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Unconventional protein sources: "date seeds"

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Egypt suffers from the same problems of other developing countries most important of which is the problem of food consumption. Protein in the Egyptian diet is mainly from plant origin. By consulting the food balance sheet for the year 1978. It was found that from the total protein intake 94.9 g/caput/day, 81.6 were derived from vegetable origin and only 13.3 gm from animal origin.

Attention has been directed towards the re-evaluation of the different natural sources of proteins in an attempt to improve the quality of the protein consumed, either by raising the production of conventional (animal and plant) protein sources, increasing the cultivated area, applying agricultural mechanisation or using unconventional protein sources from oil seeds, fish flour, and protein produced from algea, fungii and yeasts.

Date (Phoneix decatylifera) seeds were chosen for our study in order to investigate the possibility of using them as a new source of food protein.

Materials and methods

Selection and preparation of samples:

The local varieties of date seed, Balady (fresh), Amhat (merattab), Siwy (dry), Asouty (fresh), Zaghloul (fresh), Ebrimy (dry), Ramly and Agwa were obtained from local markets, while Samany variety (fresh), was obtained from Kaha (Food Processing Factory).

It should be mentioned that:

1. Fresh date has a crisp texture.
2. Merratab and Ramly are overripened fruits. They are juicy, darker in colour than the fresh ones and the skin comes off easily by hand. Agwa is the overripened fruit (Merratab) that was partially dried for preservation.
3. Ebrimy is the dried date that absorbs water on soaking. Seeds were washed, drained, dried in the air and ground for analysis.

Chemical analysis

Moisture, crude protein, ether extract, ash and phosphorus were determined according to the methods recommended by the Association of official Agricultural Chemists (1965). Soluble proteins were extracted at 20 degrees centigrade and at 100 degrees Centigrade using nitrogen free water, nitrogen was determined in both the extract and residue. Fiber was determined according to Pearson (1962). The method

used for iron determination was that of Elvehjem (1930). Calcium was determined according to Kramer (1921). Carbohydrate content was calculated by difference. The dried defatted samples were subjected to acid hydrolysis for 24 hours. The amino acids in the hydrolysates were separated by the two dimensional paper chromatography technique of Block et al. (1958). The solvents used were butanol, acetic acid and water (4 : 1 : 5) in the first run, and 0.3 % ammonia in 80 % phenol in the second run. Quantitative determination was made, whenever possible for some of the separated amino acids using the method of Giri et al. (1952). Tryptophan was not determined as it is destroyed by the acid hydrolysis.

Biological evaluation of the seed's protein:

The following basal diet of Campbell (1961) was used.

Corn starch	80 g
Cotton seed oil	10 g
Cellulose	5 g
Salt mixture	4 g
Vitamin mixture	1 g

The salt mixture used was that of Hubbel et al. (1937) and the vitamin mixture was that of Campbell (1961). The dried powdered defatted samples were added at the expense of starch to produce 5 % level of protein intake in case of date seeds and 10 % protein level incase of apricot kernels. Casein was used in the standard diet at both 5 % and 10 % levels.

Determination of the protein efficiency ratio (P.E.R.):

The method used was that of Campbell (1961). Weanling albino rats of a single strain of 20–23 days old were used. The rats were divided into groups of 6 animals for each diet. The groups were equalised as nearly as possible with respect to sex and weight. Diets and water were supplied ad libitum. The experimenet was extended for four weeks at the end of which calculations of the protein efficiency ratio (P.E.R.) were made for each rat. P.E.R.'s for experimental diets were recalculated as percentage of that for casein.

Determination of the net protein ratio (N.P.R.):

A control group of rats of nearly equal weight and age as in P.E.R. experiment was fed a protein free diet for 10 days to determine the loss in weight corresponding to the maintenance requirements of the rats. An approximate estimate of N.P.R. was done for the four weeks period from the loss in weight of group means over 10 days period.

Blood analysis:

At the end of the experimental period rats were killed by chloroform and blood samples were taken by cardiopuncture. The total serum protein was determined by Kjeldahl method according to the A.O.A.C. (1965). Serum samples of 0.2 ml were subjected to electrophoretic separation of proteins. The apparatus used was that of Elphor and the separation was carried out at pH 8.9 for 18 hours using Durrum method (1950). Dye used was bromophenol blue. Elution of the stained bands was carried out using 0.5 % sodium carbonate solution and the albumin/globulin ratio was determined colorimetrically. The free nonessential/essential amino acids ratio was determined using the method of Abdou and Awadalla (1973).

Results and discussion

Table 1 shows the percentage of flesh and seed in the different varieties of date. The percentage of flesh ranged from 83.30 to 93.50. The remainings contained peel and seed. The dates were peeled only in the overripened samples (Merratab) where the skin is loose and comes off easily by hand. It is clear from table 1 that the amount of date seed produced in 1971 was over 30,000 tons.

Table 1. The percentage composition of flesh, seed and peel of different varieties of date.

Variety of date	production in tons/year*)	flesh in g/100 g	seed in g/100 g	peel in g/100 g
Balady (fresh)	210,551	87.23	12.77	–
Amhat (Merattab)	16,328	84.10	12.30	3.60
Siwy (fresh)	19,256	88.40	11.60	–
Siwy (Merattab)		84.41	13.59	2.00
Zaghloul (fresh)	5,229	93.50	6.50	–
Samany (fresh)	11,467	93.08	6.92	–
Ebrimy (dry)	7,058	87.57	12.43	–
Asyouty (fresh)	4,383	88.61	11.39	–
Ramly	77,596	83.30	12.50	4.20
Agwa	7,301	85.57	14.43	–

*) Obtained from Ministry of Agriculture, Egypt (1970–1971).

Table 2 shows the percentage of moisture in the different samples, and the percentage of crude protein, ether extract, fiber, ash and carbohydrate in the same samples on dry weight basis.

Table 2. The percentage of macronutrients in the different samples of date seed, on dry weight basis.

Sample	crude protein	ether extract	fiber	ash	carbohydrate	moisture
Balady	7.35	9.07	16.38	1.04	66.16	14.47
Amhat	7.38	8.99	17.44	1.14	65.05	15.22
Siwy (fresh)	8.80	7.48	15.92	0.98	66.82	19.69
Siwy (Merratab)	8.24	8.33	16.31	1.04	66.08	18.27
Zaghloul	10.36	7.20	18.87	1.11	62.46	18.81
Samany	9.10	7.67	11.29	1.09	70.85	19.82
Ebrimy	7.13	6.32	15.42	1.14	69.96	10.31
Asyouty	7.17	8.23	16.04	0.99	67.57	13.23
Ramly	8.53	8.14	12.60	1.20	69.53	13.48
Agwa	8.58	9.28	10.97	0.85	70.32	15.13

It is clear from table 2 that the moisture content of date seed varried from 10.31 % for Ebrimy seed to 19.82 % in Samany seed. Crude protein content in date seed ranged from 7.13 % in Ebrimy used to 10.36 % in Zaghloul seed on dry weight basis.

It is clear from table 2 that the moisture content of date seed varied from 10.31 % for Ebrimy seed to 19.82 % in Samany seed. Crude protein content in date seed ranged from 7.13 % in Ebrimy used to 10.36 g% in Zaghloul seed on dry weight basis, values that are near to the protein content of some cereals. Eckay (1954) reported the value 5.3 g % for protein in date seed. The values obtained for ether extract, fiber and carbohydrates are in the same range as those reported by him. Using different varieties of date seed Bressani (1963) found that the protein content was 19.8–26.2 % and ash 4.4–5.8 %. The fiber and ether extract data were similar to ours.

Data regarding the solubility of protein of Samany date seed are shown in table 3. Samany seed variety was chosen as it is found in large quantities as by-products in food processing factories and it could be used in animal rations.

Table 3. Solubility of protein of Samany date seed (g soluble protein/100 g dry matter).

cold-water-extracted protein	0.66
boiling-water-extracted protein	0.57
residual protein content	7.63
total protein (calculated)	8.86
total protein (estimated)	9.10

The amount of protein extracted by boiling water was very small. Most of the protein was found in the residue.

Table 4 shows that the phosphorus content of date seed was from 26.80 to 46.80 mg%, calcium was 30.46 to 39.12 mg% and 11.77 to 15.89 mg% iron. Date seed is a reasonable good source of iron.

Samany date seed were found to contain the amino acids: cystine, lysine, methionine, valine, phenyl alanine, isoleucine, leucine, histidine, arginine, alanine, aspartic acid, serine, glutamic acid, glycine, and threonine. Tryptophan was determined as it is destroyed by the acid hydrolysis. Tyrosine and proline did not appear in the chromatograms.

Table 4. Phosphorus, calcium, and iron content of different varieties of date seed (mg/100 mg dry matter).

Variety	phosphorus	calcium	iron
Balady	26.80	34.68	13.63
Amhat	39.90	38.38	13.92
Siwy (fresh)	28.30	37.92	15.40
Siwy (Merattab)	45.50	34.77	13.96
Zaghloul	41.30	33.58	11.53
Samany	36.50	36.19	15.87
Ebrimy	46.80	35.37	11.77
Asyouty	39.00	39.12	14.65
Ramly	40.20	32.72	15.55
Agwa	27.70	30.46	15.89

Quantitative determination showed that lysine was the lowest amino acid found in date seed. It is clear that date seed is a poor protein source in general, which agrees with the findings of Bressani (1963). Results are shown in table 5.

Table 5. The concentration of some amino acids in Samany date seed.

Amino acid	mg/100 g dry	mg/100 g N
Aspartic acid	180	124
Glutamic acid	200	138
Serine	72	50
Alanine	60	41
Lysine	37	26
Arginine + histidine	80	55
Leucine + isoleucine	128	88

Table 6 shows the P.E.R. and N.P.R. values of date seed protein at 5 % protein level and casein at 5 % and 10 % protein level. The date seed protein level was used because of the low protein content of date seed, which is within the range recommended by Mitchell et al. (1926), for evaluating protein for maintenance, casein was used at the same level as a standard (control) diet. As shown in table 6. the N.P.R. for casein at 5 % protein level is higher than that at 10 % protein level. This is in agreement with the results obtained by several other investigators including Mitchell (1924). At this point, reference should be made to the view of Osborne et al. (1919) who stated "Economy of food can be effected only by supplying the young animal with as much food as it will eat, economy of protein only by reducing the nutritive ratio below that at which normal growth can be maintained". This economy of dietary proteins should not be based on the so-called protein efficiency ratio as it does not credit the dietary protein with all of its functions in the body i.e. the replacement of endogenous losses and the maintenance requirements.

The rats fed on date seed diet lost weight, which means that its protein biological value is not high enough to fulfill all the requirements even for maintenance. In this case food intake was markedly reduced. The N.P.R. for date seed was found to be 56.43 % of that of casein.

Table 6. The P.E.R. and N.P.R. values for casein and Samany date seed.

Protein source and protein level	casein level 10 %	casein level 5 %	date seed level 5 %
Average food intake g/rat	250.67	229.33	129.67
Average change in body weight g/rat	70.50	26.33	-10.67
P.E.R±S.D.	2.81±0.153	2.29±0.142	-
P.E.R as % of casein value	100	-	-
N.P.R.±S.D.	3.95±163	4.82±0.409	2.72±88
N.P.R. as % of casein value	100	100	56.43

It is clear from the chemical analysis of date seed that it is relatively low in protein and high in fiber, biological studies gave evidence for the low biological value of its protein. There is no sign of toxic effects of date seed on the rats.

Table 7 shows the average total proteins, albumin/globulin ratio and the free nonessential/essential amino acids in serum of rats fed on date seed diet. The results are in agreement with those obtained for the P.E.R. values which are based on the effect of on growth.

Table 7. The average total proteins, albumin/globulin ratio and the free non-essential/essential amino acids in serum of rats fed on date seed.

Protein	total protein g% ± S.D.	albumin/ globulin ± S.D.	non-essential/ essential amino acids ± S.D.
casein 10 %	7.25 ± 0.155	1.58 ± 0.032	2.2 ± 0.062
casein 5 %	6.91 ± 0.214	1.28 ± 0.080	2.7 ± 0.240
date seed	3.26 ± 0.351	0.79 ± 0.027	4.0 ± 0.041
protein-free diet	3.18 ± 0.193	0.77 ± 0.051	4.4 ± 0.119

Results revealed that the effect of experimental diets on growth of rats, measured as the protein efficiency ratio was parallel to the effect obtained on the blood criteria and different from that obtained regarding the effect on both weight maintenance, and growth as measured by the net protein ratio. It could be concluded that date seed cannot be used in feeding purposes because of its high content of fiber, low content of protein and low biological value.

Summary

Different varieties of Egyptian date seed were evaluated biochemically. The protein content of which was in the range of 7.13–10.36 %, while the fat content was from 6.32–9.28 %. Phosphorus, calcium and iron were determined in all experimental samples.

One variety of date seed (Samany) were used for extensive determination of the amino acids constituents by acid hydrolysis, quantitative determination of some amino acids, and evaluation of their biological value on attempt to use the seeds as new protein sources.

Weanling albino rats fed on a diet of protein level 5 % lost weight, but no toxic signs were observed. Analysis of blood serum of rats, for total proteins, albumin globulin ratio and free nonessential/essential amino acid protein, showed the date seed protein to be of low biological value.

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