Extraction and Characterisation of Protein Fraction from Date Palm Fruit Seeds

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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 40°C. 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, calyces 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]:

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

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 used 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, 40°C, 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 DSP using water/NaOH, pH 10, 55°C, 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 supernatants were mixed and precipitated at 4 °C 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.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Average</th>
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<tbody>
<tr>
<td>weight of whole fruit(g)</td>
<td>9.51*</td>
</tr>
<tr>
<td>weight of date flesh(g)</td>
<td>8.53</td>
</tr>
<tr>
<td>weight of date seed(g)</td>
<td>0.98</td>
</tr>
<tr>
<td>Flesh: whole fruit ratio</td>
<td>89.70</td>
</tr>
<tr>
<td>Seed: whole fruit ratio</td>
<td>10.30</td>
</tr>
<tr>
<td>Number of fruit in Kg</td>
<td>106.00</td>
</tr>
</tbody>
</table>

*All values are means of three determinations.

#### 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.
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 (emulsifiers, foamers or thickening/gelling agents in formulated foods.

ACKNOWLEDGMENT

The authors wish to thanks Libyan government for funding this research.

REFERENCES