CHEMICAL STUDIES ON PROTEINS AND LIPIDS OF DATE SEEDS

By

M.K.S. Shabana, A.M. Helmi, E.R. Guindi and N.Y. Attia

Fac. of Agric. Moshtohor, Biochem. Dept., Zagazig Univ.

ABSTRACT

The amounts of crude protein of date seeds were 7.56, 6.12 and 6.69% for Zagloul, Samani and Haiani respectively. Four different protein fractions were extracted from the date seeds according to their solubility.

Three clear, similar electrophoretic bands were identified in the three varieties, mentioned before, while another fourth minor band was only identified in the Haiani variety.

Free amino acids were determined qualitatively and total amino acids were quantitatively determined. Glutamic acid was the most abundant one in the three varieties since it comprises 11.8, 9.7 and 11.3 mg/g protein on dry weight basis, for Zagloul, Samani and Haiani, respectively.

The crude fat contents of the seeds were 7.58, 11.30 and 6.71 in Zagloul, Samani and Haiani, respectively.

GLC analysis of the oils showed the presence of five even number saturated fatty acids between $C_{10:0}$ - $C_{18:0}$. Two unsaturated fatty acids were identified as oleic and linoleic acids. Lauric acid was the most abundant saturated fatty acid with 23.65, 22.07 and 31.04%, while oleic predominated as unsaturated one with 42.07, 37.58, and 38.96 for Zagloul, Samani and Haiani, respectively. Consequently, Egyptian date seed oils could be classified as Semidry oil.

The unsaponifiable matter showed the presence of 15 fractions of sterols and hydrocarbons in variable quantities. B-Sitosterol was the most abundant sterol with 24.57, 34.36 and 32.75% for Zagloul, Samani and Haiani, respectively.

INTRODUCTION

The chemical components of the by-product date seeds as proteins and lipids and their potential use in animal feeding is of a major interest. Ali (1968), studied the protein content of palm date seeds of Egyptian varieties Amhat and Ramli. He found that the total nitrogen content was nearly 1.0%. He identified 13 amino acids in the protein hydrolyzate of the two varieties.

Hussein and El-Zeid (1975), studied the protein composition of Khalas date seeds grown in Saudi Arabia. Their reported data were so close to those obtained by Salem and Hegazi (1971) on Egyptian dry date seeds.

The free amino acids of date seeds were extracted according to El-Dash and Johnson (1970), and it was qualitatively determined using descending partition paper chromatography technique as described by Block et al. (1958). Quantitative determination of total amino acids extracts was carried out according to Moore et al. (1958) using Beckman amino acid analyzer Model 121.

The amount of crude oils in the three date seed varieties and the routine physical and chemical properties were determined according to the standard methods of A.O.A.C. (1975).

Identification and determination of the fatty acids content of each oil as methyl esters were carried out using G.L.C. technique according to A.O.A.C. (1975). Pye Unicam apparatus was used with Duel flame ionization detector, G.C.V. Column with polyethylene adipate 10% was used; detector temp. 220°C., injection temp. 220°C, Column temp. 190°C, nitrogen flow rate 45 ml./min. and chart speed 2 min./cm.

A set of standard methyl esters of saturated and unsaturated fatty acids was used as authentic standard reference for comparison.

The unsaponifiable matter of each oil was fractionated and identified according to the standard method of A.O.A.C. (1975) using G.L.C. analysis under the following conditions:

Column used OV 17, injection temp. 300°C, detector temp. 300°C, column temp. 270°C, air flow rate 330 ml/min and chart speed 2 min/cm.

Various fractions were identified with the aid of authentic samples consisted of hydrocarbons (C_{20}, C_{22}, C_{24}, C_{30}, and C_{32}) and sterols, (Cholesterol, Campsterol, Stigmasterol and B-Sitosterol), under the same conditions.

The relative concentration of each fraction was calculated according to Mc Nair and Bonelli (1969).

RESULTS AND DISCUSSION

Crude protein:

The data presented in Table (1) indicate that defatted date seeds contained from 6.12 to 7.56% crude protein. Zagloul seed variety contained the highest amount, i.e. 7.56%, while Samani and Haiani contained 6.12 and 6.69%, respectively. However, these results are near to that reported before by Wally et al. (1979).

Date seed protein fractions:

Four different protein fractions were extracted from date seeds of the three varieties, albumins, globulins, prolamins and glutelins. In general, Zagloul variety showed the highest percentage of the total extracted soluble protein fractions.

Mansour (1974), reported that protein content of Zagloul seed variety was higher by 35% than of Samani variety at ripening stage. Fourteen amino acids were qualitatively identified in the protein hydrolyzate using paper chromatography technique. The seed oil content amounted to 7.2 and 7.3% for both Zagloul and Samani varieties respectively. Wally (1979); reported that protein and oil percentages of date seeds based on fresh weight were 5.22% and 8.5% respectively.

Mehran and Filsof (1975), studied the physico-chemical properties and the fatty acid content of date seeds obtained from Iranian varieties. The oil content of the seeds amounted to 8.5%, 5.0% and 6.9% for Mussafti, Kabkaab and Sayir, respectively. Six saturated fatty acids from $C_{8:0}$ to $C_{18:0}$ were identified in the three varieties with different proportions, while oleic, linoleic and linolenic averaged to 45.0, 6.9, and 0.7% for Mussafti, Kabkaab and Sayir respectively. Arachidic acid $C_{20:0}$ was found in a relative small amount, about 0.7%.

The present work aims to study chemical constituents of date seeds, i.e., proteins and lipids content which might be in value in animal nutrition as additive material.

MATERIAL AND METHODS

Sampling:

Date seed samples of Zagloul, Samani and Halani varieties (Phoenix Dactylifera, Family Palmaceae) were obtained from Moshtohor, Kaluibia, Egypt, cleaned, air dried, finely grounded and kept for further analysis.

Analytical methods:

Total nitrogen content was determined using Kjeldahl method, according to A.O.A.C. (1975).

Protein fractions, albumins, globulins, prolamins and glutelins in date seeds were extracted in different solvents as recommended by Kent-Jones and Amos (1957). The extractable protein fractions were colorimetrically determined as described by Lowry et al. (1951), using Carl Zeiss Jena Colorimeter MK Type 6/6.

Electrophoretic analysis was performed as described by Davis (1964), with slight modification. The date seed soluble proteins of the three varieties were extracted by stirring one g. of the defatted finely grounded material with 10 ml. NaCl soln. 0.1 M followed by centrifugation for 20 min. at 3500 r.p.m. to get the supernatent solution.

A mixture of 0.1 ml of the protein extract (supernatent soln.), plus one drop of bromophenol blue 0.001% was pipetted on the upper surface of gel-electrophoresis. The running process was carried out using glycine and tris buffer (pH 8.3). The gels were destained by washing with acetic acid 7.5% several times until the gel background was clear. The position of the blue protein zones and their number were recorded.

The noticed variation in the amount of these fractions in the different varieties might be attributed to that Zagloul is known as a soft variety, while Samani and Haiani are semi-dry varieties, Zeidan and Maximous (1960).

Albumins compared with other fractions existed in relatively higher amounts in both varieties, Zagloul and Samani, while glutelins existed in relatively higher amounts in Haiani variety.

Electrophoretic identification date seed proteins:

Three electrophoretic clear bands were identified in the date seed extracts of the three varieties. Another minor fourth band was identified in Haiani variety which might be referred to a qualitative difference (Fig. 1).

Amino acids of date seeds:

Paper chromatography analysis of free amino acids showed the presence of 17 amino acids (Table 2). The three varieties contained the same amino acids. Only it has to be mentioned in this aspect that the amino acid tryptophan appeared on the paper chromatographic sheets, while it disappeared during the quantitative determination with amino acid analyzer. Tryptophan is completely destroyed with prolonged boiling in HCl solution during protein hydrolysis, Cantarow and Schepartz (1962).

Saler and Hegazi (1971), and Hussein and El-Zeid (1975), identified only 13 amino acids in both date seeds of Egyptian Balady variety and (Khalas) date seeds of Saudi Arabia.

On the other hand, the quantitative determination of the amino acids content of seeds of the three varieties showed slight differences in their total quantities (Table 2). Glutamic acid is the most abundant amino acid which comprises 11.8, 9.7 and 11.3 mg/g of the defatted date seeds of Zagloul, Samani and Haiani, respectively. The essential amino acids, threonine, lysine, valine, leucine and phenylalanine were detected in the three varieties in moderate proportions.

However, such results are in harmony with those of Boulter and Barber (1963). They reported the importance of such essential amino acids in building the ribosomes and for photosynthesis process during seed germination. In general, the obtained results regarding amino acids content are in agreement with those obtained by Al-Rawi et al. (1967), Salem and Higazi (1971) and Hussein and El-Zeid (1975).

It could be deduced from the previous data that date seed powder could be successfully used as an additive matter specially for animal nutrition. Such deduction might be confirmed after the application of some biological and nutritional experiments.

Lipid content:

Crude oil:

The crude oil contents of the date seeds of Zagloul, Samani and Haiani were 7.58, 11.3 and 6.71%, respectively. Such data ar-

Table (1): Total nitrogen and protein fractions content of defatted date seeds (gm/ 100 gm dry weight) .

Date seed variety	Total nitrogen %	Crude protein %	Extractable protein fractions				Total extractable proteins %
			Albumins %	Globulins %	Prolamins %	Glutelins %	
Zagloul	1.21	7.56	2.60	0.35	1.20	1.48	5.63
Samani	0.98	6.12	1.16	0.14	0.98	0.86	3.14
Haiani	1.07	6.69	0.92	0.14	1.06	1.96	4.08

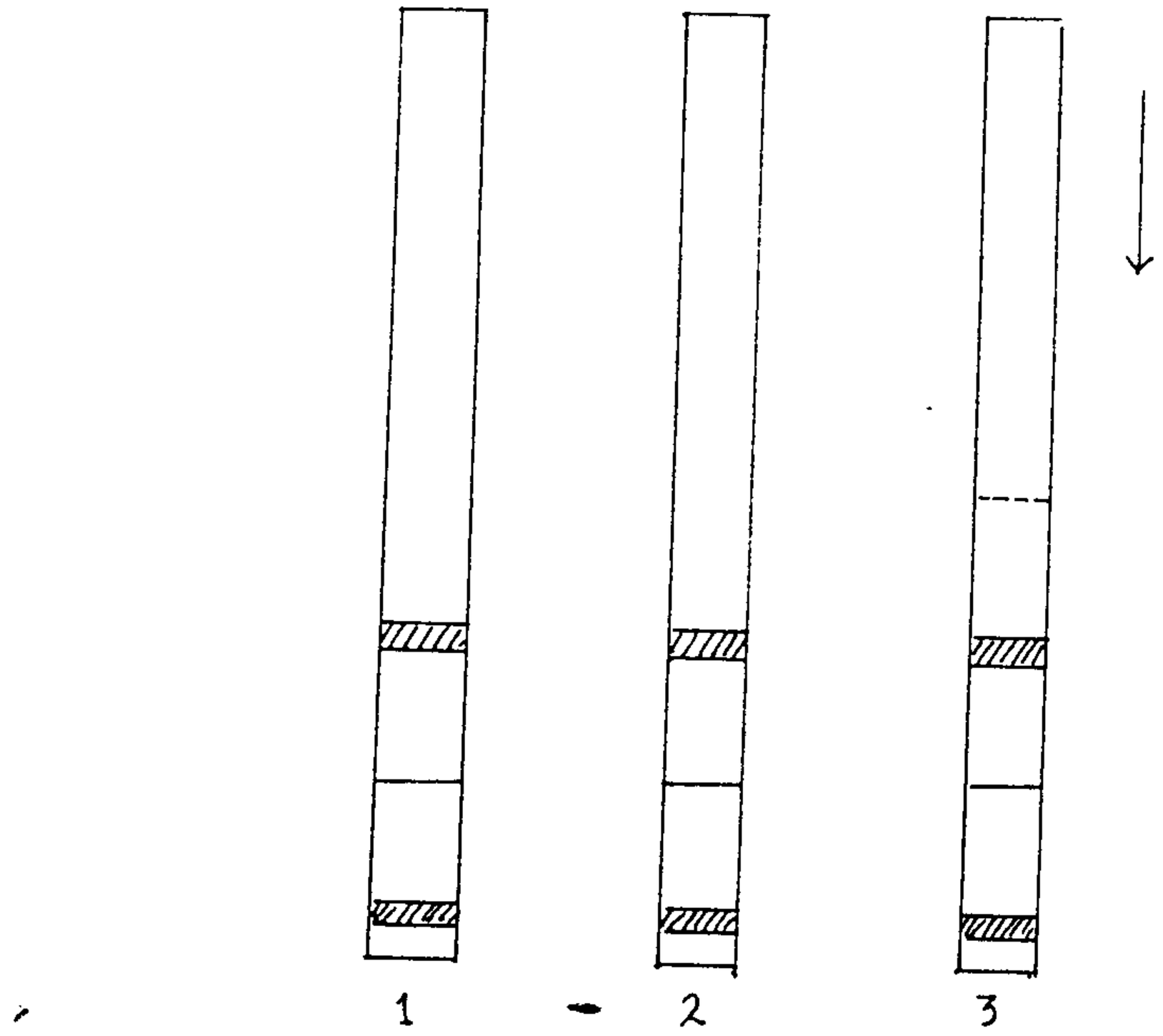

Fig. (1): Polyacrylamide gel patterns of defatted date seed proteins soluble in 0.1 M NaCl

1- Zagloul defatted proteins
2- Samani defatted proteins
3- Haiani defatted proteins

Table (2) : Amino acid content of date seed (mg/g dry weight of date seed powder).

Amino Acids	Date seed varieties		
	Zaghloul	Samani	Haiani
Lysine	3.0	2.5	3.0
Histidine	1.1	1.1	1.1
Arginine	8.0	5.9	7.1
Aspartic acid	6.6	5.1	5.4
Threonine	2.3	1.8	1.9
Serine	3.0	2.3	2.4
Glutamic acid	11.8	9.7	11.3
Proline	2.2	1.8	2.0
Glycine	2.7	2.5	2.7
Alanine	2.6	2.4	2.5
Valine	2.8	2.7	2.6
Methionine	0.8	0.7	0.8
Iso-leucine	1.7	1.7	1.7
Leucine	3.6	3.3	3.3
Tyrosine	1.1	1.0	1.0
Phenylalanine	2.7	2.0	2.1
Total	56	46.3	50.9

within limits with that reviewed before by Hussein and El-Zeid (1975) and Salem and Hegazi (1971). The extracted oils were subjected to different physical and chemical determinations and the results are listed in Table (3).

Fatty acid composition of date seed oils:

Results of G.L.C. analysis illustrated in Table (4) showed the presence of five saturated fatty acids between $C_{10:0}$-$C_{18:0}$ in oils extracted from the three varieties of date seeds, i.e., capric, lauric, myristic palmitic and stearic acids. Two unsaturated fatty acids were identified as oleic and linoleic acids. The amounts of saturated and unsaturated fatty acids differ to a little extent according to date seed varieties. The saturated fatty acids represented 51.41, 54.24 and 53.34%, while the unsaturated ones were 48.6, 45.71 and 46.65%, for Zagloul, Samani and Haiani varieties, respectively.

It is clearly shown from Table (4) that lauric acid, $C_{12:0}$ was the highest one of saturated fatty acids in the extracted oils, 23.55, 22.07, and 31.04%, while oleic acid $C_{18:1}$ was the predominant fatty acid in the date oils, 42.09, 37.58 and 38.96% of Zagloul, Samani and Haiani respectively. The ratio of saturated to unsaturated fatty acids was 1.06:1, 1.19 :1 and 1.14:1 for the three varieties as mentioned before. From such data, it can be deduced that date seed oil from these Egyptian varieties could be classified as semi-dry oil.

Unsaponifiable matter composition:

Table (5) indicates the retention time (RT) and relative retention time (RRT) of the standard authentic hydrocarbons and sterols. The unsaponifiable composition presented in Table (6), the obtained results showed the presence of 15 peaks corresponding to 15 fractions of saturated ,unsaturated hydrocarbons and sterols which varied in their retention time (RT). The relative retention time (RRT) of the sterol fractions obtained on the chromatogram was calculated in relative to B-sitosterol (6.7) which was given a value of 1.00. The different fractions were identified by comparing their RRT with those of the authentic samples under the same conditions.

Only two hydrocarbon fractions were identified as C_{24} and C_{32}, while the others were unknown.

The hydrocarbons content was 50.67, 33.95 and 38.76% for the three varieties Zagloul, Samani and Haiani, respectively.

Four sterols were obtained on the chromatogram, two of them were identified as campsterol and B-sitosterol with (RRT) 0.805 and 1.00, respectively. The other two unknown sterols showed (RRT) of 1.20 and 1.34. Samani and Haiani varieties had higher sterol contents, i.e., 66.07% and 61.23% respectively, than that of Zagloul, 48.93%. B-sitosterol occurred in the three varieties and was the most abundant sterol, 24.57, 34.36 and 32.75 for Zagloul, Samani and Haiani, respectively.

Table (3) : The physical and chemical properties of date seed oils (Zagloul, Samani and Haiani varieties).

Date seed variety	Crude Oil %	Refractive index at 25° n_D	Specific gravity d^{40}_{25}	Acid value (A.V.)	Saponification value (S.V.)	Iodine value (I.V.)	Un-sap. matter %
Zagloul	7.58	1.4655	0.8933	0.78	309.19	50.19	1.23
Samani	11.30	1.4665	0.8940	2.14	306.77	53.73	0.68
Haiani	6.71	1.4665	0.8935	2.18	325.09	52.77	1.43

Table (4) : Fatty acid contents of Zagloul, Samani and Haiani date seed oils.

Date seed variety	Fatty acids calculated by hight of peaks								
	Saturated fatty acids %						Unsaturated fatty acids %		
	Capric $C_{10:0}$	Lauric $C_{12:0}$	Myristic $C_{14:0}$	Palmitic $C_{16:0}$	Stearic $C_{18:0}$	Total	Oleic $C_{18:1}$	Linoleic $C_{18:2}$	Total
Zagloul	4.11	23.65	12.19	9.82	1.64	51.41	42.09	6.51	48.60
Samani	0.77	22.07	19.18	10.59	1.69	54.24	37.56	8.13	45.71
Haiani	1.45	31.04	10.85	8.43	1.57	53.34	38.96	7.69	46.65

Table (5 : Retention time (R) and relative retention time (RRT) for the authentic hydrocarbons and sterols.

Component	RT	RRT
n-eicosane (C_{20}).	0.1	0.014
n-docosane (C_{22})	0.2	0.029
n-tetracosane (C_{24})	0.4	0.059
n-triacontane (C_{30})	1.65	0.260
n-dotriacontane (C_{32})	2.7	0.402
Cholesterol	4.1	0.610
Campsterol	5.4	0.805
Stigmasterol	5.9	0.880
B-sitosterol	6.7	1.000

Relative retention time for B-sitosterol was given a value of 1.0.

Table (6): Unsaponifiable matter component of date seed oil(The relative percentages of the unsaponifiable matter fractions expressed in terms of total peak area).

Peak number	R.T	RRT	Identification	Fraction % Zagloul	Samani	Haiani
1	0.3	0.044	Unknown	5.12	-	-
2	0.4	0.059	C_{24}	4.49	3.22	8.76
3	0.6	0.090	Unknown	5.04	3.80	4.26
4	0.7	0.104	Unknown	4.57	3.96	8.22
5	0.95	0.14	Unknown	3.70	3.47	5.18
6	1.25	0.186	Unknown	6.38	4.96	3.66
7	1.50	0.22	Unknown	6.14	2.64	3.96
8	2.1	0.313	Unknown	2.20	-	-
9	2.3	0.34	Unknown	2.84	4.96	2.13
10	2.7	0.402	C_{32}	1.23	-	-
11	3.4	0.507	Unknown	8.82	6.94	2.59
12	5.4	0.805	Campsterol	2.55	7.43	3.81
13	6.7	1.00	B-sitosterol	24.57	34.36	32.75
14	8.1	1.20	Unknown	12.60	13.87	16.45
15	9.0	1.34	Unknown	9.21	10.41	8.22
	Total hydrocarbons		=	50.67	33.95	38.76
	Total sterols		=	48.93	66.07	61.23
	Total components		=	99.60	100.02	99.99

The ratio of total hydrocarbons to total sterols amounted (1.035:1), (0.513:1) and (0.633:1) in Zagloul, Samani and Halal respectively. However, such data showed clear quantitative differences in the unsaponifiable matter composition of date seed varieties (sterol and hydrocarbon contents) which might be used to identify the varieties of dates.

REFERENCES

Ali, F.M. (1968).
Studies on the chemical constitution of some Anacoriaceae and palm seeds.
National Research Center of Egypt U.A.R.
c.f. J. Science of Food and Agric., 22, P. 654.

A.O.A.C. (1975).
Official methods of analysis of Association of Official Agricultural Chemists. 14th Ed.
Published by Association of Official Agricultural Chemists, Washington, U.S.A.

Block, R.J., Emmett, L.D. and Gunter, Z. (1958).
A manual of paper chromatography and paper electrophoresis.
Academic Press Inc., New York.

Boulter, D. and Barber, J.I. (1963).
Amino acid metabolism in developing seed of Vicia faba.
J. New Phytol. 67, 935.

Cantarow, A. and Schepartz (1962).
(Biochemistry-third ed).
W.B. Saunders Comp., London.

Davis, B.J. (1964).
Disc electrophoresis. II- Method and application to human serum proteins.
J. Am. N.Y. Acad. Sci., 121, 404-427.

El-Dash, A. and Johnson, J.A. (1970).
Influence of yeast fermentation and baking on the content of free amino acids and primary amino groups and their effect on bread aroma, stimula.
Cereal Chem. 47, 3.

Hussein, F. and El-Zeid, A.A. (1975).
Chemical composition of Khalas dates grown in Saudi Arabia.
Egypt. J. Hort. Vol. 2, 209-214.

Kent-Jones, D.W. and Amos, A.J. (1957).
Modern Cereal Chemistry. The Northern Published Co. LTD.
Liverpool Eng. 3rd ed.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951).
Protein measurement with Fohn-phenol reagent.
J. Biol. Chem. 193, 652.

Mansour, S.M. (1974).
Varietal location effect upon the biochemical constituents of Palm fruit and seeds.
M.Sc. Thesis, Faculty of Agric., Ain Shams Univ.

McNair, H.M. and Bonelli, E.J. (1969).
Basic gaschromatography. 5th ed. 2700 Mitchell Drive Wolnut Greek.
California 94598.

Mehran, M. and Filsof, M. (1975).
Characteristic of date pit oil.
J. A.O.C.S. Bol. 52, 48.

Moore, S., Spackman, D.H. and Stein, W. (1958).
Chromatography of amino acids on sulphonated polystyrene resins.
J. Anal. Chem. 30, 1185-1190.

Salem, S.A. and Hegazi, S.M. (1971).
Chemical composition of Egyptian dry dates.
J. Sci. Food and Agric., 22, 754.

Wally, Y.A., Hussein, F. and El-Kahtani, M.S. (1979).

Zeidan, Z.I. and Maximous, S.I. (1960).
Fruit production, in Arabic.

دراسات كيماوية على بروتينات ودهون نوى البلح

مصطفى شبانـــــة　　عبد السلام حلمـــى
عزت رزق جنـــــدى　　نادية يحيى عطيـــة
كليه الزراعة بمشتهر ـــ قسم الكيمياء ـــ جامعة الزقازيـق

يهدف هذا البحث الى دارسة التركيب الكيماوى لنوى البلح باستعمال ثلاثة اصناف من البلح الزغلول والسمانى والحيانى وتشير نتائج التقديرات الكيماوية التى اجريت فى هـــذه الدراسة الـــى :ـ

كانت نسبة البروتين الخام ٧٫٥٦ ، ٦٫١٢ ، ٦٫٦٩ ٪ للاصناف زغلول ، سمانى ، حيانـــى على الترتيب ، وقد استخلصت انواع البروتينات المختلفة تبعا لذوبانها .

ويعمل التفريد الكهربى للبروتينات الذائبة فى محلول كلوريد الصوديوم ظهرت ثلاثـــة مناطق بالاضافة الى منطقة أخرى صغيرة فى الصنف الحيانى ، وقد أظهرت هذه الطريقة أن هناك اختلافات طفيفة بين الاصناف .

بتقدير الاحماض الامينية فى نوى البلح وصفيا أظهرت نتائج التحليل الورقى الكروماتوجرافـى وجود ١٧ حمض أمينى ، كما أظهرت التقدير الكمى لنتائج التحليل المائى للنوى المطحون والخالى من المواد الدهنية أن حمض الجلاتاميك هو السائد فى الثلاثة اصناف وان نسبته حوالى ١٩٫٨ ، ٩٫٧ ، ١١٫٣٪ فى الاصناف زغلول ، سمانى ، وحيانى على الترتيب .

كانت نسبة الدهن الخام ٧٫٥٨ ، ١١٫٣٠ ، ٦٫٧١ للاصناف الزغلول والسمانى والحيانـى على الترتيب .

أظهرت نتائج التحليل الكروماتوجرافى الغازى وجود خمسة أحماض دهنية مشبعة هـــى كابريك ، لوريك ميروستيك ، بالميتيك ، استيارك وكذا وجود حامضين غير مشبعين وهمـــا اوليـك ، لينوليك . كان حمض اللوريك هو الحمض الدهنى المشبع الموجود بأعلى نسبة (٢٣٫٦٥ ، ٢٢٫٠٧ ، ٣١٫٠٤) فى اصناف الزغلول والسمانى والحيانى .

أما حمض الاوليك الغير مشبع (الحمض السائـــد) كانت نسبته ٤٢٫٠٩ ، ٣٧٫٥٨ ، ٣٨٫٩٦٪ فى الاصناف الثلاثـة • ويمكن اعتبار زيت البلح من الزيـــوت نصف الجافـــة •

دلت نتائج التحليل الكروماتوجرافى الغازى على وجـــود ١٥ مركـب مـــــن الهيدروكربونات والسيترولات • وكان مركب البيتاسيتوستيرول هو المركب الســـائـــد فى الاصناف الثـــلاثة المذكـورة •