AMINO ACID PATTERN OF SOME WILD EDIBLE PLANTS

GROWING IN LEBANON

by

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ABSTRACT

Many wild leafy plants indigenous to Lebanon are consumed in appreciable amounts by the Lebanese people. Although these plants constitute a definite part of an established dietary pattern, no information is available concerning their nutritive value. This research was carried out to evaluate the quantity and quality of the protein of some of the commonly used leafy plants.

Total protein was measured by the Kjeldahl procedure and the quantitative determination of tyrosine, cystine and the essential amino acids was accomplished microbiologically. The Food and Agricultural Organization of the United Nations (F. A. O.) provisional standards were used as a basis of comparison for rating the level of the essential amino acids in the plants.

The protein content of most of the plants was found to be high (on dry matter basis) compared to that of most cereals and legumes. It is doubtful, however, that these plants are consumed to the same extent as cereals and legumes.

In general, the content of the individual amino acids in the eight plants can be rated as follows:

Lysine	: Poor – Fair
Leucine and Isoleucine	: Fair – Excellent
Threonine and Valine	: Good – Excellent
Phenylalanine, Tyrosine, Cystine, Methionine and Tryptophan	: Excellent

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INTRODUCTION

It has been established for many years that protein is a necessary constituent of the human diet. In addition to the requirement for a sufficient amount of protein in the diet, it is mandatory that food proteins provide a proper pattern of essential amino acids for synthesis of new body tissue and for maintenance.

The proteins of meat and other animal products such as milk and eggs provide the most complete amino acid patterns, and thus are the best quality. These foods, however, are relatively expensive, and in many areas of the world, people in lower income groups are unable to include them in their daily diet. As a result, the bulk of the proteins consumed by these people is the lower quality, cheaper, plant protein. It is true that suitable mixtures of proteins of vegetable origin can provide the proper amino acid pattern for growth and maintenance even in the absence of animal protein. In order to obtain this pattern, however, the vegetables must be carefully selected according to the amino acid content of each. Obviously, these highly controlled conditions are seldom met and in extreme instances of poor quality protein intake, the protein deficiency disease, Kwashiakor, may develop.

The Middle East is a good example of an area where a high proportion of plant protein is eaten as compared to animal protein. In Lebanon, more specifically, there are many wild leafy plants which are consumed in appreciable quantities and which are readily available on the market. Little research has been carried out with regard to the quantity and quality of the protein portion of leafy plants, and no such information is available concerning the wild edible plants of Lebanon. Since these foods

costitute a definite part of an established dietary pattern, it would seem important to study the relative nutritive value of each by determining the quantity and quality of the protein. This information would be of value for establishing food composition tables for use by local dietitians, nutritionists and health officers. These people could then recommend mixtures of the various plants which, when consumed, would provide a good source of essential amino acids. The information might also indicate how the leafy plants could be used to supplement other plant proteins (legumes, cereals etc.) in order to enhance the quality.

Leaf Protein

Leaf proteins comprise the enzymatic systems responsible for photosynthesis and the wide range of other metabolic processes of which higher plants are capable (5). The occurrence and general characteristics of leaf proteins have been discussed by Osborne (30) and Bondi (5). Pirie (31) has summarized the factors which affect the amount of protein in leaves.

The concept of using leaf protein as human food is relatively new and has been discussed by Guha (15) and Pirie (32). The limited information available on the amino acid analysis of leaf protein indicates that, in general, the quality is relatively good (5, 32). According to Pirie (32) leaf protein will be a valuable food in the future.

Extraction

Normally, certain extraction procedures are carried out as the first step in the quantitative determination of amino acids in tissues. Although this procedure is not always practiced, a brief discussion of various extraction methods is in order.

Two categories of extraction will be considered:

1. The extraction of non-proteinaceous products such as sugars, chlorophyll and lipids.

2. The extraction of protein from leafy tissues.

In regard to the first point, Tristram (41) has described a procedure, one step of which is the removal of chlorophyll with hot alcohol. In contrast, Plaisted (33) has discussed a method whereby hot alcohol is

used for the extraction of proteins from leaves. It would appear that
in the former method of extraction, an accurate determination of protein
and amino acids would not be achieved since a certain amount of these
materials would be lost in the alcohol. Hearn (16) has discussed the
extraction of lipids from leafy tissues but made no mention of the sol-
vents used.

With regard to protein extraction, there have been two aims in
most of the work (22):

1. To obtain pure preparations.

2. To obtain large yields.

The preparation of native protein from leaves has proved to be
much more difficult than similar preparations from seed materials (5).
Osborne (30) has reviewed this topic from the time of Beccari in 1745 up
to 1924, and has pointed out most of the workers who attempted to extract
proteins from seeds, fruits, and leaves· The first serious attempt to
isolate protein in quantity from leaves was made almost simultaneously
by Osborne and Wakeman who used spinach leaves and by Chibnall and
Schryver who worked with cabbage (30). Osborne made perhaps the most
outstanding contribution to the area of protein isolation when he intro-
duced the salting-out procedure. The modern methods of salting-out pro-
teins for technical and scientific purposes are based on the fundamentals
of Osborne's original method (5).

Various methods have been proposed for the extraction of leaf
proteins:

1. FRESH LEAVES

Chibnall (7) has described a method for the extraction of proteins
from leafy tissues employing the "Colloidal Mill". Large scale extraction

of protein from fresh leaves has been discussed by several authors (6, 15, 23). Guha (15) claims that by using 2% sodium carbonate solution, 60% of the leaf protein can be obtained for human consumption and the remaining residue can then be fed to cattle. According to Guha, this method has been practiced and found to be satisfactory.

2. DRIED LEAVES

For the extraction of protein from dried leaves, Lugg and Weller (23) suggest the use of mildly alkaline buffers in the presence of alcohol. Smith and Agiza (38), in contrast, recommended the use of hot dilute mineral acids or 90% formic acid. Neither method, however, is completely satisfactory. According to Pirie (31), the factors governing the completeness of extraction are by no means clearly understood, but, as this author points out, fine grinding is obviously an advantage. Slightly alkaline conditions also tend to promote extraction, although some of the amino acids may be destroyed in the process (4, 31).

Schwerdtfeger (35) and Mackie et al. (28) have described other less effective methods of extraction.

From this brief discussion, it appears that the complete extraction of proteins from leafy tissues is difficult to achieve and many sources of error arise. For this reason, the extraction process is omitted by some investigators and amino acid determinations are carried out after hydrolysis of the intact sample (24).

Hydrolysis

In the determination of the amino acid composition of foods and proteins, the sample is first hydrolyzed to liberate the amino acids. The various methods of hydrolysis and the advantages and disadvantages

of each method have been summarized by Block and Bolling (4). In general, the main source of error in protein analysis is the loss by destruction of amino acids during hydrolysis. According to Tristram (41), the amount of destruction during acid hydrolysis of a protein is increased by the presence of non-proteinaceous substances, especially carbohydrates and lipids. Thus hydrolytic loss is greater in leafy materials due to the presence of large amounts of fiber, mucilages, and pentosans which give rise to furfuraldehyde on acid hydrolysis.

In spite of the fact that alkaline hydrolysis completely destroys certain amino acids, this procedure is necessary for the determination of tyrosine and tryptophan (4). These two compounds are much more sensitive to acidic than alkaline conditions. The unpublished data obtained in this laboratory (Table 1) illustrate the error introduced by the use of acid hydrolysis in the assay of tyrosine. It can be seen that in six of the eight samples, a substantial loss of tyrosine resulted from acid hydrolysis.

Amino Acid Determination

In general, chemical, electrolytic, chromatographic adsorption, and microbiological methods are employed for the assay of amino acids. The details of all these procedures have been summarized by Block and Bolling (4). Since the present research involved only the microbiological method, this procedure is the one to be discussed.

Several scientists have made use of the microbiological assay method for the routine estimation of almost all the known amino acids (9, 12, 14, 37, 39). The microbiological assay, according to Lyman (24), is a "feeding trial" using a strain of microorganism which will not grow

Table 1.   Comparison of Tyrosine in Acid

And Alkaline Hydrolysates

Name of Plant		Acid	Alkaline	Loss or Gain
		mg tyrosine/gm nitrogen		per cent
MALVA	SP.	82	130	-37
CHICORIUM	SP.	82	129	-37
ERYNGIUM	SP.	98	135	-27
ARUM	SP.	137	156	-12
THYMUS	SP.	108	154	-30
ORIGANUM	SP.	91	125	-27
AMARANTHUS	SP.	143	134	+7
INULA	SP.	154	156	-1

unless a particular nutrient is provided in the medium. In an amino acid assay, the organisms are placed in a medium containing all the required nutrients except the amino acid in question. If this critical amino acid is added to the medium in graded amounts, the organisms will grow according to the concentration of this nutrient. In this way, the organisms serve as a very sensitive assay "instrument".

Dunn and co-workers (9) have pointed out the prerequisites for a satisfactory microbiological assay. According to Block and Bolling (4) the methods for all amino acids are so similar in technique that once a procedure is learned for any one amino acid, it can be applied with equal facility to any other for which suitable conditions have been found.

Lyman (24) has pointed out other advantages of the microbiological assay:

1. It is not necessary to separate out the protein before hydrolysis.

2. The procedure is relatively simple and is applicable to large numbers of samples.

3. Only very small amounts of material are required.

One distinct disadvantage of the microbiological method arises from the fact that partial or complete hydrolysis of the protein under investigation is required. As a result there is a certain amount of hydrolytic loss of amino acids. In many instances, this point is forgotten when the relative merits of the microbiological and chemical methods are discussed (4). Another disadvantage pointed out by Lyman (24) is the fact that the amino acid requirements of the lactic acid bacteria are not always fixed.

Snell (39) has discussed some of the factors which give low precision and accuracy in microbiological assays.

STATEMENT OF PROBLEM

The purpose of the research reported in this thesis was to establish the quantity and quality of the protein in eight wild leafy plants which are consumed in appreciable amounts in Lebanon.

METHODS AND MATERIALS

Selection of Samples

Samples of wild, edible plants from various parts of Lebanon were obtained either from the vegetable market in Beirut or were picked where growing. Of the plants collected, thirty five were identified (34) and of these, eight were chosen for this investigation. The families, scientific names, English names and Arabic names are shown in Table 2. The criteria for choosing these eight plants were:

1. They are all classed as "leafy".

2. Most are available on the market and thus are consumed in larger quantities than the others.

3. They are consumed in one or more of the following forms: fresh, salad, pickled, cooked and dried.

4. Some are used only in specific areas while the others are eaten throughout Lebanon.

The samples of 1961 were chosen for the determination of amino acids.

Preparation of the Samples

The leaves of the plants were removed, washed thoroughly and dried in an air draft oven at 37°C with the exception of <u>Malva</u> <u>sp</u>. and <u>Inula</u> <u>sp</u>. which were dried at 50°C. After drying, the samples were ground in a Wiley mill (60 mesh) and stored in covered jars.

Moisture Determination

Moisture was determined by drying the samples in a still air oven at 70°C for 24 hours (40).

Table 2.   Eight Wild Edible Plants Selected

For Amino Acid Studies.

Family	Scientific Name	English Name	Arabic Name
MALVACEAE	Malva rotundifolia*	Mallow	Khubbeyzeh
COMPOSITAE	Chicorium glandulosum	Chicory	Hindebeh
	Inula crithmoides	Golden Samphire	Hashishat Bahr
UMBELLIFARAE	Eryngium creticum	Botton Sake root	Qurs-Anni
LABIATAE	Thymus capitatus	Thyme	Zaatar Farisy
	Origanum syriacun	Thyme	Zaatar
AMARANTACEAE	Amaranthus sylvestris	Amarantus	Karboozeh
ARACEAE	Arum SP.**	Arum	Luf

* Other edible species of Malva are:
  M. nicaensis, M. sylvestris and M. parviflora

** Edible species of Arum that were analyzed:
   A. dioscorides, A. hygrophillum and A. palestinum.

Total Protein

The nitrogen content was determined by the Kjehdahl method (40) and the percent protein was calculated by multiplying by the factor 6.25.

Hydrolysis

For the determination of all the amino acids under consideration except tryptophan and tyrosine, a two gram sample of the ground leafy tissue was refluxed with 200 ml of 6N HCl for 24 hours. Most of the HCl was then removed by evaporation under vacuum at $70^{\circ}C$. To the remaining residue, 50-75 ml of distilled water was added and the mixture was neutralized with 4N NaOH to pH 6.8. A suitable dilution was then made (200-250 ml) and the solution filtered through Whatman No. 1 filter paper. A portion (25-50 ml) of the resulting filtrate was diluted to 250 ml and aliquots of this solution were used for the amino acid determinations.

For the tryptophan and tyrosine assays, an alkaline hydrolysis procedure was employed (19). A small quantity (16 ml) of 4N NaOH, to which 0.1 gm of L-cysteine hydrochloride had been added, was placed in a covered 100 ml beaker and autoclaved at 15 psi for 1 hour. While still hot, another 0.1 gm portion of cysteine hydrochloride was added to the solution along with 0.5 gm of tryptophan for the standard 0.5 gm of the sample to be hydrolyzed. Autoclaving was then continued for 15 hours. The addition of cysteine hydrochloride was necessary to prevent the destruction of tryptophan (19).

Assay Organisms

Leuconostoc mesenteroides P-60 (ATCC No. 8042) was used for the assay of lysine, phenylalanine, tyrosine and cystine. Streptococcus lactis R (ATCC No. 8043) was used for the assay of threonine, tryptophan and

methionine. Lactobacillus arabinosus 17-5 (ATCC No. 8014) was used for the assay of valine, leucine and isoleucine.

Although L. mesenteroides in hydrogen peroxide--treated peptone medium is recommended for the methionine assay (25), high blank readings were obtained when this procedure was used. Highly satisfactory results were obtained, however, with S. lactis R in the methionine determinations.

Reviving Lyophilized cultures

Each lyophilized culture was obtained in a vial sealed inside a glass tube. The outer tube was removed by heating the pointed end in a Bunsen flame and adding a drop of water to crack the glass. The tip of the container was then shattered by a sharp blow from a pencil or a similar object after which the inner vial containing the organisms could be removed.

The organisms were transferred to growth medium aseptically by rinsing the vial with 0.5 ml of appropriate sterile broth and pouring the mixture into a culture tube containing 10 ml of the same broth. After incubation overnight at 37°C, the organisms were sub--cultured daily for one week before being used.

The organisms were maintained as stab cultures by weekly transfer to a solid medium of the following composition:

Tryptone	1%
Agar	1%
Filtered tomato juice	20%
Peptone[1]	1%
Glucose[1]	1%

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1. The peptone and glucose can be replaced by 1% peptonized milk (27).

Preparation of Inoculum

Culture tubes containing sterilized growth medium[2] were inoculated from the stab cultures and incubated at $37^{\circ}C$ for 18 hours. The cells were then spun down (3500 rpm for 15 minutes), the supernatent medium was thoroughly decanted and the cells were resuspended, without washing, in 10 ml of isotonic NaCl solution (0.9%). This cell suspension served as the inoculum in the assay procedure.

Basal Media

The basal medium used for the determination of valine, leucine and isoleucine was the same as that described by Kuiken et al. (21) and amended by Lyman et al. (27). For the threonine, tryptophan and methionine assays the media described by Lyman et al. (20, 21, 25) were used. For lysine and phenylalanine the medium used was that described by Lyman et al. (27). The medium used for tyrosine was that described in the A. O. A. C. Handbook, 1960. This same medium, with the addition of ammonium chloride, was used for the cystine assay.

Assay Procedure

Each assay was carried out at six different test levels chosen so that at least three values obtained would be within the range of the standard curve. The range of concentrations for determining each standard curve depended upon the amino acid used. The ranges were as follows:

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2. The growth medium was the same as the solid medium with the exception that agar was not included (27).

Amino Acid	Micrograms/tube
L-cystine and L-tyrosine	0-20
DL-tryptophan	0-25
L-leucine, L-isoleucine, DL-methionine and DL-phenylalanine	0-50
DL-valine	0-100
L-lysine	0-120
DL-threonine	0-125

Certain factors had to be considered before the standard curves of several of the amino acids were plotted. Since the L-form of trypto-phan in the sample is racemized during hydrolysis with NaOH, the amount of DL-standard is used directly without dividing by two. The D-isomers of phenylalanine, threonine and valine are completely inactive and it is necessary, therefore, to divide the weight of the DL-standards by two. Lysine hydrochloride was used as the standard, hence, it was necessaty to multiply by the correction factor, 0.8.

For the preparation of the assay tubes, 5 ml of basal medium was placed in the tubes after which the appropriate amount of either the stand-ard amino acid solution or the solution of hydrolized sample was added. The volume of each tube was then made up to 10 ml with distilled water, and mixed thoroughly by shaking. The standard tubes as well as the assay tubes were all prepared in duplicate. The tubes were plugged with cotton and autoclaved at 15 psi for 15 minutes.

After cooling, the tubes were inoculated aseptically with two drops of previously prepared inoculum by means of a 10 ml hypodermic syringe equipped with a 25 gauge needle. The inoculated tubes were then incubated

at 37°C for a period of four days for L. mesenteroides and three days for L. arabinosus and S. lactis. At the end of the incubation period, the lactic acid produced in each tube was titrated with 0.1N NaOH using bromthymol blue as the indicator.

The standard curves for each amino acid were drawn by plotting the average volume of 0.1N NaOH used for titrating each set of duplicate tubes against the concentration of the amino acid in the tubes. The concentration of each amino acid in the sample was determined by extrapolation from the appropriate standard curve. Any values in the very high or very low ranges were discarded.

Calculations

The amino acid concentration (mcg per ml) was calculated for those test levels which were within the range of the standard curve, and the average of these values was taken.

The following calculations were then made for each amino acid:

1. % Amino Acid (oven dry basis):

$$\frac{\text{Average conc.(mcg/ml) x dil. factor x 100}}{\text{wt. of oven dry sample (mcg)}}$$

2. mg amino acid/gm N (oven dry basis):

$$\frac{\text{\% amino acid x 1000}}{\text{\% nitrogen}}$$

The leaves of eight wild edible plants were analyzed microbiologically for lysine (Lys), phenylalanine (Phe), tyrosine (Tyr), cystine (Cys), methionine (Met), tryptophan (Tryp), threonine (Thr), valine (Val), isoleucine (Iso) and leucine (Leu). Total protein was also determined on each sample. The data obtained have been reduced to tabular form and are presented in Tables 3-7 inclusive.

Table 3.   Total Nitrogen And Protein Content of Eight

Wild Edible Plants Growing in Lebanon.

Name of Plant		Nitrogen	Protein Nx6.25	Nitrogen	Protein Nx6.25
		per cent*		per cent*	
		1960		1961	
MALVA	SP.	5.56	34.75	5.14	32.66
CHICORIUM	SP.	3.55	22.19	4.07	25.41
ERYNGIUM	SP.	2.19	13.69	3.40	21.23
ARUM	SP.	3.20	20.00	3.94	24.62
THYMUS	SP.	1.74	10.88	2.09	13.07
ORIGANUM	SP.	2.22	13.88	2.17	13.58
AMARANTHUS	SP.	4.77	29.81	4.96	31.06
INULA	SP.	1.14	7.12	1.61	10.06

* Values are expressed on oven dry basis

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ORIGANUM	SP.	2.22	13.88	2.17	13.58
AMARANTHUS	SP.	4.77	29.81	4.96	31.06
INULA	SP.	1.14	7.12	1.61	10.06

* Values are expressed on oven dry basis

Table 4. Amino Acid Contents of Eight Wild
Edible Plants Growing In Lebanon.

Name of Plant		Lys	Phe	Tyr*	Cys	Met	Tryp*	Thr	Val	Iso	Leu
					per cent**						
MALVA	SP.	.760	.580	.670	trace	.608	.555	1.078	2.076	1.205	1.382
CHICORIUM	SP.	.546	.653	.525	trace	.747	.522	.731	1.496	1.277	1.296
ERYNGIUM	SP.	.410	.491	.460	trace	.570	.384	.532	1.169	.975	.990
ARUM	SP.	.604	.696	.613	.060	.782	.588	.705	1.533	1.319	1.262
THYMUS	SP.	.296	.370	.322	trace	.393	.267	.362	.721	.670	.616
ORIGANUM	SP.	.294	.242	.272	trace	.310	.239	.437	.597	.422	.483
AMARANTHUS	SP.	.867	.802	.667	trace	1.051	.550	1.174	1.655	1.344	1.484
INULA	SP.	.231	.211	.252	trace	.267	.234	.438	.579	.410	.434

* Determined on the alkaline hydrolysates.
** Values are expressed on oven dry basis.

Table 5.   Amino Acid Content of Eight Wild Edible Plants Growing In Lebanon.

mg / gm nitrogen

Name of Plant	Lys	Phe & Tyr	Cys & Met	Tryp	Thr	Val	Iso	Leu
MALVA SP.	148	243	118	108	210	404	234	269
CHICORIUM SP.	134	290	184	129	180	368	314	319
ERYNGIUM SP.	121	280	168	113	156	344	287	291
ARUM SP.	153	333	213	149	179	389	335	321
THYMUS SP.	142	331	188	128	173	345	321	295
ORIGANUM SP.	122	236	143	100	201	275	194	222
AMARANTHUS SP.	175	296	208	110	236	333	271	229
INULA SP.	144	287	166	145	272	360	254	270
F.A.O. Standard	270	180	90	90	180	360	270	306

* Results are expressed on oven dry basis

Table 6. Amino Acid Content of Eight Wild Edible Plants Growing In
Lebanon Compared With The F.A.O. Provisional Standards.

Name of Plant	Lys	Phe & Tyr	Cys & Met	Tryp	Thr	Val	Iso	Leu
			per cent *					
MALVA SP.	55	135	131	120	116	112	87	88
CHICORIUM SP.	50	161	204	143	100	102	116	104
ERYNGIUM SP.	45	156	187	126	87	96	106	95
ARUM SP.	57	185	238	166	99	108	124	105
THYMUS SP.	52	184	209	142	96	96	119	96
ORIGANUM SP.	45	131	158	111	112	76	72	72
AMARANTHUS SP.	65	164	231	123	131	93	100	98
INULA SP.	53	160	184	161	151	100	94	88

* Per cent Amino Acid: $\dfrac{mg\ a.a\ /gm\ nitrogen}{F.A.O.\ Standard}$ x 100

Table 6.   Amino Acid Content of Eight Wild Edible Plants Growing In

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Name of Plant		Lys	Phe & Tyr	Cys & Met	Tryp	Thr	Val	Iso	Leu
					per cent *				
MALVA	SP.	55	135	131	120	116	112	87	88
CHICORIUM	SP.	50	161	204	143	100	102	116	104
ERYNGIUM	SP.	45	156	187	126	87	96	106	95
ARUM	SP.	57	185	238	166	99	108	124	105
THYMUS	SP.	52	184	209	142	96	96	119	96
ORIGANUM	SP.	45	131	158	111	112	76	72	72
AMARANTHUS	SP.	65	164	231	123	131	93	100	98
INULA	SP.	53	160	184	161	151	100	94	88

* Per cent Amino Acid: $\dfrac{\text{mg a.a /gm nitrogen}}{\text{F.A.O. Standard}}$ x 100

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Table 7. Rating of The Amino Acid Contents of Eight

Wild Edible Plants Growing In Lebanon.*

Name of Plant	Lys	Phe & Tyr	Cys & Met	Tyrp	Thr	Val	Iso	Leu
MALVA SP.	F	E	E	E	E	E	G	G
CHICORIUM SP.	F	E	E	E	E	E	E	E
ERYNGIUM SP.	P	E	E	E	G	G	E	G
ARUM SP.	F	E	E	E	G	E	E	E
THYMUS SP.	F	E	E	E	G	G	E	G
ORIGANUM SP.	P	E	E	E	E	G	F	F
AMARANTHUS SP.	F	E	E	E	E	G	E	G
INULA SP.	F	E	E	E	E	E	G	G

* The ratings are based on the F.A.O. provisional standards.
  E : Excellent   G : Good
  F : Fair   P : Poor

Extraction

Several extraction procedures were tried in an attempt to isolate the leaf proteins prior to amino acid analysis. One method was that of Schwerdtfeger (35) in which the ground leafy material was mixed with pure quartz and placed in a column 40 x 1.5 cm. The solvents, which included water, potassium sulfate, propanol, potassium hydroxide in methanol, and sodium dodecyl sulfate, were then passed through the column. Some of the solvents, however, did not flow even under pressure and the method was abandoned.

The methods of Mackie et al. (28) (using borate buffer) and of Plaisted (33) (using hot alcohol) were tried. Both methods were rejected, however, because most of the protein remained in the residue after extraction.

A fourth method involved precipitating the protein with trichloroacetic acid (T. C. A.). It was impossible, however, to separate the precipitated protein from the residue and this method was rejected also.

Since the protein extraction methods did not prove promising, it was decided to try removing the non-proteinaceous materials in the hope that only the protein would remain after extraction. The method of Tristram (41) was followed using acetic acid, alcohol, citric acid, alcohol, and ether in that order. The acetic acid extracted mucilage in large amounts, especially from Malva sp., so that filtration was impossible even with suction.

Because of the difficulties encountered in the extraction methods, it did not seem possible to achieve the quantitative isolation

of the protein from the samples. The extraction process was eliminated, therefore, and the amino acid analyses were carried out on the hydrolyzed intact sample as is recommended by Lyman (24).

Amino Acid Determinations

Difficulties were encountered in two of the assay procedures, and it was found necessary to adopt certain modifications.

Lyman et al. (25) recommended the use of L. mesenteroides in hydrogen peroxide-treated peptone medium for the methionine determination. High blank readings were obtained, however, when this procedure was used, possibly because the hydrogen peroxide did not destroy all the methionine in the peptone. As an alternate method, S. lactis in synthetic medium was used with highly satisfactory results.

The A. O. A. C. (1960) procedure was tried for the cystine determination. In this method, cysteine and L-tyrosine are included in the basal medium and ammonium chloride is omitted. The method proved unsatisfactory, however, because high blank readings were obtained. There were two possible explanations for the high readings:

1. The L-tyrosine may have been contaminated with cystine, as has been stated by Block and Bolling (4).

2. Perhaps the organisms synthesized cystine from cysteine.

The basal medium was modified by omitting both cysteine and ammonium chloride. In this instance, zero readings were obtained for all levels of the standard. These results indicate that perhaps the organisms have a cysteine requirement.

The second modification tried was the omission of cysteine and the addition of ammonium chloride as suggested by Lyman and his associates (27)

for the use of L. mesenteroides. In this instance, satisfactory results
were obtained for the standard curve, and this method was adopted for all
the cystine assays.

It appears, then, that the assay organism (L. mesenteroides) has
a specific requirement for inorganic nitrogen in the absence of cysteine.
These findings rule out the possibility stated above that the L-tyrosine
was contaminated with cystine. Had this been true, high blank readings
would have been obtained.

Protein Content

The protein content of the eight wild plants was determined on
the samples collected in both 1960 and 1961. These data (Table 3) show
that the protein content of most of the plants is high, compared with
most cereals and legumes. The exceptions are Thymus sp., Origanum sp.
and Inula sp. which compare favorably with cereal grains.

The slight variation between the two years are probably due to
factors such as: time of picking, age of the plant, locality, whether
growing in sun or shade, etc.

Amino Acid Content

The percentages of the ten amino acids investigated in each of
the wild plants are shown in Table 4. These data were used for establish-
ing and evaluating the amino acid pattern in each plant.

It can be seen (Table 4) that, in all the plants, very little
cystine was found. Although cystine is not essential, it is a precursor
of methionine, and was determined because it represents a potential
source of the latter. The fact that only trace amounts of cystine were

found is not significant since a high level of methionine was present in all the samples.

Tyrosine, another non-essential amino acid, was measured because of its role as a precursor of phenylalanine.

In Tables 5, 6, and 7, the values for cystine and methionine are combined as are the values for tyrosine and phenylalanine. This is in keeping with the form used by the F. A. O.

Amino Acid Pattern

The F. A. O. reference amino acid pattern was established by a study group from that organization, and the calculations for the amount of each amino acid in the pattern are based on the work of Albanese et al. (1). This reference pattern represents the amount (mg per gm of nitrogen) of each essential amino acid, plus cystine and tyrosine, which should be present in a protein if it is to promote optimum growth and maintenance. The F. A. O. pattern and the pattern for each of the plant proteins studied are presented in Table 5. These data show that, in all the plants, lysine is the only essential amino acid which is present in critically low amounts. Thus, it can be considered the limiting amino acid in each plant.

The data in Table 6 represent the percentage of each amino acid present in each plant compared with the F. A. O. reference pattern. The F. A. O. values are considered to be 100.

In comparing the amino acid content of the eight samples with the F. A. O. provisional standard for amino acids, the following rating system was adopted (12): when a sample contains 100% or more of the quantity of the specific amino acid, the sample is rated as an

" EXCELLENT" source of the amino acid. Similarly, a sample containing 75-99% of an amino acid, is rated as a "GOOD" source. A "FAIR" source of a specific amino acid contains 50-74%, whereas "POOR" sources contain less than 50%. According to this classification, the ratings of the amino acids in each sample have been deduced from Tables 5 and 6 and are presented in Table 7.

It appears that, in general, all the plants investigated are relatively good sources of all the essential amino acids except lysine. Since nothing is known, however, concerning the availability of the amino acids in these plants, this information must be obtained before the true nutritive value of the protein can be assessed.

Supplementation

The idea of supplementing vegetable proteins with synthetic amino acids has received attention (3, 8, 36), as well as the idea of mixing selected vegetables so as to enhance protein quality (32). Meat and other animal products, of course, are excellent supplements for improving the quality of vegetable proteins.

From a practical point of view, the plants investigated in this research are deficient in lysine. This deficiency could be overcome by serving Leban (yoghourt), which is high in lysine, with the vegetable. Certain legumes such as chickpeas and lentils are also good sources of lysine, andif eaten along with the wild plants, they would improve the quality of the protein. Since all of the foods mentioned above are very popular with the Lebanese people, there would be no problem of acceptance of the mixtures.

Suggested Further Research

This investigation was restricted to eight leafy vegetables out of at least 65 wild plants which are consumed in Lebanon. The edible proteins, modes of consumption and the names of 30 of these plants have been established (34).

It would be of value to continue the research on the protein and amino acid content of all of these wild edible plants. Also, it would be of importance to study the other chemical components of the plants, especially the vitamins, minerals, carbohydrates and any toxic substances which may be present.

Certain vegetables, such as Brassica genus (cabbage), are known to contain goitrogenic substances. Also, it has been found that goitre is prevalent among Tasmanian children fed cow's milk, which carries with it goitrogens derived from kale (10). Since there is a high incidence of goitre in Lebanon (17), it would be of importance to investigate the possibility of the presence of goitrogenic agents in the wild edible plants.

Other related research would include:

1. The collection and identification of all wild edible plants growing in Lebanon.

2. The investigation of modes of preparation of these plants.

3. The determination of the frequency of consumption of the wild plants.

4. Contraindication of using these plants.

5. The development of methods for preserving the plants.

6. The study of the effects of preparation and / or preservation on the nutritive value of the plants.

7. The availability of the essential amino acids.

8. The availability of iron in these plants, since there is a high incidence of anemia in Lebanon.

SUMMARY

This investigation was carried out to study the relative nutritive value of eight wild edible plants growing in Lebanon. These plants are consumed in appreciable amounts and constitute a part of an established dietary pattern of the Lebanese people.

The protein content was determined and found to be high in comparison with most cereals and legumes.

The ground leaf preparations were analyzed for lysine, phenylalanine, tyrosine, cystine, methionine, tryptophan, threonine, valine, isoleucine and leucine. An amino acid pattern was established and compared with the F. A. O. standard reference pattern. In general, the amino acid pattern of the eight plants was found to be good except for lysine, which was low in all the plants.

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