the hilly red and lithomorphic soil of Shengzhou area of Zhejiang province in China in October 2014. Bovine serum albumin (BSA), sodium dodecyl sulfate (SDS), *n*-hexane and ethanol were obtained from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China), and 8-anilino-1-naphthalenesulfonic acid (ANS), pancreatin (8 USP), pepsin (2500 U/mg), amino acid standard, protein molecular weight marker, derivatization reagents O-phthalaldehyde (OPA), fluorenylmethyloxycarbonyl chloride (FMOC-Cl), 5, 5′-dithio-bis-2-nitrobenzoic acid and β-mercaptoethanol (β-ME) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA); all other reagents and chemicals were of analytical grade.

2.2. Preparation of protein extracts from T. grandis flour

After husking, the seeds were milled into flour. Then, the flour was defatted by mixing it with *n*-hexane/ethanol (9:1 v/v) in a 1:3 ratio (w/v) at room temperature and the mixture was continuously stirred for 2 h. The mixture was separated by an Aspirator Filter Pump (SHB-III, Zhengzhou the Great Wall Instrument Co., Ltd., China). The filtrate was discarded, and the residual pellet was air-dried at room temperature until it was dry and free of solvent odor. The dry powder was regarded as defatted *T. grandis* flour.

The defatted *T. grandis* flour was dispersed in water at a ratio of 1:10 (w/v) and stirred at 30 °C for 2 h; then, the mixture was centrifuged at 10,000*g* for 20 min at 20 °C. The precipitate was redissolved in water at a ratio of 1:5 (w/v) and centrifuged again. Two supernatants were mixed and adjusted to pH 4.0 by using 2 M HCl to precipitate the protein isoelectrically; this mixture was then centrifuged at 10,000*g* for 20 min at 4 °C. The precipitates were dissolved and neutralized with 2 M NaOH and stirred for 30 min. The solution was then freeze-dried. The protein samples from the Shengzhou I *T. grandis* flour (STF) and Dazinaiyou *T. grandis* flour (DTF) were defined as SZPI and DNPI, respectively.

The protein contents of SZPI and DNPI were determined using the Kjeldahl method (N × 6.25). In addition, the chemical compositions of the two *T. grandis* cultivars, defatted *T. grandis* flour and protein extracts were determined according to the Association of Official Analytical Chemists procedures (AOAC, 2000).

2.3. Amino acid composition

The amino acid analysis was done according to the method described by Rezig et al. (2013) with some modifications. First, 100 mg samples of the proteins were placed into individual dry tubes, and 8 ml of 6 M HCl was added to each tube. Then, the tubes were air-exhausted to create vacuums and heated in an oven at 115 °C for 22 h. The samples' tryptophan contents were determined by using NaOH instead of HCl. To the sample with a volume of 1 μl, OPA and FMOC-Cl were mixed with 5 μl sodium borate buffer (pH 10.4) in reaction vessels before injection. The amino acid contents were analyzed by an Agilent 1100 HPLC system (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a quaternary pump (G1311A), online vacuum degasser (G1322A), variable wavelength detector (G1314A) and a C18 ODS Hypersil column (4.0 mm × 250 mm, 5 μm particle size; Agilent Technologies, Inc.). The solvent A was 27.6 mmol/L sodium acetate solution-tri methylamine-tetrahydrofuran (500:0.11:2.5, pH 7.2) and the solvent B was 80.9 mmol/L sodium acetate solution-acetonitrile-methanol (1:2:2, pH 7.2). The gradient was programmed as follows: 0 min, 8% B; 17 min, 50% B; 20.1 min, 100% B; 24.0 min, 0% B. The column was equilibrated at 1 ml/min with a temperature of 40 °C and the monitoring wavelength was 338 nm except for proline at 262 nm. The amino acid content was computed by the Agilent data processing software and expressed as g amino acid/100 g of protein sample.

2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on a discontinuous buffered system according to the method of Laemmli (1970) with some modifications by using 12% separating gel and 4% stacking gel. The protein samples (2 mg/ml) were solubilized in a sample buffer that contained 2% (v/v) β-ME while the samples for non-reducing condition were added in the buffer without β-ME. Then, the samples were heated in boiling water bath for 5 min before electrophoresis. For each sample, 12 μl was applied to each lane. Electrophoresis was performed at 10 mA and then at 20 mA. The gel was stained with 0.1% Coomassie Brilliant blue (R-250) for 1.5 h and was destained in decolouring solution (methanol/acetic acid/water, 20:30:350) for 8 h.

2.5. Thermal properties

The thermal denaturation properties of the proteins were assessed using the differential scanning calorimetry (TA Instruments, New Castle, DE) as Rezig et al. (2013) reported. Dry protein samples with masses of 3.0–4.0 mg were mixed with water (1:1, w/v), placed in a pan and heated in a calorimeter cell at a constant rate of 5 °C/min from 20 °C to 110 °C. Peak temperatures (T_d, maximum temperature of denaturation) and denaturation enthalpies (ΔH, area below the curve in J/g of the protein sample) were calculated from thermograms using Universal Analysis 2000 (TA Instruments, Waters LLC, USA).

2.6. Free sulfhydryl group (SH) and disulfide bond (SS) contents measurements

The SH contents were determined according to the method of Beveridge, Toma, and Nakai (1974) with a few modifications. Protein samples (30 mg) were dissolved in 15 ml of Tris-Gly (pH 8.0) and 1 ml of protein solution was extracted from each tube and added to 4 ml of Tris-Gly buffer (0.086 M Tris, 0.09 M Gly, 0.5% (w/v) SDS and 4 mM EDTA, pH 8.0) containing 8 M urea to determine its SH content. Next, 50 μl of Ellman's reagent