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Physicochemical and functional properties of protein isolate produced from Australian chia seeds

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ABSTRACT

Protein was isolated from Australian chia seeds and converted to powders using spray, freeze and vacuum drying methods, to investigate the effect of drying methods on physicochemical and functional attributes of chia-seed protein isolate (CPI). It was found that there was no significant difference in the proximate composition; however vacuum dried CPI (VDCPI) had the highest bulk density and oil absorption capacity, whereas spray dried powder (SDCPI) demonstrated the highest solubility, water absorption capacity and lowest surface hydrophobicity. Solubility of all powders was higher at elevated temperature and alkaline pH. Foaming capacity and foam stability of CPI were found to increase with increasing pH and protein concentration. SDCPI was the least denatured and VDCPI the most denatured, demonstrating the poorest solubility and foaming properties of the latter. These findings are expected to be useful in selection of a drying process to yield chia seed protein powders with more desirable functionality.

1. Introduction

The demand for proteins is increasing rapidly, especially for the human diet, due to increased awareness of their nutritional and functional values. The demand for plant proteins is very high due to their higher availability in nature, lower cost of production and also due to the preference of some consumers based on their religious or other beliefs and dietary requirements. There is an increased interest in academia and industry in finding proteins from alternative sources with better functional attributes. Seed proteins are drawing increased attention as they are relatively abundant, easily digestible and contain adequate levels of essential amino acids. Plant proteins are also shown to have protective effects against some chronic degenerative diseases (Blagrove & Gillespie, 1975; Krajcovicova-Kudlackova, Babinska, & Valachovicova, 2005). Therefore, concentrates and isolates of various plant proteins are used to fully or partially replace meat and dairy proteins in many processed foods. Generally, proteins are used in various food formulations in order to improve their nutritional performance. Proteins, essential amino acids and health promoting polypeptides are also used to impart desirable sensory characteristics such as structure, texture, flavour, and colour in formulated food products. On the other hand, protein content in chia seeds is higher than most of the traditionally utilized grains; they contain approximately 19–23% (w/w), which is higher than that of wheat (14%, w/w), corn (14%, w/w), rice (8.5%, w/w), oats (15.3%, w/w), and barley (9.2%, w/w) (Sandoval-Oliveros & Paredes-López, 2013). Essential amino acids such as leucine, isoleucine and valine comprise 42.2–42.9% of total amino acids in chia seeds. This proportion of essential aminoacids in chia seed is higher than that in common oil seeds such as soyabean and sunflower (Olivos-Lugo, Valdivia-López, & Tecante, 2010). Chia seed is also rich in non-essential amino acids, such as glutamic acid, arginine and aspartic acids. Glutamic acid is known to modulate immoregulatory response and enhances athletic performance; hence, it is regarded as an important amino acid in the diet (Olivos-Lugo et al., 2010). Arginine is also known for its role in preventing heart disease.

The functional attributes of food proteins originate from their molecular size, charge distribution and three-dimensional structure. The structure-function relationships of proteins determine the way they interact with themselves and other ingredients in complex food systems (Joshi et al., 2012). The important functional properties of protein in food include hydration, water- and fat-binding, gelling, emulsifying, foaming and rheological behaviors. These properties are also influenced by environmental factors and processing conditions (Shevkani, Singh, Kaur, & Rana, 2015).

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Hydrophobic, steric, and electrical interactions affect the structure and physicochemical characteristics of proteins (Anandharamakrishnan, Rielly, & Stapley, 2008). Surface hydrophobicity affects the emulsifying, foaming, gelation and whipping properties. Solubility affects the protein functionality. The solubility of proteins depends on whether they are in their native or denatured state. It is also heavily influenced by pH and ionic strength, as these two parameters greatly affect the net charge and electrostatic repulsion between protein molecules. At the isoelectric point (pI) the net charge is zero, and the electrostatic repulsion among molecules is lowest, hence aggregation occurs most rapidly. The repulsive forces are much higher and the aggregation of protein is less likely to occur at pH values much higher or lower than the pI (Anandharamakrishnan et al., 2008).

The demand for chia seed is increasing in the functional food market as it is one of the richest sources of plant based omega-3 (alpha-linolenic acid). Once the oil is extracted from chia seeds, substantial quantity of protein-rich meal is left behind as the by-product. Chia seed meal can be a valuable source of protein, soluble and insoluble fibers and anti-oxidants as it is comprised of 17–24% protein, 18–30% fibers, 25–40% oil and 5–6% of mucilaginous gum (Sandoval-Oliveros & Paredes-López, 2013; Timilsena, Adhikari, Barrow, & Adhikari, 2016; Timilsena, Adhikari, Kasapis, & Adhikari, 2016; Timilsena, Wang, Adhikari, & Adhikari, 2016). Current total world production of chia seed is about 30,000 tons per annum (Daniells, 2013). Despite their newly realised nutritional importance, characteristic features of chia seed protein are not well understood. An extraction process, thermal, physicochemical, nutritional attributes and some functional attributes such as foaming capacity and water and oil holding capacities of Mexican chia seed have been reported (Bushway, Wilson, Houston, & Bushway, 1984; Olivos-Lugo et al., 2010; Vázquez-Ovando, Betancur-Ancona, & Chel-Guerrero, 2013). Aspects of digestibility, thermal and functional aspects of chia seed proteins have also been studied (Monroy-Torres, Mancilla-Escobar, Gallaga-Solórzano, Medina-Godoy, & Santiago-García, 2008; Sandoval-Oliveros & Paredes-López, 2013). It is reported that chia seed protein contains all nine essential amino acids in appreciable concentration and possesses good digestibility (49.4–78.9%) and good water holding, oil holding, foaming and gelling capacities (Olivos-Lugo et al., 2010; Sandoval-Oliveros & Paredes-López, 2013). Despite the popularity of whole or intact chia seeds in food formulations including bakery and cereal bars, the chia seed protein is not yet industrially utilized in food formulations. This is due to limited information on its structural composition and techno-functionality. Increased understanding of these characteristics will significantly broaden its industrial application.

Proteins are extracted by solubilizing in water and converted into powder using spray, freeze or vacuum drying (Haque, Timilsena, & Adhikari, 2015; Joshi, Adhikari, Aldred, Panozzo, & Kasapis, 2011). The process parameters involved in the extraction, purification and drying induce a certain degree of change in the protein structure and ultimately affect the techno-functionality and nutritional value of proteins (Ghodsvali, Khodaparast, Vosoughi, & Diosady, 2005). Therefore, the knowledge of the factors affecting the functional properties of chia seed protein helps to use less astringent processing techniques to produce it with better functional characteristics. However, there is no information in the literature which quantifies and explains the effect of drying methods on the physicochemical and functional properties of chia seed protein. Therefore, this study was undertaken to investigate the effect of drying methods on the physicochemical and functional properties of chia seed protein isolate (CPI) powder produced by spray, freeze and vacuum drying methods. Fourier transform infra-red spectroscopy (FTIR) was also used to quantify and explain the changes in the protein structure. This change of structure is then interpreted in terms of the difference in the functional properties of CPI powders.

2. Materials and methods

Chia seeds (*Salvia hispanica* L.) were obtained from a Coles™ supermarket in Melbourne, Australia and stored at room temperature until use. All chemicals were of analytical or food grade and were used as received from Sigma-Aldrich, Australia. The electrophoresis gel, staining solutions and the molecular weight standards were purchased from Biorad, Australia. The bicinchoninic acid (BCA) assay reagents were obtained from Fisher Scientific, Australia.

2.1. Preparation of chia seed protein isolate

Chia seed protein isolate (CPI) was extracted and purified from defatted chia seed flour according to the previously reported method (Timilsena, Wang et al., 2016) with some modification. In brief, 200 g chia seeds were finely ground using a coffee grinder and the oil was extracted using Soxhlet extraction method. The mucilage from the defatted chia seed flour was removed by treating with sodium carbonate according to Karaca, Low, and Nickerson (2011)'s method. For this, 100 g of defatted chia seed flour (meal) was mixed with 0.5 M $NaHCO_3$ solution, containing 0.02% w/v of sodium azide, at 1:10 ratio and stirred for 18 h at room temperature. Subsequently, the dispersion was filtered and washed with water (5 × 100 ml) to remove any traces of mucilage. This defatted and demucilaged chia seed meal was used for preparation of protein isolate.

The defatted and demucilaged chia seed meal was dispersed in alkaline water (pH 12.0) using a meal-to-water ratio of 1:20 to extract the protein. This slurry was stirred at ambient temperature for 1 h using a magnetic stirrer to facilitate protein solubilization. This slurry was centrifuged (Allegra 64R Centrifuge, Beckman Coulter Inc., USA) at 10,000*g* for 30 min and the supernatant containing the dissolved protein was collected. This supernatant was further filtered through Whatman No. 1 filter paper to remove any insolubles. This filtrate was acidified to a pH of 3.0 (isoelectric point) in order to precipitate the dissolved protein. The protein precipitate was recovered by centrifugation and washed with Milli-Q water (3 × 50 ml). The precipitate was re-suspended in Milli-Q water at pH 7.0 and stirred for 10 min to solubilize the protein. This protein solution was dried using spray, freeze and vacuum drying and the protein powders obtained from these three drying methods were collected. Spray drying was carried out according to Haque, Chen, Aldred, and Adhikari (2015)'s method using a laboratory scale spray dryer (Mobile Minor™ GEA, Niro, Denmark). In order to prevent thermal stress on protein, the inlet temperature of 180 °C and outlet temperature 80 °C were selected and the feed flow rate was adjusted between 300 and 360 ml/h. This outlet temperature was well below the denaturation temperature of chia seed proteins (T_d = 97.2 °C), as shown in our previous study (Timilsena, Wang et al., 2016). Freeze drying was carried out using a laboratory-scale freeze-dryer (Triad™ freeze dryer, Labconco, USA). The samples were pre-frozen at −20 °C for 3 h followed by primary drying at 0 °C for 48 h and secondary drying at 20 °C for 4 h. The collector temperature was −83 °C and a vacuum of 16 Pa were maintained in the drying chamber. The samples were dried until the sample temperature reached the shelf temperature (20 °C). Vacuum drying was carried out according to Joshi et al. (2011)'s method using a laboratory vacuum oven (VD, Binder GmbH, Germany). The drying was carried out at 60 °C and 60 kPa pressure for 24 h. The spray dried CPI powder (SDCPI) was collected, packed in an air-tight container and stored at 4 °C. The