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PROTEINS OF THE AVOCADO (PERSEA AMERICANA MILL).

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In this paper are described the isolation and composition of some proteins of the avocado. Data are given concerning the proteins of a type of vegetable tissue that has been very little investigated. Aside from the work of Smith (1) on the orange there has been to our knowledge nothing published regarding the nature and properties of the proteins of fruits.

In addition to the scientific aspects connected with a study of the proteins of the avocado, the growth of the avocado industry in the United States during recent years, and the increasing popularity of this fruit as an article of food lend further interest and importance to a consideration of the proteins from the standpoint of their biological value.

Proteins of three different types have been isolated from the fresh avocado. One was removed by extracting the fresh fruit with 10 per cent sodium chloride solution. This protein was separated from the saline extract by three different methods; heat coagulation (Preparation I), precipitation with ammonium sulfate (Preparation II), and acidification with acetic acid (Preparation III). The close agreement between these preparations obtained by different methods, with respect to their properties and composition (Table I), strongly indicates that they represent one and the same protein. This protein behaves in most respects like a globulin. It is soluble in salt solution, from which it can be precipitated by addition of ammonium sulfate to 67 per cent of saturation, or by addition of acetic acid to pH 3.9. On the other hand, it cannot be precipitated from a 5 per cent sodium

chloride solution by dilution with 25 volumes of water, nor have we been able to separate it by dialysis.

Another protein fraction was obtained which was insoluble in sodium chloride solution but soluble in an alcoholic solution of sodium hydroxide. By slightly acidifying the alkaline solution with acetic acid the protein separated as a flocculent precipitate (Preparations IV and V).

The slightly acid alcoholic filtrates from Preparations IV and V yielded on dilution with water another protein fraction (Preparations VI and VII).

TABLE I.

*Elementary Composition of Proteins.**

Preparation No.	N	C	H	S	Moisture.	Ash.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	15.27	53.12	8.56	2.82	6.52	0.42
II	15.37	52.61	8.05	2.87	3.92	1.03
III	15.30	53.03	8.10	2.97	3.60	0.59
IV	13.41	56.49	7.06	2.02	4.04	1.12
V	13.44	56.58	7.13	2.06	5.01	4.91
VI	16.32	55.52	7.40	1.90	3.92	2.86
VII	16.14	55.34	7.43	1.88	6.40	1.49

* The percentages are calculated on an ash- and moisture-free basis, and with the exception of those for sulfur, moisture, and ash, represent average results of duplicate analyses.

The method of preparing the fraction obtained by acidifying the alkaline alcoholic extract (Preparations IV and V) suggests a type of protein having somewhat the properties of glutelins. However, in view of the enzymic activity present in the pulped fruit of the avocado, it is possible that some of the salt-soluble protein originally present had become denatured, and thus escaped extraction with the salt solution, to be later dissolved from the residue by the alcoholic alkali extractant. It is also possible that this fraction represents a type of protein hitherto uncharacterized, and which does not fit into the generally accepted classification of proteins.

It is of interest to note that Preparations VI and VII were separated from an alcoholic solution, although practically no protein could be extracted with alcohol from the fresh avocado.

These preparations may represent a secondary product formed by the action of the alkali upon an original protein of more complex structure.

TABLE II.

*Distribution of Nitrogen in Avocado Proteins as Determined by the Van Slyke Method.**

Nitrogen.	Preparation No.			Amino acids expressed in percentages of proteins.†			
	I	V	VI	Amino acid.	I	V	VI
	per cent	*per cent*	*per cent*				
Total N	15.27	13.44	16.32	Arginine	7.94	4.46	12.94
Amide "	6.03	8.91	5.35	Histidine	0.59	2.04	0.99
Humin N absorbed by lime	3.15	7.49	5.06	Lysine	7.06	6.71	3.95
				Cystine	2.03	1.84	1.80
Humin N in amyl alcohol-ether extract	0.16	0.11	0.44	" ‡	1.48	0.11	
				Tryptophane§	2.12	0.38	1.07
				Tyrosine‖	7.01	4.92	2.81
Arginine N	16.74	10.67	25.51				
Cystine "	1.55	1.60	1.28				
Histidine "	1.04	4.10	1.64				
Lysine "	8.86	9.57	4.64				
Amino N of filtrate	60.47	55.38	48.12				
Non-amino N of filtrate	2.88	3.04	6.90				

* Nitrogen figures are corrected for the solubilities of the phosphotungstates of the basic amino acids.

† Except as indicated, the percentages are calculated from the average results of duplicate analyses by the Van Slyke method.

‡ Determined by Dr. M. X. Sullivan according to the Sullivan colorimetric method.

§ Determined by the method of May and Rose.

‖ Determined by the colorimetric method of Folin and Looney.

The three different proteins isolated are clearly differentiated from one another both by their elementary composition (Table I) and by the distribution of nitrogen (Table II).

The avocados[1] used in the work described in this article were grown in California and were mostly of the Fuerte variety. The

[1] The avocados were furnished by the Calavo Growers of California, and were of the grade that are marketed under the trade name "Calavo."

edible portion contained about 71 per cent water, 20 per cent oil, and 0.38 per cent nitrogen, equivalent to 2.37 per cent crude protein (N × 6.25). The proteins actually isolated amounted to 0.67 per cent of the fruit, and represented 28.5 per cent of the total nitrogen. In its content of nitrogen the avocado leads almost all fresh fruits.

A meal prepared by drying the fresh fruit at room temperature, and extracting the oil with ether was found to be unsuitable for a study of the proteins on account of changes that had occurred in the proteins, caused apparently by the action of enzymes. It was therefore decided to abandon the idea of preparing a meal, and to work directly with the fresh fruit.

It was found that three successive extractions of the fresh pulp with 10 per cent sodium chloride solution removed 39 per cent of the total nitrogen, and that 32 per cent of this nitrogen was contained in the coagulum formed by heating the salt extract.

The residue that had been extracted with sodium chloride solution still contained 39 per cent of the original nitrogen of the meal that was extractable by boiling 0.1 N, 60 per cent alcoholic solution of sodium hydroxide. By neutralizing the alkaline extract with acetic acid a precipitate formed which contained 26 per cent of the total nitrogen of the meal.

By successive extractions of the fresh avocado with 10 per cent sodium chloride solution and alcoholic sodium hydroxide, 78 per cent of the total nitrogen was removed, 58 per cent of which was protein nitrogen precipitable by tannic acid.

Preparation of Proteins.

The edible portion of the fruit was finely comminuted and extracted with 10 per cent sodium chloride solution. The clearly filtered, light brown salt extracts were neutral to litmus, but on standing they gradually turned darker and became acid in reaction, with the separation of a small quantity of protein, which completely dissolved when the solution was neutralized with 0.1 N sodium hydroxide. When slowly heated the sodium chloride extracts yielded a flocculent precipitate at 68°. Continued heating of the extract up to boiling gave no further significant amount of coagulum.

Heat-Coagulable Fraction. Preparation I.—The salt extracts

were heated to boiling, and the coagula were thoroughly washed, first with distilled water which had been slightly acidified with acetic acid, then with boiling distilled water, and finally with boiling 95 per cent alcohol. These washings were repeated many times. The protein was then dried in the usual way with absolute alcohol and ether. The final product consisted of a light, cream-colored powder which weighed 22 gm. This quantity represented the yield obtained from 5 kilos of avocados.

Fraction Precipitated by Ammonium Sulfate. Preparation II.—To the clear, filtered, sodium chloride extract of 3422 gm. of avocados was added enough ammonium sulfate to make the extract 67 per cent saturated with this salt. The precipitate which separated was dissolved by addition of water. It had been previously found that this protein could not be separated by dialysis. An attempt to separate the protein by heat coagulation gave a finely divided precipitate which could not be removed by filtration or centrifugalization. Addition of 5 volumes of 95 per cent alcohol, however, caused the separation of a flocculent precipitate. The thoroughly washed and dried product weighed 4 gm.

Fraction Precipitated by Acidification of Sodium Chloride Extract with Acetic Acid. Preparation III.—Acidification of a 10 per cent sodium chloride extract of the avocado with acetic acid gave the maximum precipitation at pH 3.9. The washed and dried precipitate obtained in this way from 1618 gm. of avocado weighed 4 gm.

Alcoholic Alkali-Soluble Fraction. Preparations IV, V, VI, and VII.—The residue remaining after exhaustive extraction of 7 kilos of avocado pulp with 10 per cent sodium chloride solution was boiled for 10 minutes with a 0.1 N, 60 per cent alcoholic solution of sodium hydroxide. The alkaline extract was readily filtered through a folded filter paper, and the clear filtrate was acidified with acetic acid. The flocculent precipitate which separated was washed many times with distilled water which had been slightly acidified with acetic acid, and was finally washed with hot 50 per cent alcohol. After drying in the usual way, 7 gm. of light material were obtained (Preparation IV).

In a similar way, Preparation V (12 gm.) was obtained from 5040 gm. of avocado.

The slightly acidified, 60 per cent alcoholic filtrate from Prepara-

tions IV and V still contained considerable protein which was precipitated by addition of several volumes of water. The dried and purified precipitates (Preparations VI and VII, respectively) consisted of light colored powders. Preparation VI weighed 4 gm.

The elementary composition of the different protein fractions obtained as described is given in Table I.

Preparations I and IV were obtained from a lot of avocados of the Taft variety. The other preparations were made from the Fuerte variety.

Analyses of Proteins by Van Slyke Method.—The general properties and elementary composition of the six preparations described indicate that we were dealing with three different proteins. No essential difference was observed between Preparations I, II, and III, although prepared by different methods. Preparations IV and V were practically identical in their behavior and composition, but distinctly different from the preceding. Preparations VI and VII were alike, but differed from all the others. As representatives of the three proteins, Preparations I, V, and VI were analyzed by the Van Slyke method. The results are given in Table II.

Determinations of Tyrosine, Tryptophane, and Cystine.

The determination of tryptophane was carried out essentially by the colorimetric method of May and Rose (2). Tyrosine was determined colorimetrically by the method of Folin and Looney (3). In addition to the values found for cystine by the Van Slyke method, there are also included in Table II figures for this amino acid obtained colorimetrically. In connection with a comparative study of methods for the estimation of cystine, Dr. M. X. Sullivan of the Hygienic Laboratory, estimated the cystine in Preparations I and V according to the colorimetric method developed by him (4) which has been shown to be highly specific. In view of the well known uncertainty regarding values for cystine as determined by the Van Slyke method, and the high degree of specificity shown for the Sullivan method, the percentages obtained by the latter method are to be regarded as expressing more accurately the actual amount of cystine present in the proteins. This view is strongly supported by correlations observed in connection with the results

of feeding experiments carried on in this laboratory with certain cystine-deficient proteins, and the cystine content of these proteins as determined by these two methods.

SUMMARY.

Three different types of protein have been isolated from ripe avocados, *Persea americana* Mill. By extraction of the edible portion of the fruit with 10 per cent sodium chloride solution a protein was obtained which behaved in most respects like a globulin. In the saline solution it coagulated at 68°, and was precipitated both by addition of acetic acid and by making the solution 67 per cent saturated with ammonium sulfate. It contained 15.31 per cent nitrogen.

By slightly acidifying with acetic acid an alcoholic alkali extract of the residue remaining after the extraction with sodium chloride, a second protein containing 13.42 per cent nitrogen was obtained. Dilution of the slightly acid, alcoholic filtrate with water caused the separation of a third protein fraction. This protein contained 16.23 per cent nitrogen. Elementary composition and distribution of the nitrogen by the Van Slyke method in the proteins were determined. Cystine, tryptophane, and tyrosine were determined colorimetrically.

BIBLIOGRAPHY.

1. Smith, A. H., *J. Biol. Chem.*, **63**, 71 (1925).
2. May, C. E., and Rose, E. R., *J. Biol. Chem.*, **54**, 213 (1922).
3. Folin, O., and Looney, J. M., *J. Biol. Chem.*, **51**, 421 (1922).
4. Sullivan, M. X., *Pub. Health Rep., U. S. P. H. S.*, **41**, 1030 (1926); *J. Biol Chem.*, **74**, p. xiv (1927).