Technical paper

Protein and amino acid profiles of Tunisian Deglet Nour and Allig date palm fruit seeds

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Abstract — Introduction. The fruit of the date palm (*Phoenix dactylifera* L.) is an important crop of the arid and semi-arid areas of the world. It has always played a genuine economic and social part in the lives of the people of these areas. Nevertheless, there are few studies related to the composition and the functional properties of the different components (fibres, fats and proteins) of the date seeds. In order to obtain more information on the nutritive value and the chemical composition of the pits of the most important and most valuable Tunisian date palm varieties (Deglet Nour and Allig), we studied their profiles of proteins and amino acids. **Materials and methods**. Date seeds of two Tunisian cultivars, Deglet Nour and Allig, were separately milled in a heavy-duty grinder to pass 1–2 mm screens; then, they were preserved at – 20 °C until analysis. The soluble proteins from defatted date seeds were extracted with water at pH 10, and were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). **Results and discussion**. Amino acid profiles revealed that date seeds from the Deglet Nour and Allig varieties contained the majority of essential amino acids: lysine, isoleucine, leucine, methionine, threonine, valine and phenylalanine. Seventeen amino acids were detected in the seeds of the two studied cultivars. Glutamic acid was the major amino acid in Deglet Nour and Allig date seeds, representing 17.83% and 16.77%, respectively, of the total amino acids. Date seeds appeared to contain a number of proteins with molecular weights ranging from 22 kDa to 70 kDa. Three prominent bands appeared at 32 kDa, 60 kDa and 70 kDa.

Tunisia / *Phoenix dactylifera* / fruits / seeds / chemical composition / proteins / amino acids

Protéines et acides aminés présents dans les noyaux des dattes tunisiennes Deglet Nour et Allig.

Résumé — Introduction. Le fruit du palmier dattier (*Phoenix dactylifera* L.) est une production importante des régions arides et semi-arides du monde. Il a toujours joué un rôle économique et social clef pour les populations de ces régions. Néanmoins, peu d'études ont porté sur la composition et les propriétés fonctionnelles des divers composants (fibres, graisses et protéines) des noyaux de datte. Afin d'obtenir davantage d'informations sur la valeur nutritive et la composition chimique des noyaux des variétés de dattiers les plus importantes et les plus intéressantes en Tunisie (Deglet Nour et Allig), nous avons étudié leurs profils de protéines et d'acides aminés. **Matériel et méthodes**. Des noyaux de datte de deux cultivars tunisiens, Deglet Nour et Allig, ont été séparément broyés dans un moulin à usage industriel afin de pouvoir passer au travers d'un tamis à mailles de 1–2 mm ; puis ils ont été conservés à – 20 °C jusqu'aux analyses. Les protéines solubles des noyaux de datte dégraissés ont été extraites avec de l'eau à pH 10, et elles ont été analysées par électrophorèse en gel de polyacrylamide contenant du laurylsulfate de sodium (SDS-PAGE). **Résultats et discussion**. Les profils d'acides aminés ont indiqué que les noyaux de dattes Deglet Nour et Allig contenaient une majorité d'acides aminés essentiels : lysine, isoleucine, leucine, méthionine, thréonine, valine et phénylalanine. Dix-sept acides aminés ont été détectés dans les noyaux des deux cultivars étudiés. L'acide glutamique a été le principal acide aminé dans les noyaux de dattes de Deglet Nour et d'Allig, représentant, respectivement,17,83 % et 16,77 % de l'ensemble des acides aminés. Les noyaux de datte ont semblé contenir un certain nombre de protéines avec des poids moléculaires s'étendant de 22 kDa à 70 kDa. Trois bandes importantes sont apparues à 32 kDa, 60 kDa et 70 kDa.

Tunisie / *Phoenix dactylifera* / fruits / graine / composition chimique / protéine / acide aminé

1. Introduction

The date (*Phoenix dactylifera* L.) has always played an important part in the economic and social lives of the people of arid and semi-arid regions of the world. The fruit of the date is composed of a fleshy pericarp and seed.

Date seeds are by-products, which could easily be recovered in the technological or biological date-processing industries [1]. The seed represents 10–15% of the total weight of the fruit [2]. In some date-processing countries, such as Tunisia, date seeds are discarded or used as fodder for domestic farm animals. Tunisia is considered to be one of the main date-producing countries; the mean annual yield of date fruits is about 125000 t. From this, around 12500 t of date seeds could be used because of their high fat, fibre and protein contents [3–5].

The published work on the valorisation of date pits has been especially focused on their incorporation in animal feeds [6–15] or on their biological transformation [1, 16–23]. From a practical point of view, the majority of these research tasks were not concretised in industrial projects [5]. The chemical composition of date pits shows their richness in fat (10–12%), proteins (5–6%) and fibres (75–80%) [3, 24–26]. This by-product of date-processing industries could be regarded as an excellent source of food ingredients with interesting technological functionality [3, 4].

A limited number of studies were carried out regarding the composition and functional properties of the various components of the date pits such as fibres, fats and proteins. The cracking approach proves to be interesting for the production of components with high added value considering their nutritional and/or techno-functional potential. Fractionation must obligatorily be justified by the study of the composition of each fraction and the development of its functional properties. In a previous work, we studied the main chemical composition, and functional and sensorial properties of the oily fraction of date pits. The results indicated the potential functional and economic utility of date seeds as a new source of oil and antioxidant compounds [4, 5, 27–29]. In order to contribute to further information about the nutritive value and chemical composition of date seeds, we studied the protein and amino acid profiles of pits from the most important and valuable varieties in Tunisia: Deglet Nour and Allig.

2. Materials and methods

2.1. Preparation of powdered date seeds

Date palm fruits were obtained from Deglet Nour and Allig date palms of the National Institute of Arid Zones (Degach, Tunisia). For each cultivar under investigation, seeds were directly isolated from 50 kg of date fruits collected at the Tamr stage (full ripeness), then kept at 10 °C for a week. The seeds were soaked in water, washed to get rid of any adhering date flesh, then air-dried. Their relative weight percentage compared with the weight of the fresh fruits was about 11.32% for the Deglet Nour variety and about 10.7% for the Allig variety. The air-dried seeds were further dried at about 50 °C. Date pits, of each variety, were separately milled in a heavy-duty grinder to pass 1–2 mm screens and then preserved at – 20 °C until analysis.

The chemical composition of date seeds from Deglet Nour and Allig varieties was previously analysed in our laboratories [4].

Lipid extraction was carried out as described by Besbes *et al*. [4] with a SER 148 Solvent Extractor (Velp Scientifica, Italy) equipped with six Soxhlet posts. The extraction was carried out over 30 min, with thimbles immersed in boiling petroleum ether, and 60 min of reflux washing. After removing the solvent, using a Rotavapor apparatus, the obtained defatted seeds were used for the analysis of protein and amino acid profiles.

2.2. Analysis of date seed amino acids

Defatted date seeds (150 mg) were hydrolysed with concentrated HCl (6N) at 110 °C for 24 h. Then, the hydrolysate was analysed by a BioChrom 20 Plus amino acid

Table I.
Amino acid composition of defatted date seed powder (mg·g^{-1} of dry matter).

Date palm variety	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val	Total	Protein
Allig	0.23	0.53	0.42	0.11	0.96	0.24	0.09	0.13	0.30	0.26	0.06	0.19	0.16	0.18	0.14	0.05	0.24	4.29	5.72
Deglet Nour	0.25	0.63	0.48	0.11	1.07	0.27	0.11	0.15	0.33	0.28	0.06	0.21	0.18	0.19	0.16	0.04	0.26	4.78	6.00

analyser according to the method of El-Adawy *et al.* [30]. Amino acids were analysed by chromatographic ionic exchange and detected colorimetrically using ninhydrin reagent. All amino acids were detected at 570 nm except proline and hydroxyproline, which were detected at 440 nm. The amino acid concentrations were calculated from the standard curves.

2.3. Analysis of soluble proteins

2.3.1. Extraction procedure

Defatted and powdered date seeds (40 g) were suspended in 400 mL distilled water, then adjusted to pH 10 using 0.1 M NaOH. The suspension was stirred for 40 min at room temperature, then centrifuged at 4000 rpm for 20 min. The residue was again mixed with 200 mL distilled water, readjusted to pH 10, and centrifuged following the same process. The supernatants of both centrifugations were blended, then lyophilised and used for the analysis of the protein profile by electrophoresis [sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)].

2.3.2. Chemical analysis of lyophilised soluble extracts

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean standard deviation.

Dry matter was determined according to the Association of Official Analytical Chemists (AOAC) [31].

Nitrogen content of defatted samples was determined by the Kjeldahl method, following the method of the AOAC [31]. Protein content of each sample was calculated by multiplying the total nitrogen content by a factor of 6.25 [4]. Protein yield was calculated after the determination of protein content in the powdered seed and in the lyophilised supernatant.

Carbohydrate content was estimated by difference of mean values, *i.e.*, [100 - (moisture% + ash% + protein% + lipids%)] [26, 32].

Ash content was determined after incineration at 550 °C, for 8 h, using a muffle furnace (NABER, Germany). It was expressed as percent of dry weight [31].

2.3.3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Hydrosoluble proteins of defatted date seeds were analysed on polyacrylamide gel (10%) according to the method of Laemmli [33]. The protein bands were visualised with alkaline silver nitrate.

3. Results and discussion

3.1. Amino acid profile of date seed proteins

Date seeds of the Deglet Nour and Allig varieties have similar amino acid profiles (*table I*). Seventeen types of amino acids were detected and identified. Glutamic acid (Glu) was the predominant amino acid, followed by arginine (Arg), aspartic acid (Asp), leucine (Leu), lysine (Lys), valin(Val), glycine (Gly), alanine (Ala) and phenylalanine (Phe). These observations were in accordance with those reported by Salem and Hegazi [34] and by Al-Rawi *et al.* [35] in their studies of the amino acid composition of Egyptian and Iraqi date seeds, respectively. Glutamic acid presented the largest amount,

Table II.
Chemical composition of lyophilised water extract obtained from defatted date seeds.

Date variety	Dry matter	Protein	Carbohydrate	Ash	Protein yields
		($g \cdot 100\ g^{-1}$ of dry matter)			(%)
Allig	97.30 ± 0.06	14.26 ± 0.10	83.00 ± 0.37	2.43 ± 0.26	30.40 ± 0.23
Deglet Nour	98.11 ± 0.02	16.95 ± 0.22	80.00 ± 0.72	3.12 ± 0.50	36.40 ± 0.45

varying from 17.83% for Deglet-Nour seeds to 16.77% for the Allig seeds. These values were in agreement with those found by Salim and Ahmed [36] for Saudi Arabian date seeds.

The essential amino acids (lysin, leucine, threonine, methionine, valin, isoleucine and phenylalanine) were present in the date seeds of the two studied varieties, except tryptophan. The disappearance of tryptophan could be attributed to its destruction during acid hydrolysis and could also account for the loss of cysteine [36].

Date seed proteins are of a relatively important biological value by reference to the standard egg proteins, considering their wealth in essential amino acids [37].

3.2. Chemical composition of soluble water extract from defatted date seeds

The freeze-dried soluble extracts of defatted date pits from the Deglet Nour and Allig varieties contained 14–17% protein, 80–83% carbohydrate and 2–3% ash (*table II*). This composition shows, as for the raw material used, that the extract resulting from Deglet-Nour defatted date seeds contained slightly higher protein and ash contents than those of the Allig extract. This could explain the difficult diffusion of proteins and minerals for the Allig extract.

The soluble extracts from Allig and Deglet Nour seeds contained more proteins than the powdered defatted date seeds [14.26–16.95 $g \cdot 100\ g^{-1}$ (dry matter basis) against 5.75–6.18 $g \cdot 100\ g^{-1}$ [4], respectively]. Protein yield was about 30–36% of the total date seed proteins (*table II*). Therefore, a large portion of date seed proteins was insoluble. These insoluble fractions are likely to be composed of high-molecular-weight polypeptides that are highly aggregated and / or cross-linked by disulphide bridges [3].

Albumins and globulins represent the two major classes of proteins. Albumins are soluble in water, while globulins are soluble in salt solutions [38]. Hamada *et al*. [3] tried to extract proteins from date cores using various solvents such as salt solutions, ethanol solutions and acetic acid solutions. They reported that a large fraction of protein was not dissolved under these conditions.

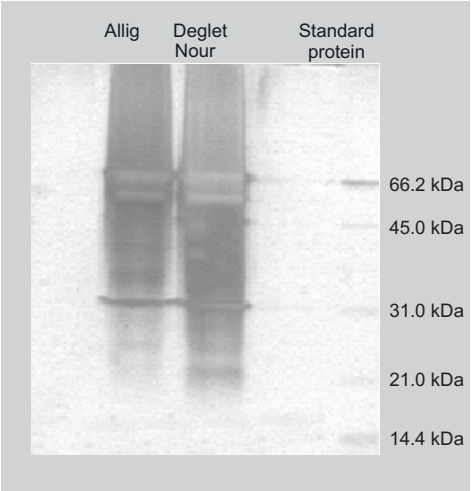

Figure 1.
Electrophoretic profiles of soluble proteins from date seeds on a 10% polyacrylamide gel by sodium dodecyl sulphate (SDS-PAGE) compared with the profile of a standard protein.

3.3. Electrophoretic profile of date seed soluble proteins

The SDS-PAGE protein profiles of the Allig and Deglet Nour extracts from the defatted date seeds were similar and revealed three principal bands (*figure 1*). The prominent protein bands have a molecular weight around 70 kDa, 60 kDa and 32 kDa when

compared with the bands of a standard protein. These proteins could be albumins, globulins, glutelins and / or prolamines, which are soluble in alkaline solutions [3].

Water-soluble albumins constitute the major proteinic fraction of date flesh. The electophoretic study in SDS medium of several varieties of Eastern dates (Buraringa, Barneh, Fardh, Hilali and Zabad) revealed two principal proteinic fractions whose molecular weights are 32 kDa and 72 kDa, respectively. On the other hand, only one proteinic band of 30 kDa was identified for Californian cultivars [38].

4. Conclusion

According to our previous results on date seed oils [4] and those of the present study, date seeds are rich in many nutritional compounds, which would justify their use as a possible valuable source for human nutrition. The proteins of date cores would have to be studied more in order to reduce their low solubility, to improve the output of extraction and to study their techno-functional properties for justifying their use as an ingredient in food formulations.

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Proteinas y aminoácidos presentes en los huesos de los dátiles tunecinos Deglet Nour y Allig.

Resumen — Introducción. El fruto de la palmera de dátiles (*Phoenix dactylifera* L.) es una importante producción de las regiones áridas y semi áridas del mundo. Siempre desempeñó un papel económico y social clave para las poblaciones de estas regiones. Sin embargo pocos estudios se refirieron a la composición y a las propiedades funcionales de los diversos componentes (fibras, grasas y proteinas) de los huesos de dátiles. Con el fin de obtener más información sobre el valor nutritivo y la composición química de los huesos de variedades de los dátiles más importantes y más interesantes en Túnez (Deglet Nour y Allig), estudiamos sus perfiles de proteinas y de aminoácidos. **Material y métodos**. Se molieron por separado en un molino de uso industrial los huesos de dátiles de dos cultivares tunecinos, para que pudiesen pasar a través de un tamiz de mallas de 1–2 mm; después de conservaron a – 20 °C hasta los análisis. Se extrajeron con agua de pH 10 las proteinas solubles de los huesos de dátil desengrasado, y se analizaron mediante electroforesis en gel de poliacrilamida que contenía laurilsulfato sódico (SDS-PAGE). **Resultados y discusión**. Los perfiles de aminoácidos indicaron que los huesos de dátiles Deglet Nour y Allig contenían la mayoría de aminoácidos esenciales: lisina, isoleucina, leucina, metionina, treonina, valina y fenilanalina. Se detectaron diecisiete aminoácidos en los huesos de los dos cultivares estudiados. El principal aminoácido en los huesos de los dátiles Deglet Nour y Allig fue el ácido glutámico, representamndo, respectivamente, el 17,83 % y el 16,77 % del conjunto de los aminoácidos. Los huesos de los dátiles resultaron contener un cierto número de proteínas con pesos moleculares extendiéndose desde 22 kDa hasta 70 kDa. Aparecieron tres bandas importantes con 32 kDa, 60 kDa y 70 kDa.

Túnez / *Phoenix dactylifera* / frutas / semilla / composición quimica / proteínas / aminoácidos