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IDENTIFICATION OF DATE (*PHOENIX DACTYLIFERA*) CULTIVARS BY PROTEIN PATTERNS

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Key Word Index—*Phoenix dactylifera*; Palmae; dates; protein; isoelectric points; M_rs; electrophoresis.

Abstract—The water-soluble proteins extracted from the soft tissue of ripe dates (cultivars Rcziz, Marzban, Mowahed, Kholass, Hatmy, Shishy, Shahl and Gir) collected in Saudi Arabia and one cultivar (Muskade) from Iraq were freed from some of their acidic polysaccharides by treatment with 33% propan-2-ol. The best protein separations and the most characteristic patterns with which to identify the cultivars were obtained after PoroPAGE or PAGIF, with both tube and thin-layer techniques. The native proteins showed characteristic M_rs in the range of 20, 22, 24 and 27 k and differentiation by isoelectric points either in the range pH 4–5 or pH 8–9. The main subunits (M_r 25 and 85 k) were used for final discrimination in SDS-PoroPAGE or in SDS-PAGE.

INTRODUCTION

Information on the chemical composition of dates (*Phoenix dactylifera* L.), especially about their proteins, is limited. Ejlali *et al.* [1] studied four cultivars grown in Iran and found that the cv. Sayer had the highest protein content. Auda *et al.* [2] reported that the protein content of ripe dates of three cultivars (Khastawi, Khadhrawi and Zahdi) from Iraq was 2.3, 2.0 and 1.9‰ respectively, on the basis of Kjeldahl nitrogen determinations. The same method was applied by Kamel and Kramer [3], who obtained values of 3% protein for dried dates, and 5.2–10.6% crude protein for date pits [4]. Hasegawa and Maier [5] purified polyphenol oxidase from an extract of Deglet Noor dates by ammonium sulphate precipitation followed by column chromatography on DEAE-cellulose.

We have attempted to separate date proteins [6]. After establishing the best method for the extraction of date proteins, we went on to study the suitability for cultivar differentiation of the patterns given by various methods of electrophoresis. The study was performed with eight cultivars from different plantations in Saudi Arabia and one from Iraq.

RESULTS AND DISCUSSION

The protein content of date extracts was determined after Kjeldahl digestion using albumin as a reference protein. The calculated amount of proteins in the cultivars ranged from 0.45 to 0.88 g protein/100 g fresh pulp, with Hatmy having the highest protein content and Muskade the lowest (Table 1).

Dates of the cv. Muskade were used to find the best method for extraction and separation of proteins. The original extract when separated by PAGE, SDS-PAGE and by PoroPAGE showed poor separation of proteins due to unknown, viscous compounds which were co-extracted and which interfered with the separation. It seemed most likely that acidic polysaccharides formed the bulk of the viscous material since its components moved slowly to the anode during PAGIF and did not stain like proteins. These compounds interfered less in PAGIF when the samples were applied near the anode and the separation was improved when the viscous components were at least partially removed by precipitation with propan-2-ol.

A good comparison of cultivars was possible when the water extract from the pulp either with or without urea was treated with propan-2-ol (added slowly and with vigorous shaking) to a final concentration of 33%. Most of the viscous material was precipitated and most of the proteins remained in solution. The precipitate contained

Table 1. Protein content of date cultivars and the subunit distribution (apparent M_r of the protomers) after SDS-PAGE

		$M_r \times 10^4$	
Cultivar	N × 6.25*	2.5	8.5
Rcziz	0.73	+ + +	+ + +
Marzban	0.76	+ + +	+ + +
Mowahed	0.73	—	+ + +
Kholass	0.75	+ + +	+ + +
Hatmy	0.88	+	+ + +
Shishy	0.66	—	+ + +
Shahl	0.50	+ + +	+
Gir	0.81	+	+ + +
Muskade	0.45	--	+

*Calculated as g protein/100 g fresh pulp.
+ + +, strong band; +, weak band.

between 5 and 9 mg N per 100 ml extract. The precipitation of proteins with different concentrations of ammonium sulphate did not work as well. All steps were checked by PAGIF.

Comparison of protein patterns after PAGIF

PAGIF with Servalyt T at pH 4 9 gave reasonable patterns, but addition of 6 M urea and even more so 8 M urea greatly improved the separation, especially for fractions having pIs between pH 5.3 and 6.6. Random samples of individual ripe dates from different plantations of the cv. Muskade yielded the same patterns in PAGIF, showing that their genetic background must be the same (Fig. 1). After this test within one cultivar, nine different cultivars were compared by PAGIF either in tubes or in thin layers, with or without urea, using commercial reference proteins or the proteins from a mature potato cv. Maritta as marker for the pIs.

The results obtained by the thin-layer technique (TL-PAGIF) showed that the more acidic proteins were quite similar for all cultivars while some in the basic region were different. The cvs. Rcziz, Mowahed, Hatmy, Shishy and Gir could not be differentiated by their pIs using this technique, and the cv. Kholass resembled them. The cv. Muskade harvested in Iraq seemed to be similar, but not identical, to this group. Marzban gave a similar pattern to Shahl, both of which were different from the others. The differences were seen particularly in the basic region around pH 7.4, 7.9, 8.35, 8.50 and 8.70 (Fig. 2A).

The results were different in some cases when using the same conditions and samples but performing PAGIF in tubes (Fig. 2B). The cvs. Rcziz and Mowahed gave similar protein patterns which differed from those of cvs. Hatmy, Shishy and Gir. The differences between these cultivars were observed particularly in the acidic region. A band with pI 4.6 not seen in TL-PAGIF was found in cvs. Rcziz, Mowahed and Shahl but not in the other cultivars. Muskade seemed to be similar to Rcziz, Mowahed, Hatmy, Shishy and Gir, which differed in two protein bands of pI 4.6 and 4.9 in the acidic region. The cvs. Marzban and Shahl are similar but not identical as in TL-PAGIF, since Shahl had two prominent acidic proteins with pI 4.6 and 4.9 and Marzban one at pH 4.9 (Fig. 2B).

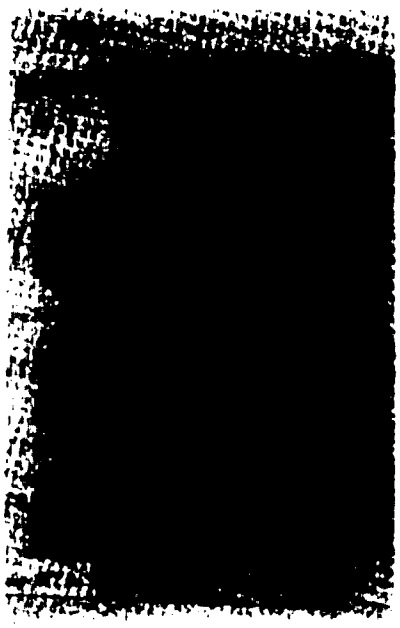

Fig. 1. Reproducibility of protein patterns given by untreated extracts of different dates from the same cultivar (Muskade). PAGIF: thin-layer (0.18 mm), Servalyt T at pH 4–9 for 2510 V hr. M, sap from potato tuber cv. Maritta.

Combining the results of PAGIF in thin layers and in tubes, we could identify each cultivar since each had some unique characteristic(s) in its PAGIF pattern (Table 2). It

Fig. 2. Protein patterns given by extracts (20 µl each, prepared as in Fig. 1) from nine cultivars of dates: 1, Rcziz; 2, Marzban; 3, Mowahed; 4, Kholass; 5, Hatmy; 6, Shishy; 7, Shahl; 8, Gir; 9, Muskade; M, sap of cv. Maritta. PAGIF: thin-layer (A) as in Fig. 1, but in 6 M urea; the same conditions but done in tubes (B), 500 V hr was used.

Table 2. Characterization of cultivars by the pIs of proteins. PAGIF in tubes and in thin layers

pI	Rcziz	Marzban	Mowahed	Kholass	Hatmy	Shishy	Shahl	Gir	Muskade
				(A) PAGIF in tubes with 6 M urea					
4.2	—	+	+	+	—	—	—	—	—
4.6	+ + +	—	+ + +	—	—	—	+ + +	—	—
4.9		+ + +		+ + +		—	+ + +	+	+ + +
				(B) PAGIF in thin layers and tubes with 6 M urea					
7.4	+	+ + +		+ +		—	+ + +		+
7.9	+	+ + +					+ + +	—	+
8.35	+	+ + +		+		—	+ + +		+
8.5	+	+ +	—	—		—	+ +	—	+
8.7		+	—	—		—	+	—	—

should be emphasized that pIs in aqueous solutions do not correspond with pIs in urea solutions.

Protein patterns after PoroPAGE

The M_rs of the native proteins in the original extract (viscous material not removed) were estimated by porosity gradients in 5–25% PAA both with and without 1 M urea, using oligomers of serum albumin as marker proteins. The separation in 1 M urea or in the absence of urea was better than that in 6 M urea. The cultivars showed different M_r distributions. The cvs. Rcziz, Marzban, Mowahed, Kholass and Shahl gave a strong band of M_r 268 k. This band was not given by cvs. Hatmy, Shishy and Muskade, although cv. Gir contained it in low concentration. The patterns differed also for proteins in the lower M_r range. The cvs. Marzban and Shahl had proteins of 20, 22, 24 and 27 k. In cv. Kholass the latter two bands were missing and the other cultivars did not have proteins in this range. Cultivars Rcziz, Marzban, Mowahed, Kholass, Hatmy and Gir had a protein of 134 k in contrast to cvs. Muskade, Shishy and Shahl. Cultivars Rcziz, Marzban, Mowahed, Kholass and Muskade had stronger bands of 50 and 52 k compared to the other cultivars.

After removing the viscous compounds by precipitation with 33% propan-2-ol, the same electrophoretic technique was applied. Without urea, the protein bands of 268 k and of 20, 22, 24 and 27 k were missing after removing the propanol precipitate. On the other hand, all the cultivars showed a strong band of 134 k, except cv. Shahl (weak band) and Muskade (no band). Cultivars Rcziz, Marzban, Mowahed and Kholass each showed a band around 80 k barely visible in the others (Fig. 3). With 1 M urea, a strong band appeared of M_r very close to 134 k in cvs. Hatmy and Gir only.

Subunits of proteins

Protomers (SDS-loaded subunits) of date proteins were separated in SDS-PoroPAGE (15–30% PAA) or in SDS-PAGE (15% PAA with 10% sucrose or glycerol). The separation was better in 15% PAA compared with the use of only 7.5% PAA. The main subunit of 85 k was found in all cultivars as a strong band except in cvs. Shahl and Muskade, and as a weak band after SDS-PoroPAGE (Fig. 4); the other (25 k) was found only in cvs. Rcziz, Marzban, Kholass and Shahl as a strong band and as a weak band in cvs. Hatmy and Gir. It was not seen in cvs. Mowahed, Shishy and Muskade. The ratio of these two main subunits allowed for a further differentiation (see Table 1).

CONCLUSION

Different samples from one cultivar taken from different plantations showed the same protein pattern. Hence it must be concluded that such a pattern is a characteristic feature of the respective cultivar. Ripe dates have to be used since during maturation tissue proteins tend to change, as discussed in refs. [7] and [8]. But it should be emphasized that this change is not as obvious when real protein patterns are investigated compared to 'proteins' based on N determinations which include changes in the degree of amidation.

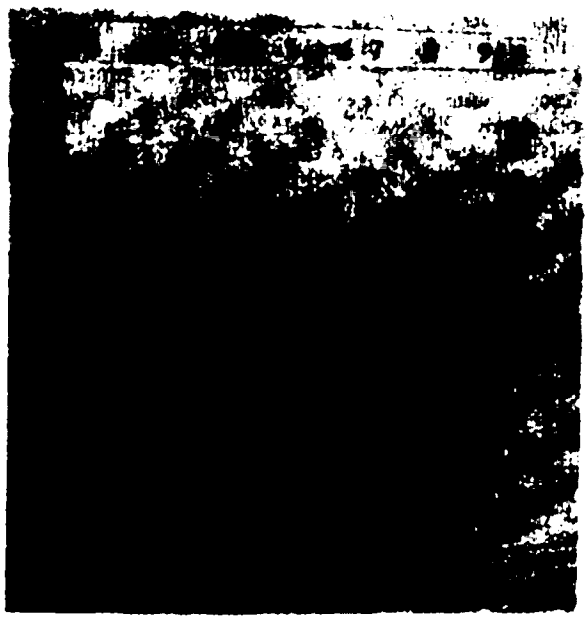

Fig. 3. M_r distribution of nine cultivars of dates (sequence as in Fig. 2) after precipitation with 33% propan-2-ol. PoroPAGE with 5–25% PAA in Tris-borate buffer, pH 8.9, without urea for 16 hr at 300 V. S, bovine serum albumin as M_r marker (multiples of 67 k).

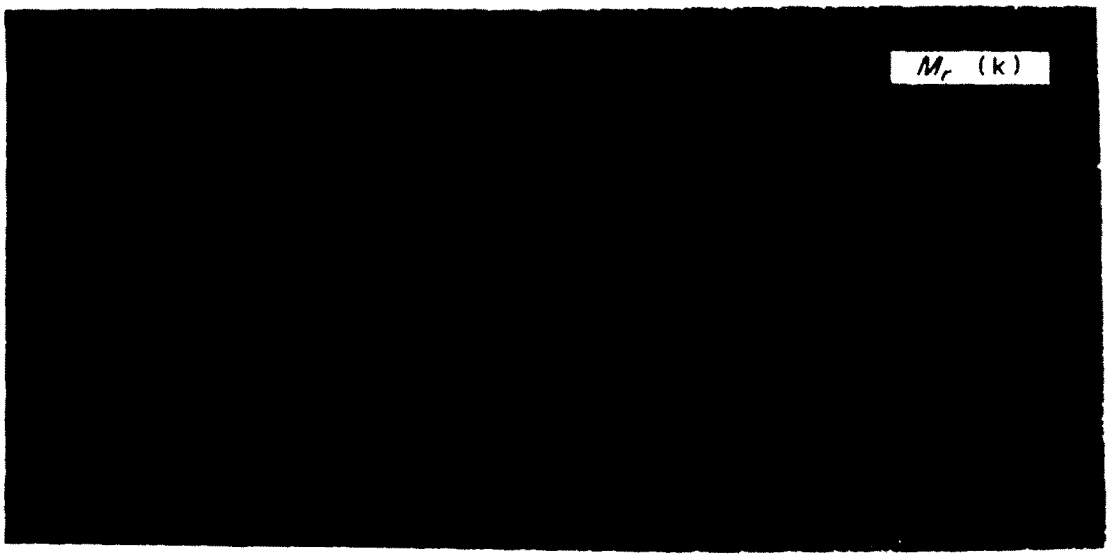

Fig. 4. Protein subunits of date cultivars. Sequence as in Fig. 2; preparation as in Fig. 3. The SDS-PoroPAGE was done in 15–30% PAA and Tris–borate buffer, pH 8.9, with 0.1% SDS for 17 hr at 300 V. M, SDS protein markers (lysozyme, 14.3; chymotrypsin A, 25.7; alcohol dehydrogenase, 37.0; bovine serum albumin, 67.0 and phosphorylase b, 97.4 k) from bottom to top.

Different cultivars from the Haza region (Saudi Arabia) yielded protein patterns characteristic of the respective cultivar, regardless of the plot in which they were grown. However, further effects due to open pollination were not investigated. This leads to the conclusion that the pericarpic tissue of a ripe date is a fairly safe material for differentiation of cultivars.

The quality of the electrophoretic separation of proteins to identify cultivars depends, particularly for PAGE and SDS-PAGE, on removing the material which is precipitated by propan-2-ol treatment. The protein patterns of aqueous date extracts were suitable for differentiation by PoroPAGE (5–25% PAA) or by PAGIF (pH 4–9), the latter in 6 or 8 M urea. SDS-PAGE and SDS-PoroPAGE allowed for some further differentiation due to the different ratios of the two main subunits (25 and 85 k).

It is noteworthy that the results of PAGIF differed depending on whether the separation was done in tubes or in thin layers (TL). Both were reliable and reproducible, but the TL technique yielded less differentiation in spite of more (*ca* 43) bands which were mostly identical among the cultivars in the acidic region. The differences seen here were particularly in the basic region between pH 7.4 and 8.7. The patterns in tubes (only *ca* 20 bands) differed mainly in the acidic range, indicating that a combination of both PAGIF types should be applied.

EXPERIMENTAL

Materials. The cv. Muskade from Iraq was purchased from the market and eight cvs., Rcziz, Marzban, Mowahed, Kholass, Hatmy, Shishy, Shahl and Gir, were collected in Saudi Arabia (Hasa region).

Sample preparation. Dates were peeled and the skin and seeds discarded. From the remaining pulp, 4 g was taken and ground with 5 ml H_2O in a porcelain mortar and then centrifuged at 20000 *g* at 3° for 40 min. The clear supernatant was purified in different ways in order to remove non-protein compounds: (1) Seven-fold concn and dialysis against H_2O overnight in a collodion bag (SM 13200) using all-glass apparatus (SM 16304, Sartorius Membranfilter, Göttingen) at 500 mm Hg. (2) Precipitation by propan-2-ol to remove non-protein material. The final concns of PrOH ranged from 20 to 75%. The residues and the supernatant were concentrated as in method (1) and then analysed. Similar treatments were done with 23% propan-2-ol in 3.46 M urea, 33% propan-2-ol in 3.00 M urea, and 41% propan-2-ol in 2.65 M urea. (3) $(NH_4)_2SO_4$ was added to the supernatant up to 43, 60 and 75% saturation, respectively. The ppt. was centrifuged down at 20000 *g* at 3° for 40 min and dialysed first against 1% and then against 0.1% glycine each time for 30 min. The concn was done as in method (1).

The total protein content of the samples was estimated by comparing bands in different electrophoretic systems [9] with bands of reference proteins of known concn.

Determination of proteins. Total N was determined after Kjeldahl digestion according to the method of ref. [10].

Electrophoresis. Vertical electrophoresis in 3 mm slabs was performed in either a PANTA-PHOR or a MONO-PHOR apparatus. The POOMA-PHOR was used particularly for slabs of 1 or 2 mm thickness (equipment from Labor-Müller, Hann. Münden). Tris borate buffer (0.125 M), pH 8.9, in 6% PAA was applied in standard PAGE. SDS-PAGE was done in 7.5% PAA with 10% glycerol in Tris borate buffer, pH 8.9, containing 0.1% SDS as in ref. [11] and in 15% PAA with 10% glycerol or with 10% sucrose. SDS-PoroPAGE was performed in 15–30% PAA in Tris–borate buffer, pH 8.9, with 0.1% SDS, according to ref. [9].

PoroPAGE was done with 5–25% PAA in Tris–borate buffer, pH 8.9, with 1–7% sucrose in 6 M urea and in the absence of urea, or in a steeper sucrose gradient, 1–30%, essentially according to ref. [12]. Oligomers of serum albumin were used as M_r markers.

PAGIF was done in the MONO-PHOR apparatus in thin layers (0.18 mm) of 5% PAA with 3% Servalyt T at pH 4–9 in

6 M or 8 M urea or in the absence of urea according to refs. [13], [14] and [9] for about 3300 V hr. PAGIF in tubes was performed in 6% PAA and 1% Servalyt T, pH 4–9, with 6 M urea for about 500 V hr.

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