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Extraction and Characterisation of Protein Fraction from Date Palm Fruit Seeds

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***Abstract*—**Date palm (Phoenix dactylifera L.) seeds are waste streams which are considered a major problem to the food industry. They contain potentially useful protein (10-15% of the whole date's weight). Global production, industrialisation and utilisation of dates are increasing steadily. The worldwide production of date palm fruit has increased from 1.8 million tons in 1961 to 6.9 million tons in 2005, thus from the global production of dates are almost 800.000 tonnes of date palm seeds are not currently used [1]. The current study was carried out to convert the date palm seeds into useful protein powder. Compositional analysis showed that the seeds were rich in protein and fat 5.64 and 8.14% respectively. We used several laboratory scale methods to extract proteins from seed to produce a high protein powder. These methods included simple acid or alkali extraction, with or without ultrafiltration and phenol trichloroacetic acid with acetone precipitation (Ph/TCA method). The highest protein content powder (68%) was obtained by Ph/TCA method with yield of material (44%) whereas; the use of just alkali extraction gave the lowest protein content of 8%, and a yield of 32%.

***Keywords*—**Date palm seed, Phoenix dactylifera L., extraction of date palm seed protein

I. Introduction

PROTEINS are valued by the food manufacturer for their functional properties (emulsification, gelation, foaming etc.) and for their nutritional value. Of late there has been concern over the sustainability of some food protein sources such as fish meal protein, and the rising cost of others such as egg proteins and soy proteins. This has led to the investigation of alternative protein sources for food use which can be used to either partially or fully replace more expensive proteins.

There is also an advantage in using material that was previously considered to be waste to recover useful functional proteins. Such waste includes the peel, skin and seeds of fruits and vegetables, materials which are either discarded or used in low value commodities such as animal feed. One waste product we are investigating to see if useful protein can be extracted is the seed of the date palm fruit.

There is limited published work focused on date seeds even though they contain potentially useful quantities of protein and fat/oil, in this study we attempted to recover a high proportion of the soluble protein from date seed. Previous studies by [2]; suggest that at least 53% of the total protein can be recovered using simple aqueous extraction techniques. We tried to improve on this by using methodologies that are standard in the protein industry, and are commonly used for protein powders derived from other seed products (e.g. soya bean protein). Protein extraction from seeds is problematic due to the relatively low proportion of protein and the high proportion of complex carbohydrates.

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II. Materials And Methods

A. Materials

Date palm fruit (Phoenix dactylifera L.) at Tamr stage (complete maturity) of the commercially available date palm *Deglet Nour* variety were purchased from a local supermarket. The seeds were manually isolated, soaked in water, washed to remove any remains date flesh, air dried for a week, and then were further dried overnight at 40ºC. The seeds were ground into a fine powder and defatted by extraction with hexane using a Soxhlet apparatus. The defatted powder was dried to form a date seed powder (DSP). All chemicals and solvents used in this study were Analar grade.

B. Methods

1. Physical analysis

Firstly, calyxes were removed to measure the physical characteristics including: weight of whole fruit, flesh and seed, flesh: whole fruit ratio and seed: whole fruit ratio. The weight of one hundred fruits and seeds was measured, and then the average of single fruit and seed were calculated.

2. Chemical analysis

Proximate analysis of date palm fruit (DPF) and date seed powder(DSP) including moisture and total solids, Total ash, Crude fat, Protein, Crude fibre were carried out according to[3]. The percent yield of protein was calculated using a formula published by [4]:

$$\text{Percent yield} = \frac{\text{Extracted sample (g)} \times \%\ \text{Protein in extracted sample}}{\text{Starting sample (g)} \times \%\ \text{Protein in starting sample}} \times 100$$

Total carbohydrate was calculated by difference as total percent value using the following formula: Total carbohydrates = 100 – (%moisture + % ash + % protein + % fat + % crude fibre).

All analytical determinations were carried out in triplicate and the final data were expressed on a dry weight basis.

C. Laboratory preparation of date palm seed protein extract

Five methods were use to extract protein from DSP.

- *Method 1*

Method 1 was based on the procedure for concentrating the protein from soybean proposed by [5] with some modifications. This involved solubilisation of non-protein contaminants in defatted DSP using water/HCl, pH 4.5, 40ºC, 60min, centrifugal separation, neutralization of the sediment with 1M NaoH and freeze drying to form a protein concentrate.

- *Method 2*

Method 2 was based on the method by [6]. This involved solubilisation of non-protein contaminants in DPSP using water/NaOH, pH 10, 55ºC, 60min, centrifugal separation, washing of the sediment with distilled H2O, centrifugation,

resolubilization of pellet in 1M NaoH at pH 7 and freeze drying to form a protein concentrate.

- *Method 3*

Method 3 was based on method by[7]. DPSP was mixed with water/NaOH, pH 10, room temperature, 60min, and then centrifugally separated. The supernatant was kept for further treatment. The pellet was mixed with distilled water/NaOH, pH 10.0 centrifuged. The two supernatants were combined and ultrafiltered (10 kDa membrane) and freeze dried.

- *Method 4*

The same as method 2 except that after the pellet was solubilised the slurry was then ultrafiltered (10 kDa membrane) and freeze dried.

- *Method 5*

Method 5 was based on a method from [8], [9]. DPSP was mixed with cold acetone, vortexed and centrifuged. The pellet was washed with cold acetone twice and allowed to dry at room temperature. The pellet was ground to fine powder, rinsed with 15% w/v TCA in acetone, vortexed and then centrifuged. This was repeated 3x. Finally, the pellet is washed with cold 15% w/v TCA in water and another three 3x with cold 80% v/v acetone.

The pellet was suspended in a 1:1 mixture of Tris-buffer, pH 8.0 and dense SDS buffer (2%w/v SDS, 5%w/v sucrose 0.1M Tris-HCL, pH 8.0, 5% v/v β-mercaptoethanol), vortexed and centrifuged.

The pellet was re-suspended in the same buffer and centrifuged. Both supernatents were mixed and precipitated at 4 ºC overnight with five volumes of cold 0.1M ammonium acetate in methanol, and centrifuged The pellet was then washed three times with 0.1M ammonium acetate in methanol and centrifuged as above followed by the same process with cold 80% v/v acetone.

The pellet was mixed with aqueous 24%w/v TCA, vortexed, precipitate on ice for 30min, and centrifuged. The pellet was washed with cold acetone, incubated 15min on ice and centrifuged and dried.

III. Results and Discussion

A. Some physical measurements

The physical characteristics of date palm fruit provides important information to the date industry that helps trading, processing and storage of dates.

Table I summarizes the physical measurements, for the date variety used in this study. The data indicate that seed and flesh comprised 10 and 90% respectively of the mass of the whole fruit.

The results were higher than those reported by [10], which is most likely due to a difference in the variety of dates used, as well as differences in agriculture treatments and environmental conditions.

TABLE I
SOME PHYSICAL MEASUREMENTS OF DATE PALM (FLESH&SEED)

Measurements	Average
weight of whole fruit(g)	9.51*
weight of date flesh(g)	8.53
weight of date seed(g)	0.98
Flesh: whole fruit ratio	89.70
Seed: whole fruit ratio	10.30
Number of fruit in Kg	106.00

*All values are means of three determinations.

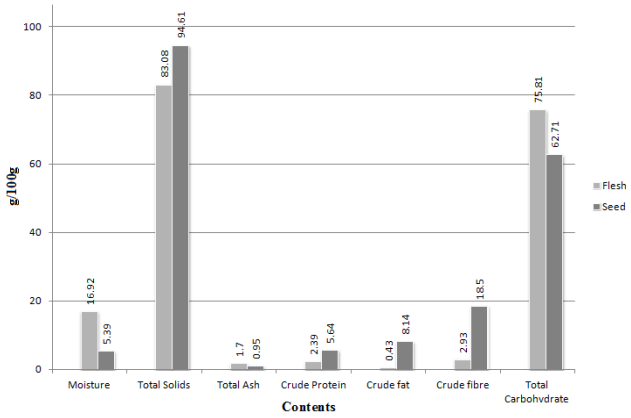

Fig. 1 proximate analysis of date palm (flesh and seed)

B. Proximate Composition of DPSP and DPF

Figure (1) presents the chemical composition of date palm flesh and seed. The total solids, crude protein, crude fat and crude fibre of date seed were higher than those in date flesh, whereas, moisture, total ash and total carbohydrate were lower than those in date flesh. Crude protein and crude fat were higher in the seed than in the flesh. The results of both (seed and flesh) contents were agreement with those reported by [11], [12] and [13], higher than those reported by [14], [15] and lower than those reported by [16], [17] and [18]. Those differences are most likely due to the variability between cultivars, as well as stage of maturity [19].

C. Chemical compositions of full fat DPSP and defatted DPSP

Prior to protein extraction, removal of the oil fraction from the seed is desirable. This changes the chemical composition of the powder. The chemical composition of full fat and defatted of date palm seed is presented in Figure 2. The fraction of all non-oil components increases slightly, as expected. In addition the color of defatted DSP was lighter than the original DSP.

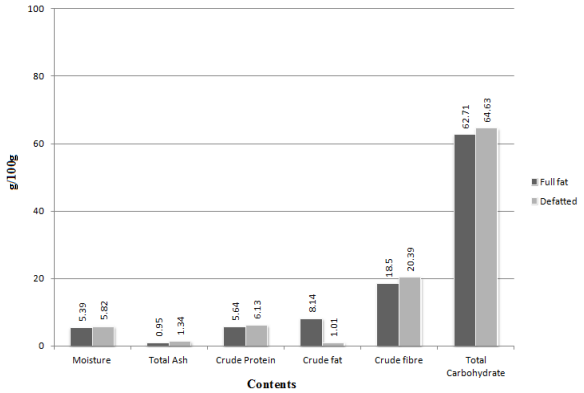

Fig. 2 Chemical compositions of full fat and defatted DSP

D. Protein extraction of date palm seed

Figure 3 presents results for the protein content and yield for the five procedures used to prepare date palm seed protein concentrates. illustrating the difficulty in removing protein from plant seeds. All methods were able to extract protein at a higher concentration than in the original powder. The protein content in DSP increased from 8.65% (Method 1) to 68.24 (Method 5) while the lowest protein extract was obtained by alkaline extraction (Method 1) The yield of protein extracted from the DSP ranged from 27-52%; 52.5%, with the highest protein yield obtained by using UF along with the alkaline extraction (Method 4) whereas, using just the alkaline extraction (Method 2) gave the lower protein yield. these The differences in protein content and yield are likely due to the presence of polysaccharides or other high molecular weight contaminants present free in solution or conjugated to the protein.

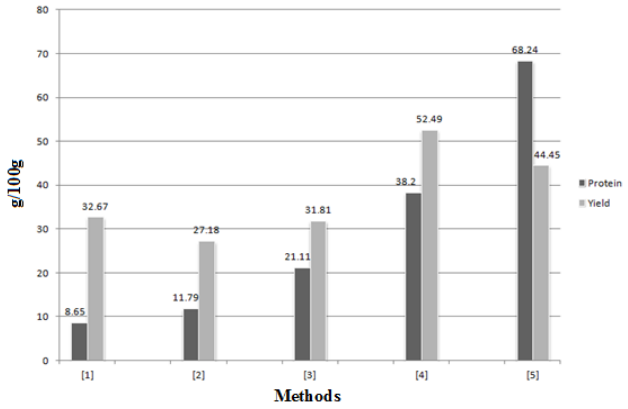

Fig. 3 Laboratory methods to extract date palm seed proteins

IV. Conclusions and Future Work

The increasing cost of foods particular food protein from animal source in the world has encouraged the food scientists to develop new source of plant proteins; date palm (seed or flesh) could be one of these sources. It is clear from the current results that protein of a relatively high quality can be recovered DSP, although it remains to be seen whether this can be done on a commercially viable scale. Any such protein could be used for animal feeds.

Additional experiments are under way in our lab to improve extraction using enzymatic methods to breakdown complex polysaccharides that interfere with the extraction of high purity powders. We are also carrying out studies to identify the proteins in of concentrated date palm protein powders using techniques such as 2D electrophoresis combined with Maldi-tof mass spectrometry. Further work will look at the functional properties of seed proteins to see if they may be of use as ingredients (emuslifiers, foamers or thickening/gellling agents in formulated foods.

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References

[1] FAO stat data base, 2007, www.FAO.org.
[2] Hamada, J.S., Hashim, I.B., and Sharif, F.A. Preliminary analysis and potential uses of date pits in foods. *Food chemistry*, 76, pp.135-137, 2002.
[3] Official methods of analysis, 15edition: Association of Official Analytical Chemists, AOAC, Washington, D.C, USA, 1995.
[4] Onsaard, E.; Pomsamud, P. and Audtum, P. Functional properties of sesame protein concentrates from sesame mel *As.J.Ag-Ind*. 3(04), pp.420-431, 2010.
[5] Wang, H.; Johnson, L. A. and Wang, T. Preparation of soy protein concentration and isolate from extruded-expel led soybean meals. *Journal of the American oil chemists society*, 81, pp.713-717, 2004.
[6] L'hocine, B. Composition and functional properties of soy protein isolates prepared using alternative defatting and extraction procedures. Journal of food science, 71, pp.137-145, 2006.
[7] Bouaziz, M., Besbes, S., Blecker, C., Wathelet, B., Deroanne, C., and Hamadi, A. Protein and amino acid profiles of Tunisian Deglet Nour and Allig date palm fruit seeds. *Fruits*, 63, pp.37-43, 2008.
[8] Wang, W.; Scali, M.; Vignani, R., Spadaford, A.Protein extraction for two-dimensional electrophoresis from olive leaf, a plant tissue containing high leavls of interfering compounds. *Electrophoresis* 24, pp. 2369 - 2375, 2003.
[9] Vidal, S. G.; Tena, M.; Liorca, L. V. L. and Salinas, J. Protein extraction from *Phoenix dactylifera* L. Leaves, a recalcitrant material for two-dimensional electrophoresis. *Electrophoresis*, 29, pp.448-456, 2008.
[10] Al-Hooti, S.; Sidhu, J.S. and Qabazard, H. Physicochemical characteristics of five date fruit cultivars grown in the United Arab Emirates. *Plant Foods for Human Nutrition*, 50, pp.101-113, 1997.
[11] Al-Showiman, S.S., & Baosman, A.A. Protein and amino acid contents of some Saudi Arabian date palm seeds (Phenix dactylifera L.) Arab Gulf J. Scient. Res., 10(2), 1-9, 1992.
[12] Al-Hooti, S.; Sidhu, J. S. and Qabazard, H.Chemical composition of seeds of date fruit cultivars of United Arab Emirates. *J. Food Sci. Technol.*, 35(1), pp.44-46,1998.
[13] Al-Shurfa, M. Y.; Ahmed, H. S. and Abou-Naji, S. E.Organic and inorganic constituents of date palm pit(seed). *Date palm J.*,1(2), pp.275-284,1982.
[14] Elleuch, M.; Besbes, S.; Roiseux, O.; Blecker, C.; Deroanne, C.; Drira, N. and Attia, H. Date flesh: chemical composition and characteristics of the dietary fibre. *Food chemistry*, 111, pp.676-682, 2008.
[15] Ahmed, I. A.; Ahmed, A. W. Chemical composition of date varieties as influenced by the stage of ripening. *Food chemistry*, 54, pp.305-309, 1995.
[16] Besbes, S., Blecker, C., and Deroanne, C. Date seeds: chemical composition and characteristic profiles of lipid fraction. *Food Chemistry*, 84, pp.577-584, 2004.
[17] Rahman, M.S., Kasapis, S., Al-Kharusi, N.S.Z., Al-Marhubi, I.M., and Khan, A.J.Composition characterisation and thermal transition of date pits powders. *Journal of Food Engineering*, 80, pp.1-10, 2007.
[18] Besbes, S.; Drira, L.; Blecker, C.; Deroanne, C. and Attia, H. Adding value to hard date (Phoenix dactylifera L.): compositional, functional and sensory characteristics of date jam. *Food chemistry*, 112, pp. 406-411, 2009.
[19] Hulm, A.C. the biochemistry of fruit and their products. Academic press,London&New York, Vol. 2, 1971.