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The major proteins of the seed of the fruit of the date palm (*Phoenix dactylifera* L.): Characterisation and emulsifying properties

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ABSTRACT

Proteins were extracted from the seeds of the fruit of the date palm. Proteomic analysis and SDS–PAGE electrophoresis of the extracted proteome suggested it is composed predominantly of the storage proteins glycinin and β-conglycinin, although over 300 proteins were detected, 91 of which were identified with confidence. In terms of protein type, the largest numbers of proteins were associated, not unexpectedly, with metabolism and energy functions, which reflected the requirements of the germinating and growing embryonic plant. The emulsifying properties of the extracted proteins were determined. Date seed protein exhibited a lower emulsifying activity than either whey protein concentrate or soy protein isolate, at each of the pH values tested. However, the stability of the emulsions produced with all three proteins was very similar at the different pH values. This combination of large emulsion droplet size and high emulsion stability properties suggested that the date proteins may adsorb as large protein oligomers.

1. Introduction

The increasing cost of proteins from animal sources such as meat, egg and dairy products has encouraged the food industry to find alternative sources of proteins, for use as functional ingredients in formulated foods. In addition, it is becoming evident that protein sources, such as fish meal or soy protein that are often used as animal feed, are unsustainable or economically not viable. Plant proteins, such as soy, legume, canola and cereal proteins, are appealing as sources of food protein because their production is more sustainable (Pimentel & Pimentel, 2003). However, plant proteins are often more difficult to extract, require large quantities of water during the extraction process and may lose functional properties during extraction (Schutyser & van der Goot, 2011). Loss of functional properties occurs due to loss of solubility, brought about by denaturation of the protein under the extreme conditions (acid or alkaline and heating) required to extract the proteins from the polysaccharide-containing plant matrix (Schutyser & van der Goot, 2011).

The fruit of the date palm, *Phoenix dactylifera* L., is one of the richest fruit-based sources of protein. Date palm is one of the major fruit crops produced in dry and semidry regions. It is an important commercial crop in different regions of the world (Al-Yahyai & Manickavasagan, 2012), and is considered the third most important palm species in the global agricultural industry, after coconut and oil palms. The seeds of the date fruit, which are a waste product from date processing, also contain 5–7% protein by weight (Aldhaheri, Alhadrami, Aboalnaga, Wasfi, & Elridi, 2004), but very little is known about the composition and the functional properties of these seed proteins. If it is possible to extract the proteins from the seeds, it might be useful as a source of protein for human or animal nutrition. Robust methods for the extraction of proteins from date seeds could facilitate the utilisation of date palm wastes, such as seeds in the human and animal diet.

Functional properties of proteins define their behaviour in a food system during production and processing. Extraction and isolation of proteins from plant seeds is only the first step to integrating these proteins into food products. If they are to be of use as food ingredients they have to prove sufficiently functional to be used in place of current food proteins, such as milk, egg and soy proteins. Studies of the functional properties of new protein sources can provide valuable information on the potential effectiveness of the proteins in food products. The important functional properties of proteins in food applications are solubility, swelling and water/fat holding capacity, emulsifying activity and emulsion stability, foaming ability and foam stability and gelling capacity.

There is a lack of information in the literature on the functional properties of proteins from date palm seed. This study aimed to investigate the extraction of protein from date seed, characterise these proteins using mass spectrometry and test their emulsifying properties.

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2. Materials and methods

All chemicals were purchased from Sigma Aldrich, Dorset, UK unless otherwise stated.

2.1. Preparation of date seed protein isolate

Dates (i.e. the fruit of the date palm *P. dactylifera* L.) were purchased from a local supermarket in Edinburgh, United Kingdom. The dates were purchased at the Tamr stage (complete maturity) and their variety was Deglet Nour that had been grown in Tunisia. Seeds were removed from 40 kg of whole dates, washed in water to remove any remaining date flesh and then air-dried for a week. The seed was found to make up 10.3% (w/w) of the total mass of the date fruit on average. The seeds were then further dried overnight at 40 °C in a drying oven. The seeds were milled using a hammer mill to a particle size that could pass through a 1–2 mm sieve screen and then stored at −20 °C until further preparation was required. The powder obtained was identified as date palm seed powder (DPSP). The composition (w/w) of the DPSP has been reported in our previous paper as protein, 5.64%, moisture, 5.39%, fat 8.14%, fibre 18.50%, ash 0.95%, carbohydrate 61.38% (Akasha, Campbell, & Euston, 2012).

Oil was extracted from DPSP using a Soxhlet apparatus. Fifteen gram samples of dried DPSP were weighed into an extraction thimble (Fisher Scientific, UK) and sealed with cotton wool. The thimble was inserted in a Soxhlet extraction flask and extracted with boiling hexane (boiling point 68 °C) for 10 h or until the solvent at the sample chamber was colourless, indicating it was free from oil and that all the oil had been extracted. The defatted DPSP was removed from the extraction thimble and left to dry overnight, to allow the hexane to evaporate. This defatted date seed powder (DDSP) was kept at −20 °C until processed further. The residual fat content and protein content of the defatted powder were reported previously (Akasha et al., 2012) as 1.01% (w/w) and 6.13% (w/w), respectively. This protein content is equivalent to a 100% yield of protein. The effect of the hexane extraction step on the functionality of the proteins was not determined. However, it is well known that the methods used to extract the proteins from the powder will also affect the functionality, so the additional effect of hexane extraction is likely to be negligible.

2.1.1. Protein Isolation

Protein was extracted from the DDSP using a phenol/trichloracetic acid (Ph/TCA) extraction procedure, based on the methods (with some modifications) proposed by Gomez-Vidal, Tena, Lopez-Liorca, and Salinas (2008) for olive and *P. dactylifera* L. leaves, respectively. Ten grams of defatted DDSP was mixed with 30 ml of ice-cold acetone, vortex mixed and then centrifuged at 10,000 rpm for 10 min at 4 °C (Beckman Avanti J26-XP centrifuge). The supernatant was decanted and discarded, with the residual pellet being washed twice with ice-cold acetone and allowed to dry at room temperature. After the pellet had dried, it was ground to a fine powder using a pestle and mortar, rinsed with 15% (w/v) TCA in acetone, vortex mixed and then centrifuged at 10,000 rpm for 10 min at 4 °C. The rinsing with TCA/acetone and centrifugation was repeated three times. The pellet was then rinsed with cold 15% (w/v) TCA in water and centrifuged. The rinsing with cold TCA and centrifugation was repeated three times. The pellet was then rinsed with cold 80% (v/v) acetone followed by centrifugation, and this was also repeated three times. The pellet was then air dried.

2.1.2. Protein purification

To purify the protein, 2 g of the dry pellet was suspended in a mixture of 10 ml of Ph/Tris-buffer at pH 8.0, and 10 ml of dense SDS buffer (2%[w/v] SDS, 5%[w/v] sucrose, 0.1 M Tris-HCl, pH 8.0, 5% [v/v] β-mercaptoethanol). The mixture was vortex mixed and the pellet was obtained by centrifugation at 10,000 rpm for 10 min at 4 °C, using a Beckman Avanti J26-XP centrifuge fitted with a JA25.50 rotor (Beckman-Coulter, High Wycombe, UK). The pellet was re-suspended in Ph/Tris-buffer and dense SDS solution, and centrifuged again under the same conditions. The pellets from both centrifugations were mixed and precipitated with five volumes of cold 0.1 M ammonium acetate in methanol, refrigerated at 4 °C overnight and then centrifuged at 10,000 rpm for 10 min at 4 °C. The pellet from this centrifugation was then washed three times with cold methanol, plus 0.1 M ammonium acetate and centrifuged as above, followed by the same process with cold 80% (v/v) acetone. Half a gram of the dried pellet was then mixed with 5 ml of cold aqueous 24% (w/v) TCA, vortex mixed and left to precipitate on ice for 30 min, followed by centrifugation at 13,000 rpm for 15 min at 4 °C. The pellet was washed with 2 ml of ice cold acetone, incubated for 15 min on ice and then centrifuged at 13,000 rpm for 15 min at 4 °C. The final pellet or date seed protein concentrate (DSPC) was air-dried in an oven at 30 °C overnight (16 h) and stored at −20 °C, until required for further analysis.

2.1.3. Protein content of DSPC

The crude protein content of the extracted DSPC and DDSP was determined by measurement of the nitrogen content using the Kjeldahl method (Lynch, Barbano, & Fleming, 1998).

The percent yield of protein from the date palm seed was determined by calculating the protein recovered in the DSPC and comparing this to the maximum possible protein recovery from the DDSP.

2.2. SDS–PAGE analysis of DSPC

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was performed on date palm seed on a 12% polyacrylamide gel (BioRad, Hemel Hempstead, UK). A sample of DSPC and soy protein isolate (SPI) was run on the gel. A protein molecular weight ladder (BioRad, Hemel Hempstead, UK) was also run on the gel, to allow molecular weight determination. Gels were stained overnight with colloidal Coomasie blue and destained (10% [v/v] ethanol and 2% [v/v] orthophosphoric acid) until the background become clear, and protein bands were visible. Gels were scanned using a BIO-RAD Molecular imager® (ChemiDocTM XRS+) and analysed using GelAnalyzer 2010a software, to estimate the molecular weight of protein bands.

2.3. Preparation of protein for liquid-chromatography tandem mass spectrometry (LC–MS/MS)

Protein preparation was carried out using a method proposed by Le Bihan et al. (2011). Ten mg of DSPC was resuspended in 50 μl of distilled water (dH_2O), followed by denaturation with 250 μl of 8 M urea and dilution with 950 μl dH_2O, prior to trichloroacetic acid (TCA) precipitation with 310 μl of 100% TCA, 1250 μl methanol and 625 μl chloroform. Samples were vortex-mixed and incubated (4 °C, 10 min) before centrifugation (4500*g*, 4 °C, 10 min). The top phase was removed before adding 1 ml methanol. The sample was vortex mixed before centrifugation (4500×*g*, 4 °C, 10 min), the supernatants were removed and the solid sample washed twice with 1 ml acetone, centrifuged at 10,000*g*, at 4 °C for 5 min, and dried under vacuum. Then, the sample was resuspended in 100 μl dH_2O.

Protein digestion was carried out using the method proposed by Le Bihan et al. (2011) on 20 μl of protein extract. Briefly, samples were denatured in 8 M urea, reduced by incubating with dithiothreithol (DDT) prior to cysteine alkylation with iodoacetamide, and overnight digestion with 60 μg trypsin at room temperature.