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Physicochemical and functional properties of protein isolate obtained from cottonseed meal

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ABSTRACT

To investigate the effect of preparation methods of cottonseed meals on protein properties, the physicochemical and functional properties of proteins isolated from hot-pressed solvent extraction cottonseed meal (HCM), cold-pressed solvent extraction cottonseed meal (CCM) and subcritical fluid extraction cottonseed meal (SCM) were investigated. Cottonseed proteins had two major bands (at about 45 and 50 kD), two X-ray diffraction peaks (8.5° and 19.5°) and one endothermic peak (94.31 °C–97.72 °C). Proteins of HCM showed relatively more β-sheet (38.3%–40.5%), and less β-turn (22.2%–25.8%) and α-helix (15.8%–19.5%), indicating the presence of highly denatured protein molecules. Proteins of CCM and SCM exhibited high water/oil absorption capacity, emulsifying abilities, surface hydrophobicity and fluorescence intensity, suggesting that the proteins have potential as functional ingredients in the food industry.

1. Introduction

Cottonseed, which is available in many temperate and tropical countries, is one of the richest sources of oilseeds mostly processed to extract oil that is used as edible fat (Zhou, Zhang, Gao, Wang, & Qian, 2015). Cottonseed meal is a co-product of the cottonseed oil processing industry. With processing, typical yields from cottonseed are 50% meal, 22% hulls, 16% oil and 7% linters, with a 5% loss (Hinze et al., 2015). The commonly used methods of lipid extraction from oil seeds are pressing and extraction with organic solvents (cold or hot). Pressing is the process of mechanically pressing liquid out of liquid containing solids, whereas extraction refers to the process of separating a liquid from a liquid-solid system (Anderson et al., 2016). Subcritical fluid extraction is one of the newly emerging clean and environment-friendly technologies for food products (Zheng, Ren, Su, Yang, & Zhao, 2013). During the oil extraction from cottonseeds, a portion of free gossypol binds with the epsilon amino group of lysine, thereby reducing the availability of lysine. Free gossypol in cottonseed meal depends on the variety of cultivars, methods of oil extraction and proportion of kernel to husk (Nagalakshmi, Rama Rao, & Panda, 2007). According to the Food and Drug Administration (FDA), a protein food product made from cottonseed is considered edible if it contains less than 0.045% free gossypol (FDA regulations, 1974). Several edible products have been developed, and cottonseed flours and protein concentrates have been accepted as functional and nutritional additives for meat products, baked goods, and cereals (Zhuge, Posner, & Deyoe, 1988). The use of cottonseed as protein source for humans does not depend only on the nutritional value of cottonseed, but also on their ability to be used as, or to be incorporated into, foods. Therefore, the functional properties of proteins rather than their nutritional value largely determine their acceptability as ingredients in various foods (Tsaliki, Pegiadou, & Doxastakis, 2002).

The functional attributes of food proteins depend on their molecular size, charge distribution, and three-dimensional structure. The structure-function relationships of proteins determine their interactions with themselves and with other ingredients in complex food systems (Joshi et al., 2012). The important functional properties of proteins in foods include hydration, water/oil combination, gelling, emulsification, foaming formation and rheological behaviours. These properties are influenced by environmental factors and processing conditions (Shevkani, Singh, Kaur, & Rana, 2015). Some of the physicochemical and functional properties of these proteins have already been reported (Mohan & Narasinga Roa, 1988; Tsaliki, Pegiadou, & Doxastakis, 2004; Tsaliki et al., 2002; Zhou et al., 2015). However, few studies have explored the influence of preparation methods of cottonseed meals in protein physicochemical and functional properties. The present work mainly aims to compare the physicochemical and functional properties of proteins isolated from hot-pressed solvent extraction cottonseed meal

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(HCM), cold-pressed solvent extraction cottonseed meal (CCM) and subcritical fluid extraction cottonseed meal (SCM). The results can be used to further enhance the use of cottonseed proteins and investigate the methods used to prepare cottonseed meals, ultimately broadening the applications of cottonseed meals.

2. Materials and methods

2.1. Materials

HCM (TianKang, XinLiang, JingGu, and YiHai), CCM (Colour cotton, Insect-resistant cotton) and SCM (Colour cotton) were provided by the Institute of Cotton Research of Chinese Academy of Agricultural Sciences. Cottonseeds undergo a series of suitable pretreatment for hot pressing, such as cleaning, conditioning, decorticating, cracking, flaking, cooking (at 160–180 °C for 20 min), extruding, and drying to optimal moisture content of 9%. TianKang cottonseed meal (protein content: 56.23%), XinLiang cottonseed meal (protein content: 58.26%), JingGu cottonseed meal (protein content: 59.62%), and YiHai cottonseed meal (protein content: 60.86%) were respectively obtained from cottonseed oil extracted via hot pressing at 110–115 °C and then leached with hexane overnight. Colour cottonseed meal (protein content: 65.19%), insect-resistant cottonseed meal (protein content: 61.89%), peanut meal (protein content: 57.87%), and soybean meal (protein content: 47.45%) were obtained from seed oil extracted via cold pressing below 60 °C by LH188 oil pressing machine (Foshan Nanhai Lihua Electronic Technology Co., Ltd, China) and then leached with hexane overnight. Subcritical fluid extraction cottonseed meal (protein content: 66.65%) was obtained from colour cottonseed oil extracted by butane four times at 47 °C for 36 min for each round by using a CBE-5L subcritical fluid extraction equipment (Henan province subcritical extraction biological technology Co., Ltd, China). The free gossypol content of all cottonseed meal was lower than 0.012%. All samples were crushed to pass through a 40-mesh screen. All chemicals were reagent grade and obtained from Sigma-Aldrich Co (St. Louis, USA).

2.2. Preparation of protein isolate

Protein isolate was extracted and purified from defatted meal according to a reported method with some modifications (Timilsena, Adhikari, Barrow, & Adhikari, 2016). Meal (2 kg) added to petroleum ether (4 l) was stirred at room temperature for 60 min, then left standing, until natural sedimentation of the meal and organic solvent separation occurred. Recovery of organic solvents was then completed, and the precipitated cottonseed meal added to petroleum ether, this being repeated three times. The meal was subsequently placed in a fume hood at room temperature for 12 h. The protein isolate was obtained from the defatted meal. The defatted meal was dispersed in alkaline water (pH 11.0) by using a meal-to-water ratio of 1:20 to extract the protein. The slurry was treated at 45 °C for 20 min by using a KQ-700DE CNC ultrasonic device (Ultrasonic Instrument Co., Ltd, Kunshan, China, 650 W). The slurry was centrifuged at 1500 × *g* for 30 min, and the supernatant containing the dissolved protein was collected. This supernatant was acidified to a pH of 4.5 to induce precipitation of the dissolved protein. The protein precipitate was recovered through centrifugation and then resuspended in alkaline water to a pH of 7.0. This protein solution was vacuum freeze-dried and then the protein samples were stored at 4 °C until further tests.

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

Protein profile was determined using SDS-PAGE according to the modified Laemmli method (Arogundade, Mu, & Akinhanmi, 2016). SDS-PAGE test was performed on a gel slab comprising 4% stacking gel and 12.5% separating gel in a SDS-Tris-glycine discontinuous buffer system. The protein solution (2 mg/ml) was mixed with sample buffer (1:4, v/v) containing either 0% or 10% β-mercapto-ethanol and then boiled for 5 min. Each lane of the gel was loaded with 8 μl of sample. Following electrophoresis, the gels were stained with 0.1% Coomassie Brilliant Blue to reveal the protein bands. After removing the free dye, the gels were photographed using a Gel Doc XRTM System (Bio-Rad Laboratories, Hercules, CA).

2.4. Protein solubility (PS)

PS was determined according to the method described by Wu, Wang, Ma, and Ren (2009). In brief, 500 mg of proteins were dispersed in 50 ml of deionized water maintained at different pH values (pH 3.0, 5.0, 7.0, 9.0, and 11.0). The mixture was stirred for 1 h and then centrifuged at 1500 × *g* for 30 min. The protein contents of the supernatants were measured using the Bradford method with bovine serum albumin as standard. PS was expressed as percentage ratio of supernatant protein content to the total protein content.

2.5. Water absorption capacity (WAC) and oil absorption capacity (OAC)

WAC and OAC were determined using the method described by Ajibola, Malomo, Fagbemi, and Aluko (2016) with some modifications. Protein samples (0.5 g) were dispersed in distilled water (or soybean oil) (5 ml) in a 10 ml pre-weighed centrifuge tube. The dispersions were vortexed for 1 min, allowed to stand for 30 min, and centrifuged at 1400 × *g* for 30 min at room temperature. The supernatant was decanted, excess water (or oil) in the upper phase was drained for 10 min, and tube containing the protein residue was weighed again to determine the amount of water or oil retained per gram of sample.

2.6. Foaming capacity (FC) and foam stability (FS)

FC and FS were determined according to the method described by Timilsena et al. (2016). Protein samples (0.5 g) were dispersed in distilled water (50 ml) previously adjusted to the specified pH (4.0, 5.0, and 7.0). Foam was formed using XHF-D H-SPEED homogenizer (Ningbo Xinzhi Inc., China) at 10,000 × *g* for 2 min.

2.7. Emulsifying activity index (EAI) and emulsion stability index (ESI)

EAI and ESI were measured according to the method described by Zhang, Yang, Zhao, Hua, and Zhang (2014) with some modifications. Soybean oil (2 ml) and 1% (w/v) proteins (6 ml) were mixed. The mixture was homogenized using XHF-D H-SPEED homogenizer (Ningbo Xinzhi Inc., China) at 10,000 × *g* for 1 min. An aliquot of the emulsion (50 μl) was pipetted from the bottom of the container at 0 and 10 min after homogenization and then mixed with 0.1% SDS solution (5 ml). The absorbance of the diluted solution was measured at 500 nm by using a spectrophotometer (UV-1200, Instrument Co., Ltd, Shanghai, China).

2.8. Measurement of surface hydrophobicity (H_0)

H_0 was measured according to Kato and Nakai's method (Kato & Nakai, 1980) with some modifications. Protein samples were prepared through serial dilution with phosphate buffer solution (10 mM, pH 7.0) to obtain protein concentrations ranging from 1.0 mg/ml to 0.02 mg/ml. For each measurement, 5 ml of diluted sample was placed into 10 ml test tubes, in which 25 μl of 8 mM ANS solution was added. The tube contents were mixed and incubated in the dark for 15 min at ambient temperature. Fluorescence intensity (FI) was measured using a fluorescence spectrometer (LS55, PE Inc., USA) at 390 nm (excitation) and 470 nm (emission), with a constant excitation and emission slit of 5 nm. The slope of FI versus protein concentration linear regression plot (r = 0.99) was used as H_0 index.