THE AMINO ACIDS OF IRAQI DATES

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This thesis, I would like to dedicate to my mother and my husband for their moral support in my effort to advance my education.

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INTRODUCTION

Dates have been cultivated since antiquity. The earliest written record pertaining to dates goes back to 3500 BC. The oldest date growing area was ancient Mesopotamia, which is present day Iraq (19). Today dates are one of the principal foods in some parts of the Middle East.

Iraqi dates have been reported to contain 1.9 - 3.0% proteins, 0.0 - 2.5% fats, 67.3 - 70.6% carbohydrates, 1.3% minerals, 13.8 - 26.1% moisture, and small quantities of vitamins B_1, and B_2, ascorbic acid, and nicotinic acid (8).

The free amino acids content of dates was first determined by Grobbelaar *et al.* in 1955. They identified 17 free amino acids.

Rinderknecht in 1959 using Grobbelaar's technique in preparing the date extracts found that the free amino acids content of dates varies with the stage of maturity of the fruit (green, yellow, red, and brown), rising to a maximum in the red stage and decreasing during the brown stage. He identified 12 amino acids. Rinderknecht did not find threonine, lysine, valine, β-alanine, and homoserine which had been reported by Grobbelaar *et al.* Also Rinderknecht found arginine in high concentration whereas Grobbelaar found only trace amounts of this amino acid.

Only one sample of dates has been analyzed for total amino acids and this analysis includes only ten amino acids (20).

The scarcity and discrepancy of the available data on the amino acid composition of dates and the fact that dates comprise a considerable part of the human diet in some areas of the world led to the present study. Iraqi dates were chosen for this study because Iraq is a very

important date producing country. It produces about 400,000 tons of dates each year, representing 50% of the total world production. Of this production about 32% is exported.

REVIEW OF LITERATURE

Dates (Phoenix dectylifera)

There are about 200 varieties of dates growing in Iraq, and they differ in shape, color, taste, and time of ripening. The most important varieties are: Hallawi, Khadhrawi, Saiyir, Dairi, and Zahidi.

The development of dates may be divided into three steps:

1- The fruit stem develops rapidly with very little enlargement of the fruit.

2- The green fruit comes to full size, but no accumulation of sugars occurs during this stage.

3- Sugars accumulate rapidly, the fruit increases in weight, and the skin of the fruit takes on a translucent appearance.

The most important sugars in dates are: sucrose, glucose, and fructose (2, 26).

Browning and Darkening of Dates

Ripe and preserved fruit exhibit a brown to dark-brown color. Vinson in 1924 attributed this color to the tannins lying beneath the skin of the fruit. Rinderknecht in 1959 (22) found that the tannins located in the epiderm and the first layer of the hypoderm might be oxidized by a polyphenolase during ripening and yield an insoluble dark product. However, continued darkening after thermal destruction of this enzyme can be observed too. Recently, Maier in 1963 (11) isolated from California dates a caffeoylchikimic acid which he considered to be a substrate of the browning enzymes.

Classification of amino acids

There are 22 amino acids that occur frequently in protein hydrolyzates. Meister in 1957 (12) classified the twenty most common amino acids as follows:

1- Aliphatic:

Monoaminomonocarboxylic:

Glycine, alanine, isoleucine, leucine, and valine.

Hydroxymonoaminomonocarboxylic:

Serine, and threonine.

Monoaminodicarboxylic (amides):

Asparagine, and glutamine.

Diaminomonocarboxylic:

Arginine, and lysine.

Sulfur containing:

Cystfiene, cystine, and methionine.

2- Aromatic:

Phenylalanine, and tyrosine.

3- Heterocyclic:

Tryptophan, histidine, proline, and hydroxproline.

Of these 20 amino acids, eight are essential for adult humans. Those are: lysine, valine, threonine, leucine, isoleucine, tryptophan, phenylalanine, and methionine. The absence of any of these amino acids will result in malnutrition. The symptoms of amino acids deficiency are: deficient muscle development and retarded growth rates in children, low resistance to tuberculosis and rheumatic fever in some population groups,

anemia among women of childbearing age and children, toxemias, premature labors among pregnant women, and low appetite (3, 9).

Qualitative and Quantitative Determination of Amino Acids

Greenstein et al. (6) described a number of methods which can be used for quantitative and qualitative determination of ∝-amino acids. These methods are:

1- Chemical methods.

Mixtures of amino acids are separated chemically and determined either gravimetrically or colorimetrically by treatment with a reagent which forms a colored solution with the amino acid. The most popular colorimetric method is the ninhydrin method, which involves the reaction between ∝-amino acids and triketohydrindene hydrate (ninhydrin). Amino acids form a violet color in this reaction, except proline and hydroxproline which give a yellow-brown color with ninhydrin.

2- Physicochemical methods.

a- Titrimetry.

This method involves the determination of the amino acid concentration in aqueous solution by titration with strong acids or alkalies.

b- Isotope dilution.

The principle of the method is the following. An amino acid containing a heavy or radioactive isotope is added to a mixture of amino acids. The labeled amino acid is isolated from the mixture and the label determined quantitatively. From the ratio of added to recovered label, the concentration of the amino acid is calculated.

c- Spectrophotometry.

The ultraviolet and infrared spectra are also used in amino acid determination.

d- Optical rotation has been used in amino acid determination.

3- Microbiological assay methods.

These methods are based on the fact that the growth of certain microorganisms is a function of the concentration of specific amino acids.

4- Biochemical methods.

Amino acids can be released by enzymatic action and subsequently determined by one of the previously described methods.

5- Chromatographic methods.

a- Paper chro matography.

Martin, Consden, and Gordon in 1944 employed two dimentional paper chromatography for the separation and determination of amino acids. For the quantitative analysis of the amino acids by paper chromatography, color densitometry can be used, or the amino acids can be eluted and determined by colorimetry.

b- Column chromatography.

Starch was used in columns by Stein and Moore in 1948, but it was later replaced by ion exchange resins. Dowex 50 is used for the separation of both neutral and acidic amino acids. The elution of the amino acids depends on the presence of cations capable of competing with the cationic form of the amino acids.

Automatic amino acid analyzers were introduced in 1958 and extensively used since.

The retention on and recovery from an anion exchange column of an amino acid can be illustrated as follows:

$$R.NH_2 + HOOC.CH_2.CH_2.CH\ NH_3.COO^- \text{------} R.NH_3^+ (^-OOC.CH_2.CH_2.CH.NH_3^+.COO^-)$$

$$R.NH_3^+ (^-OOC.\ CH_2.CH_2.CH\ NH_3^+.COO^-) + HCI \text{------} R.NH_3^+.Cl^- + HOOC.\ CH_2.\ CH_2.CH\ NH_3.COO^-$$

Amino acids of dates

The amino acids content of dates has not been extensively studied. Grobbelaar et al. in 1955 (7) attempted a complete analysis of the free amino acids of dates. Using paper chromatography, they identified the following amino acids: δ-amino butyric acid, β-alanine, alanine, arginine, aspartic acid, biakiain, glycine, glutamic acid, homoserine, 5- hydroxy-pipecolic acid, isoleucine, leucine, lysine, pipecolic acid, serine, threonine, and valine.

Later Rinderknecht in 1959 (22) also using paper chromatography reported the presence of the following free amino acids in dates: - amino butyric acid, alanine, arginine, aspartic acid, biakiain, citrulline, cystine, glycine, glutamic acid, 5-hydroxypipecolic acid, proline, and serine. He also indicated that there is a sharp rise in the glutamine, and arginine content during the red stage and a decline during the brown stage of the development of dates.

Piez et al. in 1956 (21) using both paper chromatography and ion exchange resin, determined the following amino acids as mg./1 g. dates 1.8 5-hydroxypipecolic acid, 0.13 hydroxy proline, 0.7 proline, and 0.005 pipecolic acid.

Earlier, 1954, Garther and Dobzhanky (5) reported the presence of an unknown ninhydrin-positive substance in the urine of people who consumed dates. This substance seemed to be excreted quantitatively. It was suggested by Grobbelaar in 1955 that this unknown product could be hydroxypiperidine carboxylic acid.

MATERIALS AND METHODS

Four 1-pound boxes of semidry dates of each of the 3 most important commercial varieties in Iraq were used in this investigation. These varieties were: Hallawi, Saiyir, and Khadhrawi.

Moisture Determination

One 100 g. sample was randomly selected from 2 pounds of boxed pitted dates. The sample was ground in a meat grinder and 3 g. of the ground material were used for the determination of the moisture of each variety. Drying was done in a vacuum oven at 70°C to constant weight (13).

Total Nitrogen Determination

Five grams of ground sample prepared as described above were used for total nitrogen analysis by means of the Kjeldahl-Cunning-Arnold method. Triplicate determinations were made for each variety (13).

Amino Acid Determination

1- Paper Chromatography

Paper chromatography was used only for the identification of the free amino acids of dates. Fifty grams of dates were soaked for 1 hour in 100 ml. of 70% ethanol. They were then homogenized for 5 minutes in a Waring Blendor. The slurry was filtered and the filter cake was washed with approximately 10 ml. of 70% ethanol. The filtrates were passed through a column of Dowex 50X8, 1.4 cm. in internal diameter and 40 cm. in height, and washed with 50 ml. of water followed by 50 ml. of 1N ammonium hydroxide solution for the elution of the amino acids. The eluate was concentrated in a flash evaporator

to a volume of 5 ml. Ten drops of the extract were applied to a spot in the corner of Whatman #1, (22 x 18 inch sheet) paper 4 inches from each edge; one drop was applied at a time and dried with a jet of warm air. The paper was irrigated descendingly overnight with butanol/acetic acid/water (4/1/5 volume/volume) in the long direction, then dried in a forced air oven at 40°C for 2 hours and trimed one inch from both top and bottom. Then it was sprayed with borate buffer pH 8.3 (300 ml. of 0.1 M boric acid plus 60 ml. of 0.1 N NaOH) after covering the area run by the sample with aluminum paper. After spraying, the paper was dried at 40°C for half an hour.

The second solvent was made by mixing 30 g. of m-cresol, 15 g. phenol, and 7.5 ml. of borate buffer pH 8.3; this was the mobile phase. The aqueous phase was made by mixing 250 ml. of the borate buffer of pH 8.3 with 8 ml. of the mobile phase and was placed in a tray at the top and bottom of the chromatographic cabinet. The paper was placed in the cabinet for irrigation in the short direction. It was allowed to equilibrate with the aqueous phase for 4 hours and then irrigated with the second solvent the same way as with the first solvent. The paper was then dried at 40°C for 2 hours and sprayed with a mixture of 15 ml. glacial acetic acid, 50 ml. of 0.1% ninhydrin in ethyl alcohol, and 2 ml. of collidine. It was then placed for 5 minutes in a 100°C oven for color development (10).

The amino acids were identified by applying known amino acids on a similar sheet of paper and chromatogramed under the same conditions. Only the Saiyir variety was used for the paper chromatography analysis. Several chromatograms were prepared for this variety.

2- Column Chromatography

A- Moore and Stein 1954 Method.

Four hundred and fifty grams of Dowex 50x8, 200-400 mesh was washed with 2-3 liters of 4N HCl, rinsed with distilled water and then washed with 2N NaOH (about 400 ml.) until the filtrate was alkaline. The resin was then suspended in 500 ml. of 1N NaOH and heated over a steam bath for 3 hours. The heated mixture was allowed to stand for 1 hour, then the supernatant liquid was decanted and replaced with hot 1N NaOH. This procedure was repeated 5 times. The resin was then thoroughly washed with distilled water (about 5 liters), filtered and kept as the moist sodium salt.

One hundred ml. of the resin was transferred to a Buchner funnel and was washed first with 1 liter of 1N NaOH, followed by 1 liter of 0.2N sodium citrate-acetate buffer, pH 5.0. The resin was mixed with 100 ml. of the same buffer. This suspension was used to prepare a water jacketed column of 150 cm. in height, 1 cm. in internal diameter. Hundred ml. of 0.2N NaOH containing BRIJ 35* solution was passed through the column overnight at 15-20 inches water pressure. Then 200 ml. of 0.2N sodium citrate buffer, pH 3.1 was passed through the column before being used.

The buffers were prepared as described by Moore and Stein, 1954 (7). Ten ml. of the sample was prepared as for paper chromatography and was adjusted to pH 2.5 with 5N HCl. It was applied to the

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* BRIJ 35 is an ether of polyethylene glycol and acts as a detergent (15).

column and washed down with 3 portions of 0.3 ml. aliquots of citrate buffer pH 2.2.

The column was mounted over a fraction collector, the water in the column jacket was thermostatically adjusted to 30°C and the 0.2N sodium citrate buffer, pH 3.1 was forced into the column by applying air pressure of 40 inches of water. After 90 ml. of this buffer had passed through, the temperature around the column was raised to 50°C, and after 210 ml. of the buffer had passed through, its pH was gradually increased by mixing it with 0.2N citrate buffer, pH 5.1 (17).

Two ml. fractions were obtained from the column. To each fraction 1 ml. of the ninhydrin reagent (prepared by dissolving 20 g. of ninhydrin and 3 g. of hydrindantin in 750 ml. of methyl cellusolve, and made to 1 liter volume by 4N Na acetate buffer pH 5.5) was added. The tubes were shaken well and heated for 15 minutes in a boiling bath, then diluted with 50/50 ethanol/water. The color was determined spectrophotometrically at 570 μm (440 for proline and hydroxyproline) after cooling the tubes to room temperature. The blank reading was usually chosen from tubes before or after the peak (16).

The amino acid identification was accomplished by comparison with known amino acids separated under identical conditions as the unknowns.

B- Beckman/Spinco Amino Acid Analyzer Method

This method is principally the same as the Moore and Stein method.

Many steps of the analysis are performed automatically. The analyzer is provided with three columns, the temperature of which is regulated automatically, a gradient elution system, a ninhydrin reaction system with the effluent, three color measuring devices, and an automatic recorder.

From a 100 g. sample representing 2 pounds of boxed dates, two 4 g. samples were used for the free amino acids analysis and two 0.4 g. samples were analyzed for the total amino acids for each of the three varieties (25).

1- Preparation of the sample for the free amino acid determination:

The 4.0 g. sample was mixed with 40 ml. of 1% picric acid in all glass tissue grinder and the macerate was centrifuged in a clinical centrifuge at full speed. Twenty-five ml. of the supernatant was passed through a Dowex 2X10 column, 2.0 cm. in height and 2.5 cm. in internal diameter. The resin bed and the walls of the chromotogram tube were washed with five 3.0 ml. portions of 0.2N HCI, and the effluent was concentrated to a volume of about 1 ml. in a flash evaporator and transferred into a test tube with water. The solution was adjusted to pH 7.2 - 7.5 with 1N NaOH. Two tenths of 1 ml. of freshly prepared 0.5N solution of sodium sulfate was added to the concentrate and allowed to stand in the air for 4 hours at room temperature; this resulted in the auto oxidation of the glutathione

which would interfere with the separation of aspartic acid (18).

The sample was then adjusted to pH 2.2 - 2.3 with NaOH solution and subjected to the spectro-photometric analysis.

2- Preparation of the sample for the total amino acid determination:

For the total amino acid analysis the proteins and peptides of dates were hydrolyzed according to the Spackman method (25) and the Bandemer and Evans method (1).

In the Spackman method 400 mg. of ground dates were placed in a pyrex tube, 1 ml. of conc. HCI and 1 ml. of water were added, the mixture was frozen in a dry-ice-alcohol bath, the tube evacuated by means of high vacuum pump and the neck of the tube sealed under vacuum. The tube was then placed for 22 hrs. in an air circulating oven adjusted at 110°C for digestion. After digestion the top of the tube was removed and the hydrolyzate was filtered. Both the tube and filter paper were then washed with water, and the hydrochloric acid was evaporated from the sample in a water bath almost to dryness. The dried film of the hydrolyzate was removed by 0.2N Na citrate buffer, pH 2.2 and made to 10 ml. volume.

In the Bandemer and Evans method the 400 mg. sample was also mixed with 1 ml. HCI and 1 ml. water, but the tube was not sealed; instead, it was placed in an autoclave for

6 hours at 15 pounds pressure. After digestion, the sample was filtered and was made up to 10 ml. volume by adding the citrate buffer, pH 2.2.

Determination of the amino acids by the automatic analyzer:

Two or three ml. of the prepared sample were applied on the two columns of the analyzer; one for the basic amino acids and one for the neutral and acidic amino acids. The basic amino acid column was 15 cm. in height and 1 cm. in internal diameter, and was operated at 50°C. The elution of the basic amino acids was achieved with 0.35N Na citrate buffer, pH 5.28. The neutral and acidic amino acids column was 150 cm. in height and 1 cm. in internal diameter, and was also operated at 50°C. The elution of the neutral and acidic amino acids started with 0.2N Na citrate buffer, pH 3.25, and was continued with 0.2N Na citrate buffer of pH 4.25.

Thiodiglycol was added to the buffers to help in minimizing the conversion of small amounts of methionine to methionine sulfoxide during the addition of the sample to the column and during the time that the sample was standing on the long column while the basic amino acids analysis was being carried out on the short column.

The use of the detergent with the eluting buffer has been found to permit faster flow rates without concominant broading of the peaks on the effluent curves. The complete separation in the analyzer took 22 hours.

For the quantitative determination, measured quantities of all the amino acids known to be present in dates were run through the analyzer and a mathematical formula was prepared for the calculation of the unknowns.

RESULTS AND DISCUSSION

1- Moisture and Nitrogen Content of Dates

The average moisture and nitrogen contents of the three varieties of dates analyzed are shown in Table 1.

Table 1. Moisture and Nitrogen Contents of Dates.

Variety	Average moisture %	Average nitrogen % (wet basis)
Hallawi	17.1	0.286
Khadhrawi	13.4	0.260
Saiyir	19.6	0.275

2- Amino Acids Determination

a- Paper chromatography.

The free amino acids identified by paper chromatography are: aspartic acid, glutamic acid, glycine, valine, leucine, and proline.

The location of these amino acids on the paper chromatography is shown in Figure 1.

b- Column chromatography.

By means of the Stein and Moore method, thirteen peaks were obtained on the chromatogram of the free amino acids of the Saiyir variety dates (Figure 2). Of these peaks the following were identified: alanine, aspartic acid, glutamic acid, glycine,

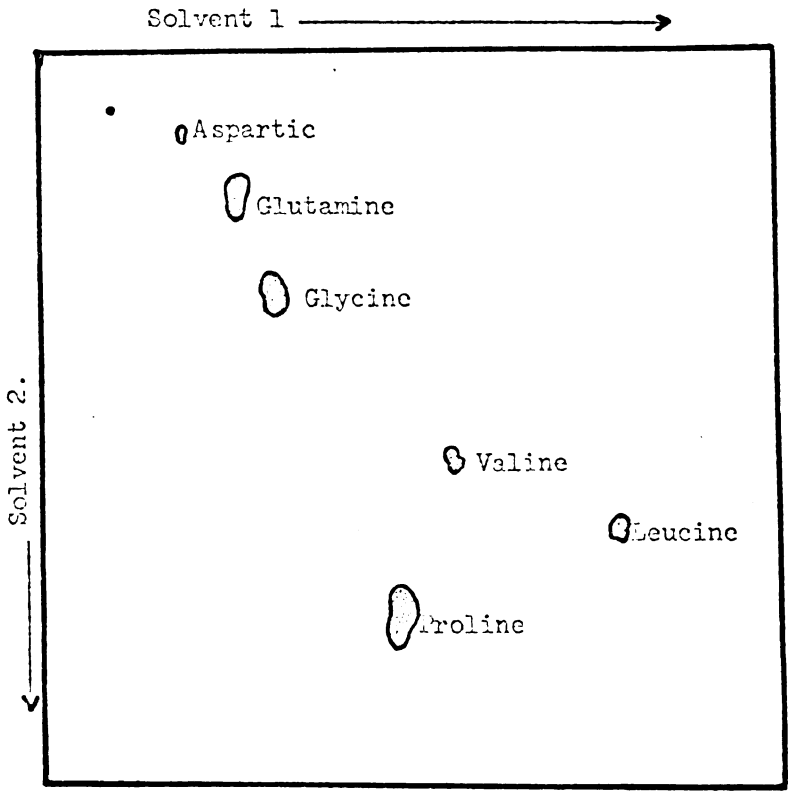

Figure 1. Paper chromatogram of the free amino acids of Saiyir var. dates. Whatman No. 1.

Solvent 1: Butanol-acetic acid-water (4:1:5)
Solvent 2: m-cresol-phenol (2:1), pH 8.3 borate buffer.

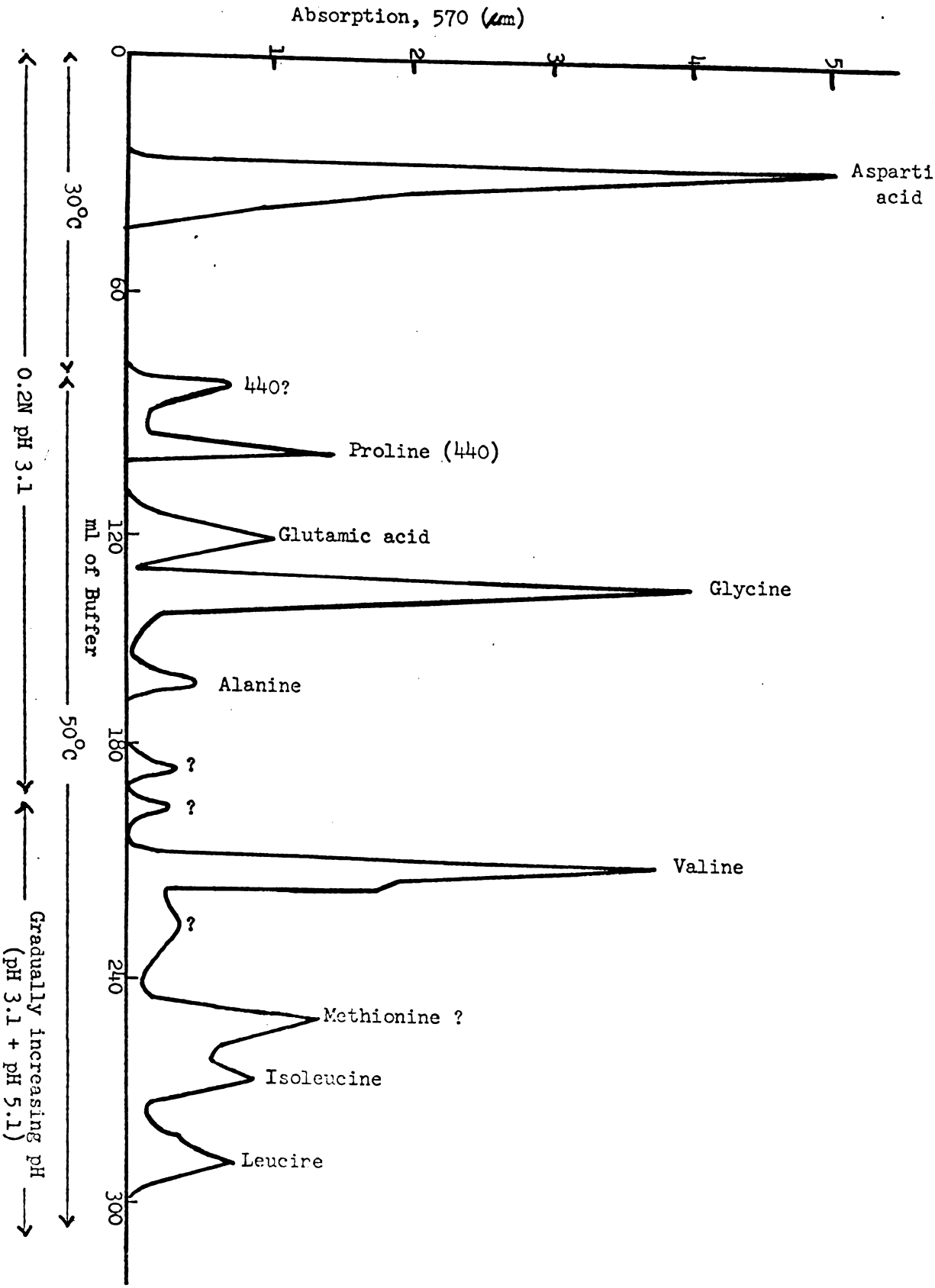

Figure 2. Free amino acids of Saiyir var. dates as separated by the Moore and Stein (1954) method.

isoleucine, leucine, methionine, proline, and valine. No further effort was made to identify the other peaks or to analyze the other two date varieties with this method because at that time the automatic amino acid analyzer became available and this instrument was used for the complete analysis.

Using the Beckman/Spinco amino acid analyzer the free and total amino acids were determined in duplicate samples of each of the three varieties. The results are summarized in Tables II to VII.

Two automatically recorded charts are also included, one from the analysis of the free amino acids of variety Khadhrawi, and one from the analysis of the total amino acids of the same variety, to illustrate the relative portion and size of the peaks which represent amino acids.

Tryptophan and cystine were destroyed during acid hydrolysis and do not appear in the results of this analysis. Instead, a peak corresponding to ammonia appears in all the charts.

Concerning the methods of analysis used in this work, the following can be said.

Paper chromatography did not resolve all the amino acids that could be identified with column chromatography. Paper chromatography is not recommended for a thorough study of amino acids, especially when quantitative results are desired.

Table II. Free Amino Acid Content of Dates, Var. Hallawi.

Amino Acids	moles/100g sample			Average	Average	Average
	Sample 1	Sample 2	Average	mg/100g sample	mg/100g dry sample	mg/g nitrogen
Alanine	199.1	165.0	182.0	16.2	19.6	101.0
Arginine	10.8	12.0	11.4	1.7	2.0	10.1
Aspartic Acid	40.4	47.0	43.7	5.8	7.0	36.1
Glutamic Acid	11.2	13.4	12.3	1.8	2.2	11.3
Glycine	75.3	97.0	86.1	6.5	7.8	42.5
Histidine	27.2	45.7	36.4	5.6	6.8	35.1
Isoleucine	19.1	21.1	20.1	2.6	3.2	16.5
Leucine	10.2	9.8	10.0	1.3	1.6	8.2
Lysine	13.7	15.5	14.6	2.1	2.6	13.4
Methionine	25.6	19.9	22.7	3.4	4.1	21.1
Phenylalanine	458.5	434.0	447.3	73.9	89.1	459.3
Proline	326.6	311.6	319.1	36.7	44.3	228.4
Serine	27.9	39.3	33.6	3.5	4.3	22.2
Threonine	7.8	20.2	14.0	1.7	2.0	10.3
Tyrosine	19.2	18.8	19.0	3.4	4.2	21.6
Valine	29.8	15.0	22.4	2.6	3.2	16.5
Total	1302.4	1285.3	1294.7	168.8	203.8	1050.5

Table III. Free Amino Acids Content of Dates, Var. Khadhrawi.

Amino Acids	moles/100 g sample			Average	Average	Average
	Sample 1	Sample 2	Average	mg/100g sample	mg/100g dry sample	mg/g nitrogen
Alanine	226.8	239.1	232.9	20.7	23.5	79.6
Arginine	6.7	11.3	9.0	1.3	1.5	5.0
Aspartic Acid	48.5	39.3	43.9	5.8	6.7	22.3
Glutamic Acid	16.7	14.5	15.6	2.3	2.7	8.8
Glycine	88.4	81.3	84.8	6.4	7.3	24.6
Histidine	37.6	39.2	38.4	6.0	6.9	23.1
Isoleucine	20.3	17.0	18.6	2.4	2.8	9.3
Leucine	7.7	7.4	7.5	1.0	1.1	3.8
Lysine	8.0	8.7	8.4	1.2	1.4	4.6
Methionine	9.0	11.2	10.1	1.5	1.7	5.8
Phenylalanine	445.9	388.7	417.3	68.9	79.6	265.0
Proline	226.4	177.5	212.0	24.4	28.2	93.8
Serine	64.0	58.2	61.1	6.4	7.4	24.6
Threonine	21.2	26.9	24.0	2.9	3.3	11.2
Tyrosine	15.3	17.2	16.2	2.9	3.4	11.2
Valine	20.5	22.2	21.4	2.5	2.9	9.5
Total	1263.0	1179.7	1221.2	156.6	180.7	602.3

Table IV. Free Amino Acids Content of Dates, Var. Siayir.

Amino Acids	moles/100 g . sample			Average	Average	Average
	Sample 1	Sample 2	Average	mg/100g sample	mg/100g dry sample	mg/g nitrogen
Alanine	137.9	162.7	150.3	13.4	16.7	48.7
Arginine	18.1	12.2	15.2	2.2	2.8	8.6
Aspartic Acid	19.1	32.7	25.9	3.4	4.3	12.4
Glutamic Acid	13.3	29.6	21.4	3.1	3.9	11.3
Glycine	69.1	92.4	80.8	6.1	7.5	22.2
Histidine	31.7	41.9	36.8	5.7	7.1	20.7
Isoleucine	22.4	29.1	25.7	3.4	4.2	12.4
Leucine	10.3	11.4	10.9	1.4	1.8	5.1
Lysine	6.4	10.6	8.5	1.2	1.6	4.4
Methionine	8.4	13.4	10.9	1.6	2.0	5.8
Phenylalanine	454.0	450.4	452.2	74.7	92.9	271.6
Proline	296.6	247.5	272.0	31.3	38.9	113.8
Serine	35.5	56.2	45.9	4.8	6.0	17.5
Threonine	12.6	18.7	15.6	1.9	2.3	6.9
Tyrosine	34.1	21.2	27.6	5.0	6.2	18.2
Valine	23.1	19.5	21.3	2.5	3.1	9.1
Total	1192.7	1249.5	1221.0	161.7	201.3	588.1

Table V. Total Amino Acids Content of Dates, Var. Hallawi.

Amino Acids	moles/100 g sample			Average	Average	Average
	Sample 1#	Sample 2*	Average	mg/100g sample	mg/100g dry sample	mg/g nitrogen
Alanine	1180.0	1196.5	1185.3	105.6	127.4	369.2
Arginine	286.1	241.9	264.0	38.9	46.9	136.0
Aspartic Acid	977.0	959.6	968.3	128.9	155.5	450.7
Glutamic Acid	1059.8	1081.8	1070.8	157.5	190.0	550.7
Glycine	1237.6	1369.4	1303.5	97.8	118.0	342.0
Histidine	123.3	147.1	135.2	21.0	25.3	73.4
Isoleucine	343.5	311.3	327.4	42.9	51.8	150.0
Leucine	629.9	649.3	639.6	83.9	101.2	293.4
Lysine	366.7	321.0	343.9	50.3	60.6	175.9
Methionine**	112.3	136.3	124.4	18.6	22.4	65.0
Phenylalanine	336.5	307.1	321.8	53.2	64.1	186.0
Proline	915.3	1001.8	958.6	110.4	133.1	386.0
Serine	535.7	676.1	605.9	63.7	76.8	222.7
Threonine	410.1	454.1	432.1	51.5	62.1	180.1
Tyrosine	87.5	72.7	80.1	14.5	17.5	50.7
Valine	573.0	503.1	538.0	63.0	76.0	220.3
Total	9174.3	9329.1	9298.9	1101.9	1329.2	3852.1
Bound A. A.	7871.9	8043.8	8004.2	933.1	1125.4	2801.6

Sample 1 was hydrolyzed by the method of Bandemer et. al.
* Sample 2 was hydrolyzed by the method of Spackman.
** Methionine includes Methionine Sulfoxide.

Table VI. Total Amino Acids Content of Dates, Var. Khadhrawi.

Amino Acids	moles/100 g sample			Average	Average	Average
	Sample 1#	Sample 2*	Average	mg/100g sample	mg/100g dry sample	mg/g nitrogen
Alanine	1020.9	1139.5	1080.2	96.2	111.6	370.0
Arginine	312.4	266.3	289.4	42.7	49.3	164.2
Aspartic Acid	1039.5	986.0	1012.8	134.8	155.7	518.5
Glutamic Acid	1126.5	1262.7	1194.6	175.8	203.0	676.2
Glycine	1233.5	1346.5	1290.0	96.8	111.8	372.3
Histidine	143.5	143.4	143.5	22.3	25.7	85.8
Isoleucine	361.9	289.5	325.7	42.7	49.3	164.2
Leucine	642.9	605.8	624.4	81.9	94.6	315.0
Lysine	393.6	339.5	366.6	53.6	61.9	206.2
Methionine**	66.3	85.5	75.9	11.3	13.1	43.5
Phenylalanine	289.7	273.3	281.5	46.5	53.7	178.8
Proline	835.6	796.5	816.0	93.9	108.5	361.2
Serine	566.0	673.3	618.2	65.0	75.0	250.0
Threonine	409.1	427.9	418.5	49.9	57.6	191.9
Tyrosine	42.3	62.9	52.6	9.5	10.1	36.5
Valine	542.2	453.5	497.9	58.3	67.4	224.2
Total	9025.9	9152.1	9087.8	1081.2	1248.9	4158.5
Bound A. A.	7762.9	7972.4	7866.6	924.6	1068.2	3556.2

Sample 1 by the method of Bandemer et. al.

* Sample 2 by the method of Spackman.

** Methionine includes Methionine Sulfoxide.

Table VII. Total Amino Acids Content of Dates, Var. Saiyir.

Amino Acids	moles/100 g sample			Average	Average	Average
	Sample 1#	Sample 2*	Average	mg/100g sample	mg/100g dry sample	mg/g nitrogen
Alanine	941.0	821.5	881.3	78.5	97.6	285.5
Arginine	370.8	238.1	304.5	44.8	55.7	162.9
Aspartic Acid	969.1	815.3	892.2	118.8	147.6	432.0
Glutamic Acid	1519.6	969.6	1244.6	183.1	227.6	665.8
Glycine	1299.6	1133.2	1216.4	91.3	113.5	332.0
Histidine	123.1	123.9	123.5	19.2	23.8	69.8
Isoleucine	371.0	249.9	310.5	40.7	50.6	148.0
Leucine	639.7	546.4	593.0	77.8	96.7	282.9
Lysine	409.8	279.9	344.9	50.4	62.7	183.3
Methionine**	98.7	64.7	81.7	12.2	15.2	44.4
Phenylalanine	269.3	244.0	256.7	42.4	52.7	154.2
Proline	968.5	756.9	862.7	99.3	123.5	361.1
Serine	553.6	560.1	556.9	58.5	72.4	212.7
Threonine	392.8	360.7	376.8	44.9	55.8	163.3
Tyrosine	80.3	90.5	85.4	15.5	19.2	56.4
Valine	572.0	382.0	477.0	55.9	69.5	203.3
Total	9578.9	7636.7	8608.1	1033.3	1363.4	3757.6
Bound A. A.	8386.2	6387.2	7387.1	8701.7	1162.1	3169.5

Sample 1 was hydrolyzed by the method of Bandemer et. al.
* Sample 2 was hydrolyzed by the method of Spackman.
** Methionine includes Methionine Sulfoxide.

Ion exchange column chromatography performed either manually or by means of the automatic amino acid analyzer is an excellent method for both the quantitative and qualitative determinations of amino acids. But the conditions of the analysis (pressure, temperature, buffer change, colorimetry, etc.) can be controlled much better by the automatic amino acid analyzer, making the analysis more accurate and less time consuming.

The two methods which were used here for the protein hydrolysis, the autoclave method and the sealed tube method, gave similar results. Since the autoclave method is the easiest of the two, it should be prefered for this analysis.

Regarding the results of the analysis, it was found that the difference in water content among the 3 varieties studied was greater than the difference in nitrogen content.

All the essential amino acids were present in dates, except tryptophan which escaped the analysis. The proportions of these amino acids in comparison to the dietary proportions recommended by Rose (23) for the adult man appears in Table VIII.

Table VIII. Proportions of Essential Amino Acids in Dietary Allowances and in Dates (Threonine = 1.0).

Amino Acid	Allowance	Dates		
		Hallawi	Saiyir	Khadhrawi
Isoleucine	1.4	0.8	0.9	0.8
Leucine	2.2	1.6	1.7	1.6
Lysine	1.6	1.0	1.1	1.1
Phenylalanine	2.2	1.1	0.9	0.9
Methionine	2.2	0.4	0.3	0.2
Threonine	1.0	1.0	1.0	1.0
Valine	1.6	1.2	1.2	1.2

From this comparison, and disregarding tryptophan, the limiting amino acid of dates appears to be methionine.

Of the three varieties analyzed, variety Hallawi contains the highest proportion of methionine.

Table IX indicates the amino acid composition of dates as reported by previous investigators (7, 22, 23) and in this work. Rinderknecht reported 12 free amino acids, Grobbelaar et al. 17 free amino acids, In "The Amino Acid Content of Foods," 10 amino acids are reported as present. In this work 16 amino acids have been identified in both the free and bound state. Some small peaks in our flowing chromatograms were not identified. The differences between our results and those of the previous investigators may be due to stage of maturity, variety, origin of the

Table IX. Amino Acids of Dates Reported by Various Investigators.

	Free A. A.			Total A. A.	
	Ref. 22	Ref. 7	This work	Ref. 23	This work
γ-amino butyric acid	+	+	-	-	-
β-alanine	-	+	-	-	-
Alanine	+	+	+	-	+
Arginine	+	+	+	+	+
Aspartic acid	+	+	+	-	+
Biakiain	+	+	-	-	-
Citrulline	+	-	-	-	-
Cystine	+	-	-	-	-
Glycine	+	+	+	-	+
Glutamic acid	+	+	+	-	+
Histidine	-	-	+	+	+
Homoserine	-	+	-	-	-
5-hydroxypipecolic acid	+	+	-	-	-
Isoleucine	-	+	+	+	+
Leucine	-	+	+	+	+
Lysine	-	+	+	+	+
Methionine	-	-	+	+	+
Phenylalanine	-	-	+	+	+
Pipecolic acid	-	+	-	-	-
Proline	+	-	+	-	+
Serine	+	+	+	-	+
Threonine	-	+	+	+	+
Tryptophan	-	-	-	+	-
Tyrosine	-	-	+	-	+
Valine	-	+	+	+	+

dates used, and analytical methods. However, no efforts were made here for the identification of biakiain, citrulline, and pipecolic acid.

According to the data obtained, the total quantity of the free amino acids is about 1/6 of that of the bound amino acids in all three date varieties analyzed. One amino acid, namely, phenylalanine, is present in higher concentration in the extract than the hydrolyzate. Since phenylalanine is not destroyed during acid hydrolysis, the paradox can be explained by the presence of a ninydrin-positive compound which is present in the extract and emerges from the column with phenylalanine, but which is destroyed during the hydrolysis. Two compounds have been reported to have effluent volumes similar to that of phenylalanine: the dipeptide, leucyl-glycine and o-tyrosine. If the dipeptide is present, hydrolysis of the extract will reduce the phenylalanine peak and increase the leucine and glycine peaks. Such a hydrolysis has not been tried.

Taurine has also been identified in the three varieties, but it was impossible to determine its quantity due to the appearance of an unidentified compound which emerged from the column at the same time as taurine.

SUMMARY

1- Dates of three varieties (Hallawi, Saiyir, and Khadhrawi) produced and processed in Iraq were analyzed for moisture, total nitrogen, free amino acids and total amino acids.

2- Although paper chromatography was used for the identification of some of the free amino acids of the dates, the overall analysis was essentially based on gradient elution column chromatography performed by means of an automatic amino acid analyzer.

3- The following amino acids were present both in the free state and in the acid hydrolyzate of dates: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine.

4- Tryptophan and cystine require special analytical methods, and they were not determined here.

5- The ratio of free to bound amino acids differed significantly between individual amino acids. However, for all amino acids collectively, this ratio was approximately 1 to 6.

6- All the essential amino acids, except tryptophan which escaped this analysis, were found to be present in dates. The limiting amino acid, from the nutritional viewpoint, appears to be methionine.

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