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The composition and properties of date proteins

I. S. A. Ahmed, K. N. Al-Gharibi, A. S. Daar & S. Kabir*

Laboratory for Molecular Immunology, Department of Surgery, College of Medicine, Sultan Qaboos University, PO Box 35, Al-Khodh, Muscat, Postcode 123, Sultanate of Oman

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Proteins from various date cultivars were isolated by extraction with phosphate-buffered saline (PBS) and were analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Dates contained a number of proteins with molecular weights ranging from 12000 to 72000. Although there were minor protein bands, most date cultivars contained two prominent bands appearing at 30000 and 72000. Upon isoelectric focusing on thin-layer polyacrylamide gels, date proteins were resolved into five or six bands in the pH range of 4·15–5·85. Sequential extraction of date pulps, first with water and later with PBS, showed that most date proteins were water-soluble albumins. When the kinetics of protein accumulation were studied, it was observed that early green dates contained very little protein. There was a rapid increase in the protein content and also in the number of protein components at a later stage in maturation. Dates from the countries of the Middle East such as Saudi Arabia, Oman and Iran were similar in their protein profiles as they contained similar complex mixtures of proteins in the molecular weight range of 12000–72000. A date variety from the USA had very little protein and also a simple protein profile with one major band appearing at 30000. Amino acid analysis revealed that dates, irrespective of their cultivars, contained all the essential amino acids. These proteins were rich in acidic amino acids and poor in sulphur-containing amino acids such as methionine and cysteine.

INTRODUCTION

Date palm (*Phoenix dactylifera*) is considered to be one of the oldest cultivatable crops, being extensively grown in northern Africa, the Arabian Peninsula and Iran (Janick, 1977). It is also cultivated in the arid and semi-arid areas of the south-western US. The date is a one-seeded fruit, usually oblong. It has been the staple food and chief source of wealth in the irrigable desert from ancient times. It is very high in carbohydrate content (60% of dry weight), but also contains proteins (about 2%) and traces of fats (Pennington, 1989). Despite the presence of a small amount of protein, the date is richer than most other fruits in protein (Pennington, 1989). Although the date is a popular human food, very little is known about the composition and properties of date proteins. There are several date varieties. The present investigation was initiated to analyse the protein compositions of dates obtained from various cultivars. Although primarily examining several date varieties cultivated in Oman, we have also included in the study dates grown in other countries such as Saudi Arabia, Iran and the USA.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Isolation of date proteins

Mature dates were collected directly from various botanically identified date palm trees cultivated in the Sultanate of Oman. Unless otherwise stated, proteins were isolated from mature dates by extraction with phosphate-buffered saline (PBS, pH 7·4). Briefly, date pulps (100 g), obtained by removing seeds, were crushed in a Waring blendor with PBS (200 ml) containing 0·01% sodium azide. The mixture was stirred at 4°C for 20 h and centrifuged at 20000 rpm in a Beckman J-21 high-speed refrigerated centrifuge. Afterwards the supernatant was collected and analysed for proteins and carbohydrates.

Sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS-PAGE)

Unless otherwise stated, proteins were analysed on polyacrylamide gels (10%, w/v, 1 mm) using the discontinuous buffer system of King and Laemmli (1971). Protein bands were visualised by staining with either Coomassie Brilliant Blue R-250 or alkaline silver nitrate (Kabir, 1986).

Isoelectric focusing (IEF)

High-performance analytical isoelectric focusing was performed in 0·5 mm thin-layer polyacrylamide gels using a pH gradient of 4·0–6·5. Ultra-thin gels were cast between glass plates separated by a rubber gasket (0·5 mm thickness). The solutions for gel polymerisation were prepared as follows: (a) acrylamide (29·1 g) made upto 100 ml, (b) bisacrylamide (0·9 g) made upto 100 ml in water, (c) ammonium persulphate (100 mg) dissolved in 1 ml of water. Acrylamide solution (3·5 ml) was mixed with bisacrylamide (3·5 ml), ampholine (1·5 ml), ammonium persulphate (0·15 ml, 10% w/v), water (12·35 ml) and TEMED (20 μl). Polymerisation was complete in 2 h. IEF was performed on a flat bed (LKB, Sweden) cooled to 5°C. The anode and the cathode wicks were saturated with CH_3COOH (0·5M) and NaOH (0·5M), respectively, and proteins were focused at a constant power of 25 W for a period 120 min.

Amino acid analysis

Date proteins, obtained by extracting date pulps with PBS, were hydrolysed with concentrated HCl (6M) at 110°C for 24 h and analysed by a Beckman 7300 high performance amino acid analyser with integrator Beckman 7000 data system.

Miscellaneous determinations

Proteins were quantitated by the Bio-Rad's protein assay kit following the procedure of Bradford (1976). Carbohydrates were quantitated by the phenol-sulphuric acid method (Dubois et al., 1956).

RESULTS

Protein composition

In this investigation we have used nine different varieties of dates (Fardh, Bunaringa, Khasab, Khuneizy, Zabad, Hilali, Khalas, Barneh and Handhal) popularly consumed in Oman. Proteins were isolated by extracting crushed date pulps with PBS. Although these extracts were rich in carbohydrates, they also contained significant amounts of proteins (Table 1).

Table 1. Carbohydrate and protein contents of various date varieties[a]

Date variety	Carbohydrate (mg/ml)	Protein (mg/ml)
Bunaringa	185	1·03
Barneh	157	0·93
Fardh	155	1·14
Khalas	171	1·09
Khasab	145	0·82
Khuneizy	113	0·62
Handhal	143	0·79
Hilali	160	0·73
Zabad	206	1·07

[a]PBS extracts of date pulps were analysed for carbohydrate and protein contents as described in the text.

Date proteins from different varieties were analysed by SDS-PAGE under reducing conditions, bands being visualised by staining with Coomassie Brilliant Blue R-250 and alkaline silver nitrate. A few bands were detected when gels were stained with Coomassie Brilliant Blue R-250 (Fig. 1(a)). Most dates contained prominent protein bands in two regions of molecular weights, one appearing around 72 000 and the other at 30 000. There were variations in intensities among these protein bands. The protein band at 72 000 was prominent with dates from cultivars such as Barneh, Bunaringa,

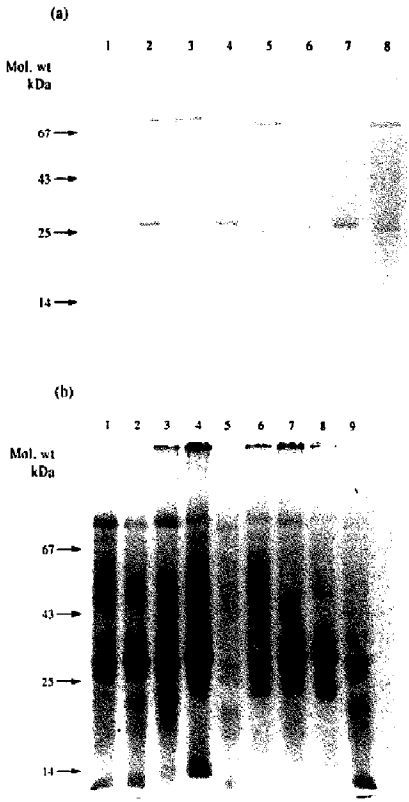

Fig. 1. Analysis of PBS-soluble date proteins on a 10% polyacrylamide gel by SDS-PAGE under reducing conditions according to the procedure of King and Laemmli (1971). (a) Bands were visualised by staining with Coomassie Brilliant Blue R-250. The positions of various date varieties are (1) Barneh, (2) Bunaringa, (3) Fardh, (4) Khalas, (5) Khasab, (6) Khuneizy, (7) Hilali, and (8) Zabad. (b) Bands were visualised by staining with alkaline silver nitrate (Kabir, 1986). The positions of various date varieties are (1) Barneh, (2) Bunaringa, (3) Fardh, (4) Khalas, (5) Khasab, (6) Khuneizy, (7) Handahl, (8) Hilali, and (9) Zabad. The positions of standard proteins such as bovine serum albumin (BSA, 67 kDa), ovalbumin (OVA, 43 kDa), chymotrypsinogen (25 kDa) and ribonuclease A (14 kDa) are represented by arrows.

Fardh, Khasab and Zabad. In some date varieties, such as Bunaringa, Khalas and Hilaly, the protein band around 30 000 was prominent. When stained with the more sensitive alkaline silver nitrate, several additional bands were detected (Fig. 1(b)). While Coomassie Brilliant Blue R-250 did not stain any protein band below the molecular weight range of 25 000, in most date varieties, a few protein bands at molecular weights lower than 25 000 were visualised upon staining with silver nitrate (Fig. 1(b)). In addition, bands also appeared in the molecular weight range of 45 000–67 000.

Extraction of dates with various agents

Albumins and globulins represent two major classes of proteins. Albumins are soluble in water while globulins are soluble in salt solutions (Smith *et al.*, 1983). To determine the amounts of albumins and globulins, date pulps from Bunaringa variety were sequentially extracted with water and PBS. Most date proteins were albumin in nature as these were soluble in water. When residues obtained after extraction with water were re-extracted with PBS, hardly any protein band was detected by SDS-PAGE.

Chaotropic agents, such as urea and guanidine–HCl, extracted significant amounts of date proteins (Bunaringa variety), the amounts being 0·96 mg/ml and 1·56 mg/ml, respectively. The SDS-PAGE protein profiles of these two extracts were similar to that obtained by extraction with water.

Isoelectric properties of date proteins

To study charge properties, date proteins from nine cultivars were subjected to isoelectric focusing on ultra-thin polyacrylamide gels in the pH range 4–6·5. There were similarities in the IEF pattern as approximately five to six bands in the pH range of 4·15–5·85 were detected in all these date proteins (Fig. 2).

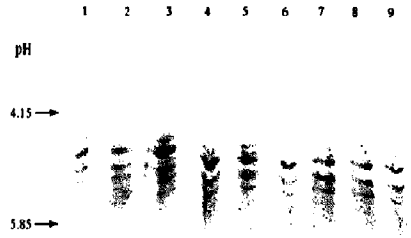

Fig. 2. Analysis of date proteins from various types by isoelectric focusing (IEF) on a ultra-thin layer (0·5 mm) polyacrylamide gel using a pH gradient of 4–6·5. Positions of date varieties: (1) Khalas, (2) Bunaringa, (3) Zabad, (4) Barneh, (5) Khalas, (6) Hilali, (7) Khuneizy, (8) Handahl, and (9) Fardh.

Accumulation of date proteins at various stages of growth

To determine the kinetics of accumulation of proteins, dates from one variety (Khalas) were harvested from the tree at different stages of growth (Fig. 3(a)). Very early fruit was deep green in colour and did not contain any detectable amount of protein (stage 1). All subsequent harvests were obtained at a regular interval of 2 weeks (stages 2–5). At the stage 2, the fruit was light green in colour and only two narrowly spaced faint protein bands in the molecular weight range of 15 000–17 000 were observed (Fig. 3(b)). At the stage 3, these two bands became very prominent and afterwards these bands became fainter although fruits were visibly becoming more mature, turning in colour to bright yellow (stage 4). No other protein band was visible at this stage. However, within the next 2 weeks, there was a rapid accumulation of proteins as dates became semi-ripe and a large number of proteins were detected (stage 5). Two bands around 24 000 and 30 000 were very prominent. In addition, several less prominent bands appeared in the molecular weight range 32 000–72 000. Bands at 14 000–17 000, previously prominent at the stage 3, became very faint.

Amino acid compositions

All the essential amino acids required for human nutrition (Ile, Leu, Lys, Met, Cys, Phe, Tyr, Thr and Val) were present in significant amounts in all the nine varieties of Omani dates studied (Table 2).

Protein profiles of dates obtained from different geographic regions

Apart from Oman, mature dates were also collected from countries such as Saudi Arabia, Iran and USA (California). While the amounts of PBS-soluble proteins in dates from Oman (1·1 mg/ml), Iran (1·2 mg/ml) and Saudi Arabia (1·3 mg/ml) were rather similar, the mature dates from one California variety contained much less protein (0·13 mg/ml). SDS-PAGE showed that, apart from minor variations, there were overall similarities in the protein profiles of dates cultivated in the countries of the Middle East such as Saudi Arabia, Oman and Iran (Fig. 4). Prominent protein bands around 72 000 and 30 000 were observed in all these dates. There were minor variations among protein bands. Thus, the band appearing at 25 000 in the Iranian date (lane 2) was weaker than those observed with Omani (lane 1) and Saudi Arabian (lane 3) dates. The date variety from California had a simpler protein profile (lane 4). Only one major band at 30 000 was detected even when the gel was stained with the sensitive alkaline silver nitrate.

DISCUSSION

Dates contain a mixture of water-soluble proteins, distributed over the wide molecular weight range of

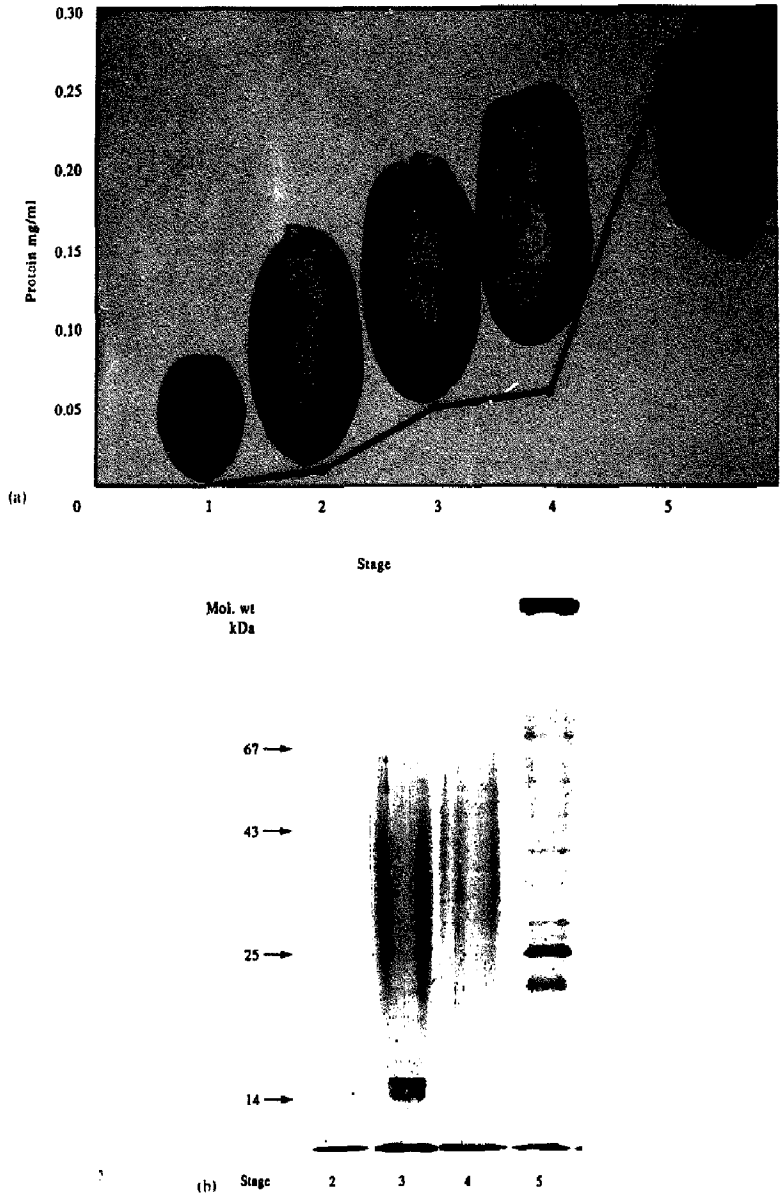

Fig. 3. (a) The kinetics of accumulation of date proteins at various stages of growth. At stage 1, the fruit was deep green. All subsequent harvests were carried out at a regular interval of 2 weeks (stages 2–5). Proteins were extracted from crushed date pulps with PBS and quantitated as described in the text. (b) Analysis of date proteins, obtained at various stages of growth, by SDS–PAGE on a 10% polyacrylamide gel. Bands were visualised by staining with alkaline silver nitrate (Kabir, 1986).

12 000–72 000. Since it is important to maintain a biological pH (i.e. pH 7·0), PBS was used to extract these proteins. Chaotropic agents such as guanidine·HCl and urea were also effective in isolating the proteins. However, these agents may denature proteins by decreasing protein stability (Creighton, 1993). Therefore, it is advisable not to use such reagents for isolating proteins if these are to be used later for biological investigations.

Dates have been consumed by the people in the Arabian Peninsula for thousands of years. The present

Table 2. Amino acid composition of date proteins from different varieties[a]

	Essential									Non-essential							
	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Val	Arg	His	Ala	Asp	Glu	Gly	Pro	Ser
Bunaringa	5·19	8·63	4·19	1·61	3·75	5·05	5·54	5·10	6·82	3·28	2·35	5·94	10·67	14·12	6·74	5·94	5·08
Handhal	4·19	8·70	3·40	1·81	3·57	5·05	5·54	5·36	8·16	3·30	1·95	5·86	11·03	13·73	6·77	6·39	5·19
Hilali	5·16	8·66	3·93	1·81	2·62	5·11	5·60	5·35	6·90	2·97	2·07	6·02	11·69	14·26	6·68	6·39	5·42
Khalas	5·03	8·68	3·76	2·09	3·43	5·19	5·69	5·10	6·64	4·70	2·37	5·72	11·11	13·05	6·33	5·79	5·34
Fardh	5·35	8·74	3·95	1·71	3·67	5·01	5·50	5·30	7·00	2·90	2·20	6·00	11·44	13·63	6·50	6·03	5·09
Khasab	5·33	8·80	3·68	2·00	1·98	5·27	5·78	5·02	7·17	4·36	2·44	5·77	11·36	13·25	6·48	6·34	4·95
Barneh	5·45	8·84	3·55	1·50	3·03	5·08	5·55	5·11	7·18	3·45	2·08	5·95	11·13	14·37	6·71	5·84	5·18
Khuneizy	5·67	9·32	3·90	1·87	3·11	5·46	5·99	5·23	6·28	3·30	2·20	6·67	10·74	12·97	5·89	6·20	5·19
Zabad	4·85	8·32	3·88	1·78	3·60	5·07	5·54	4·59	6·45	3·53	2·10	6·47	11·25	14·09	6·75	6·34	5·41

[a] Results are expressed as residues/100. Amino acids have been designated by the three-letter abbreviations.

investigation suggests that dates have always been a source of nutritionally important essential amino acids for the people of this region. Since the level of cysteine is low, little crosslinking has been detected in date proteins. This is consistent with the fact that the SDS-PAGE profile of date proteins remained unaltered whether the samples were reduced with suphydryl agents such as 2-ME or not.

When the kinetics of protein accumulation in the date were studied, it was observed that green dates obtained at an early stage contained very little protein. There was a rapid increase in the protein content as dates were progressively ripening and becoming soft. Green fruits, in contrast to mature ones, are firm, as they contain high amounts of fibres such as pectin, hemicellulose, cellulose and lignin (Hobson, 1968; Hinton & Pressey, 1974; Huber 1983; Simpson *et al.*, 1984). During maturation, activities of enzymes such as cellulase and polygalacturonase increase rapidly and these enzymes play significant roles in softening the fruit. Therefore, it is possible that we have observed large number of protein bands, representing various enzymes, as the process of ripening progressed rapidly. The SDS-PAGE profiles of date proteins at early stages of development (Fig. 3, stages 2 and 3) showed protein-type materials running as a general smear along the lanes in addition to the most well-defined bands. These might represent fragments of any proteinases or other components such as glycoproteins or protein–carbohydrate interaction products.

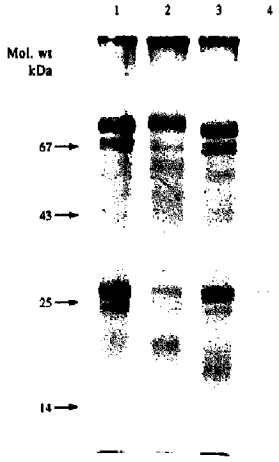

Fig. 4. Analysis of PBS-soluble proteins from dates, collected from different countries, by SDS-PAGE on a 10% polyacrylamide gel. Bands were visualised by staining with alkaline silver nitrate (Kabir, 1986). Positions: dates from Oman (lane 1), Iran (lane 2), Saudi Arabia (lane 3) and the USA (lane 4).

There were similarities in the protein profile of dates cultivated in Saudi Arabia, Oman and Iran. All these dates contained two distinct protein bands appearing at 72 000 and 30 000. In this respect we observed that the Californian variety was much lower in protein content and contained only one prominent band at 30 000. The exact cause of this difference is not understood. It is possible that the variation in the date palm cultivar may account for the observed difference in protein composition. In this respect, it has to be mentioned that dates from Oman and California contained approximately equimolar amounts of glucose and fructose suggesting that they were not different so far as carbohydrate compositions were concerned (unpublished observations).

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