

PROTEINS OF THE CANTALOUPE SEED, CUCUMIS MELO.

ISOLATION OF A CRYSTALLINE GLOBULIN, AND A COMPARATIVE STUDY OF THIS GLOBULIN WITH THE CRYSTALLINE GLOBULIN OF THE SQUASH SEED, CUCURBITA MAXIMA.

BY D. BREESE JONES AND C. E. F. GERSDORFF.

(From the Protein Investigation Laboratory, Bureau of Chemistry, United
States Department of Agriculture, Washington.)

(Received for publication, March 3, 1923.)

The extremely complex character of the protein molecule renders it difficult if not impossible by any known means at present available to determine the chemical individuality of any single protein, or to establish beyond question the identity of any two proteins from different sources. This complexity confers upon proteins such chemical and physical properties that the tests and methods commonly used to determine the identity of more simple compounds either cannot be applied or, when applied, give results which do not furnish conclusive evidence to this end. Two proteins may have the same crystal form, elementary composition, percentages of the same amino-acids, molecular weight, crystallographic and optical properties, even the same immunological reactions, and yet be distinctly different. All of these points of similarity together would not constitute indisputable proof that the proteins were identical, although they would furnish very strong evidence to that effect.

In this paper is described an investigation of the proteins of the cantaloupe seed, *Cucumis melo*. Two proteins were isolated from this seed—a globulin and a glutelin. A globulin preparation was obtained which consisted entirely of well formed octahedra. The crystal form, method of preparation, and elementary composition of this globulin so closely resembled those of the squash seed globulin as isolated and described by Osborne (1) that a comparative study of the two globulins from these seeds was

suggested, with the view of securing as much evidence as possible toward establishing either their identity or non-identity. For this purpose a crystalline globulin preparation from hulled squash seeds was prepared according to the method of Osborne (1). With but few exceptions the vegetable proteins which have been isolated in crystalline form have been globulins obtained from oil seeds, such as hemp seed, castor bean, Brazil nut, sesame seed, flaxseed, and squash seed (1). It is a rather remarkable fact that in practically all cases the crystalline globulins have been obtained as octahedra or as crystals belonging to the isometric system. The

TABLE I.

Average Results of Duplicate Analyses of Squash Seed Globulin. A Comparison of the Elementary Composition of the Cantaloupe and Squash Seed Globulins.*

	Crystallized squash seed globulin.			Crystallized cantaloupe seed globulin (Preparation XIV).	Average analysis of fourteen preparations of cantaloupe seed globulin.
	Preparation.		Average.		
	I	II			
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
C.....	52.19	52.14	52.17	52.74	52.65
H.....	6.54	6.57	6.56	6.72	6.67
N.....	18.39	18.63	18.51	18.42	18.41
S.....	1.01	0.99	1.00	1.11	1.13
O.....	21.87	21.67	21.76	21.01	21.14
Moisture.....	6.99	9.51			
Ash.....	0.56	0.52			

* Calculated on an ash- and moisture-free basis.

similarity of the globulins from the seeds of the squash and cantaloupe was first revealed by their practically identical behavior and physical properties exhibited during the process of their preparation.

Furthermore, from all of the chemical and physical data obtained in our study of the isolated and purified globulins of these two seeds, we have been unable to find any point of difference which would indicate their non-identity.

Table I gives figures showing the elementary composition of the two crystalline globulins. These figures represent the average

results of a large number of duplicate analyses. The small differences noted in their elementary composition are not greater

TABLE II.

*Distribution of Nitrogen in the Cantaloupe Seed Globulin as Determined by the Van Slyke Method.**

Sample I, ash- and moisture-free, 2.6901 gm. protein, 0.4955 gm. nitrogen.†
 " II, " " " 2.6901 " " 0.4955 " " †

	I	II	I	II	Average.
	gm.	gm.	per cent	per cent	per cent
Amide N.....	0.0376	0.0371	7.59	7.49	7.54
Humin N adsorbed by lime.....	0.0035	0.0034	0.71	0.69	0.70
Humin N in ether-amyl alcohol extract.....	0.0004	0.0004	0.08	0.08	0.08
Cystine N.....	0.0039	0.0038	0.79	0.77	0.78
Arginine N.....	0.1411	0.1404	28.48	28.33	28.41
Histidine N.....	0.0303	0.0312	6.11	6.30	6.20
Lysine N.....	0.0166	0.0172	3.35	3.47	3.41
Amino N of filtrate.....	0.2500	0.2500	50.45	50.45	50.45
Non-amino N of filtrate.....	0.0106	0.0106	2.14	2.14	2.14
Total N regained.....	0.4940	0.4941	99.70	99.72	99.71

* Nitrogen figures corrected for the solubilities of the phosphotungstates of the bases.

† Nitrogen content of protein, 18.42 per cent.

TABLE III.

Basic Amino-Acids of the Proteins.

Amino-acids.	Cantaloupe seed globulin.	Squash seed globulin.	Cantaloupe seed glutelin.
	per cent	per cent	per cent
Cystine.....	1.23	1.26	1.07
" *.....	1.31	1.42	1.11
Arginine.....	16.26	15.69	12.42
Histidine.....	4.22	4.54	2.72
Lysine.....	3.29	3.70	4.59
Tryptophane†.....	2.63	2.92	3.03

* By the colorimetric method of Folin and Looney (2).

† By the colorimetric method of May and Rose (3).

than those frequently met in the analysis of different preparations of the same protein.

A study of the distribution of the nitrogen (Tables II, III, IV, and V) in the two globulins as determined by the Van Slyke

TABLE IV.

*Distribution of Nitrogen in the Squash Seed Globulin as Determined by the Van Slyke Method.**

Sample I, ash- and moisture-free, 2.7735 gm. protein, 0.5100 gm. nitrogen. †
 “ II, “ “ “ “ 2.7735 “ “ 0.5100 “ “ “ †

	I	II	I	II	Average.
	gm.	gm.	per cent	per cent	per cent
Amide N.....	0.0392	0.0394	7.69	7.73	7.71
Humin N adsorbed by lime.....	0.0053	0.0058	1.04	1.14	1.09
Humin N in ether-amyl alcohol extract.....	0.0004	0.0001	0.08	0.02	0.05
Cystine N.....	0.0040	0.0041	0.78	0.80	0.79
Arginine N.....	0.1397	0.1404	27.39	27.53	27.46
Histidine N.....	0.0342	0.0340	6.71	6.67	6.69
Lysine N.....	0.0196	0.0197	3.84	3.86	3.85
Amino N of filtrate.....	0.2602	0.2602	51.02	51.02	51.02
Non-amino N of filtrate.....	0.0083	0.0077	1.62	1.50	1.56
Total N regained.....	0.5109	0.5114	100.17	100.27	100.22

* Nitrogen figures corrected for the solubilities of the phosphotungstates of the bases.

† Nitrogen content of protein, 18.39 per cent.

TABLE V.

Distribution of Nitrogen in the Proteins as Calculated from the Van Slyke Analyses in Terms of Percentage of the Proteins.

Nitrogen.	Cantaloupe seed globulin.*	Squash seed globulin.†	Cantaloupe seed glutelin.‡
	per cent	per cent	per cent
Amide.....	1.39	1.42	1.23
Humin.....	0.14	0.21	0.41
Basic.....	7.15	7.14	5.74
Non-basic.....	9.69	9.67	9.01
Total.....	18.37	18.44	16.39

* Nitrogen content, 18.42 per cent.

† Nitrogen content, 18.39 per cent.

‡ Nitrogen content, 16.37 per cent.

method also gave results which agree within the limits of error of this method for duplicate analyses of the same protein.

In addition to the values found for cystine by the Van Slyke method, the content of this amino-acid was also determined colorimetrically by the recently published method of Folin and Looney (2). It is of interest to note the close agreement between the results obtained by the two different methods (Table III). Tryptophane was determined colorimetrically according to the method of May and Rose (3). The percentage of tryptophane found in the two globulins, together with those of the diamino-acids, is given for comparison in Table III.

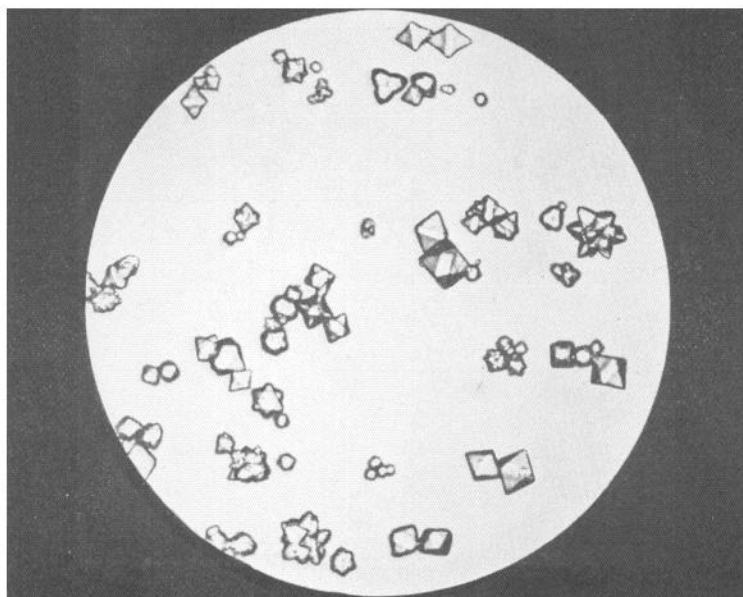


FIG. 1. Globulin from the cantaloupe seed, *Cucumis melo*. $\times 210$.
Photomicrographs by G. L. Keenan.

The globulins of both the squash seed and cantaloupe seed were obtained in the form of regular, microscopic octahedra. Samples of the globulins were submitted to Dr. E. T. Wherry and Mr. G. L. Keenan of the Bureau of Chemistry, who kindly made the crystallographic and optical examinations and prepared the photomicrographs (Figs. 1 and 2). No difference in the crystallographic or optical properties of the two globulins was detected.

In view of the practically identical results obtained by the study of the chemical and physical properties of these two globulins it was of interest to compare them with respect to their immunological relations. Through the kind cooperation of Prof. H. G. Wells of the University of Chicago, we have been enabled to secure data to make this comparison. Guinea pigs sensitized with squash seed globulin reacted anaphylactically as severely to cantaloupe seed globulin as did those sensitized with the latter,

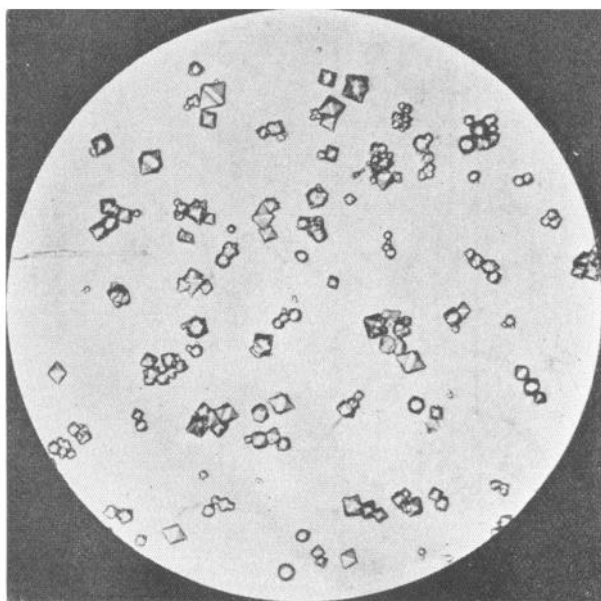


FIG. 2. Globulin from the squash seed, *Cucurbita maxima*. $\times 210$.
Photomicrographs by G. L. Keenan.

and *vice versa*. No distinction immunologically could be observed between the globulins of the two seeds. The results of these tests are given in Table VI. These results are of special significance in the comparative study of these globulins, since Wells and Osborne (4) have pointed out that it seems probable that "the specificity of the anaphylaxis reaction is determined by the chemical structure of the reacting proteins rather than by their biological origin."

TABLE VI.
*Anaphylaxis Experiments Made with the Crystallized Globulins of the Seeds of the Cantaloupe and Squash.**

Experiment No.	Sensitizing dose (1 mg.).	Intoxicating dose (50 mg.).	Result.
1	Squash seed globulin.	Squash seed globulin.	Died, 2 hrs.
2	" "	" "	" 1.75 "
3	" "	" "	" 70 min.
4	" "	" "	" 2 hrs.
5	" "	" "	Moderately severe.
6	Cantaloupe seed globulin.	" "	" "
7	" "	" "	" "
8	" "	" "	Died, 25 min.
9	" "	" "	" 40 "
10	" "	" "	Moderately severe.

* These experiments were made by Prof. H. Gideon Wells, University of Chicago.

The collective data obtained in this investigation offer very strong evidence that the crystalline globulins of the seeds of the squash and cantaloupe are identical. This is of interest in connection with the fact that these globulins were obtained from the seeds of plants which, although belonging to the same botanical family, Cucurbitaceae, are not only specifically different, but which belong to distinct genera.¹

The cantaloupe seed globulin used in the aforementioned comparative studies was obtained from a meal prepared from hulled cantaloupe seeds containing 58.16 per cent of protein ($N \times 5.68$). All the protein preparations used in the other general studies were obtained from a press-cake remaining after expression of the oil. This press-cake² included the hulls of the seeds, and contained 4.08 per cent of nitrogen, which when multiplied by the conventional protein factor 6.25, is equivalent to 25.50 per cent of protein. When the factor 5.68³ is used, however, the more accurate protein content of 23.17 per cent is obtained. Although the globulin prepared from the press-cake containing the hulls could be obtained largely in the form of crystals, it was found that when meal from the hulled seeds was used the globulin could be readily obtained wholly as octahedra.

No difference in the composition of the proteins, whether obtained from meal containing the hulls or from meal without hulls, was observed.

By extraction of the finely ground meal with 5 per cent sodium chloride solution, a maximum yield of 12.07 per cent of globulin was isolated, based on the weight of the meal used. The residue further yielded on extraction with 0.5 per cent aqueous sodium hydroxide 5.78 per cent of glutelin.

The cantaloupe seed globulin was prepared by five different methods, all of which yielded preparations which gave the same results on analysis.

¹ This information was furnished by Dr. W. E. Safford, Economic Botanist, Bureau of Plant Industry, United States Department of Agriculture.

² The cantaloupe seed press-cake was furnished by the Oil, Fat and Wax Laboratory, of the Bureau of Chemistry, United States Department of Agriculture.

³ This factor is based on the relative quantities of globulin and glutelin found in the meal, and their respective nitrogen contents.

Careful examination failed to reveal the presence of an albumin or of more than one globulin in the seed.

EXPERIMENTAL.

Proteins of the Cantaloupe Seed.

Preliminary Experiments.—Small quantities of finely ground meal prepared from cantaloupe seed press-cake were thoroughly mixed with various solvents, in the ratio of 10 cc. of solvent to 1 gm. of meal, and the mixture was allowed to stand at room temperature, with occasional stirring, for 1 hour. Nitrogen determinations made on aliquot portions of the extracts⁴ showed that the maximum quantity of protein was extracted by a 5 per cent sodium chloride solution (Table VII). Further experimentation showed that an extraction period of 15 minutes was as effective as that of 1 hour.

Exhaustive extractions were made by successively extracting the meal five or six times with sodium chloride solution. After the last extraction the residue was thoroughly washed with distilled water, and then extracted twice for $\frac{1}{2}$ hour periods with 0.5 per cent aqueous sodium hydroxide. Nitrogen determinations were made on aliquot portions of each extract. The results of these experiments are given in Table VII.

Precipitation tests showed that practically all of the protein could be precipitated from the sodium chloride extract of the meal by making the extract 0.25 saturated with ammonium sulfate, precipitation beginning at 0.2 of saturation. Increasing the concentration of ammonium sulfate up to complete saturation caused the further separation of but a mere trace of material. The unusually narrow range of precipitability with ammonium sulfate indicates the presence of only one globulin in the cantaloupe seed.

Experiments were made to ascertain as nearly as possible the total content of globulin and glutelin in the meal. For this purpose 200 gm. of the meal were exhaustively extracted with 5 per cent aqueous sodium chloride, and the joint extracts slightly acidified with acetic acid and boiled. The coagulum obtained,

⁴ Acknowledgment is due to Mr. S. Phillips of this laboratory for the Kjeldahl nitrogen determinations made in the course of this investigation.

TABLE VII.
Extraction Experiments.

Preliminary* extractions.			Maximum extractions.					
Solvent †	Protein extracted.		Extraction No.	Solvent. ‡	Protein extracted.			
	(N × 6.25)				Experiment I §		Experiment II.	
	per cent	(N × 5.41) †			per cent	(N × 6.25)	per cent	(N × 5.41) †
Distilled water.....	2.02	1.75	1	5 per cent NaCl.....	11.71	10.14	12.33	10.67
0.5 per cent NaCl.....	3.73	3.23	2	5 “ “.....	2.79	2.42	4.96	4.29
1.0 “ “.....	5.53	4.79	3	5 “ “.....	1.60	1.38	0.47	0.41
1.5 “ “.....	5.79	5.01	4	5 “ “.....	0.60	0.52	0.11	0.10
2.5 “ “.....	7.85	6.80	5	5 “ “.....	0.58	0.51		
4.0 “ “.....	8.42	7.29	6	5 “ “.....	0.47	0.41		
5.0 “ “.....	15.97	13.83	7	0.5 “ “ NaOH.....	6.14	6.14	5.92	5.92
7.0 “ “.....	15.71	13.60	8	0.5 “ “.....	0.26	0.26		
10.0 “ “.....	15.84	13.71		Total.....	24.15	21.78	23.79	21.39

* The extractions were carried out at room temperature, for a period of 1 hour each, with frequent stirring.

† Solvents were used in the proportion of 10 cc. per gram of meal.

‡ This factor is based on the known nitrogen content of the globulin. The factor for glutelin is 6.25.

§ The extractions were carried out at room temperature, for a period of $\frac{1}{4}$ hour each, except for the last two which were for $\frac{1}{2}$ hour each.¶ The extractions were carried out at room temperature, for a period of 24 hours each, except for the last two which were for $\frac{1}{4}$ hour each.

|| These extracts when subjected to tests for protein gave negative results.

after a thorough washing with distilled water and drying, weighed 25.48 gm. (corrected for ash and moisture). This is equivalent to 12.07 per cent of the meal, or 52.09 per cent of the protein content of the meal, as calculated from the nitrogen ($N \times 5.68$). Since previous examination had shown that the meal contained no albumin, these figures may be considered as representing the globulin content of the meal. The residue remaining from the above sodium chloride extractions was thoroughly extracted at room temperature with 0.5 per cent aqueous sodium hydroxide, and the dissolved glutelin isolated in the same manner as described in the case of the globulin. The yield of glutelin obtained was 11.55 gm., which represents 5.78 per cent of the meal, or 24.94 per cent of the total calculated protein.

Preparation of the Globulin.—For each preparation of the globulin from 200 to 400 gm. of meal were extracted with 5 per cent salt solution. Owing to the presence of rather coarse particles of hulls in the meal, the extracts were readily obtained perfectly clear by one filtration by suction through mats of filter paper pulp.

Preparations of the globulin were obtained by seven different methods. In all cases the precipitated proteins were finally washed and dried in the usual way.

1. Preparations X, XI, and XII were obtained in 3.65 per cent yield by directly dialyzing the salt extract against chilled running water for from 10 to 15 days. Two of these preparations consisted in part of octahedral crystals.

2. Dilution of the extract with 10 volumes of distilled water gave Preparation VIII, in 3.5 per cent yield.

3. Slight acidification of the extract with acetic acid, immediately followed by dilution with 10 volumes of water, resulted in somewhat greater yields of protein (5.75 per cent) than did dilution with water without acidification (Preparations IV, V, VI, and VII).

4. Preparations I, II, and III were obtained by making the extract 0.4 saturated with ammonium sulfate. The precipitate was removed by filtration, redissolved by addition of water, and the solution filtered in order to remove small quantities of undissolved material. The globulin was then precipitated from the clear solution by dialysis. The yield was 4.72 per cent.

5. By boiling the extract after first acidifying with acetic acid, Preparation IX was obtained in 8.27 per cent yield.

6. Dialysis of the extract against dilute alcohol yielded Preparation XIII in 5 per cent yield. This preparation was almost wholly composed of very small crystals. The extract was first dialyzed for 2 days against 35 per cent alcohol, and the dialysis continued for 10 days against 60 per cent alcohol. Every 2 days the old alcohol was replaced by fresh alcohol. Treatment of the precipitated protein with 5 per cent sodium chloride solution at 32°C. showed that most of it had become denatured during the dialysis against alcohol. The insoluble portion was composed largely of octahedral crystals. No further examination was made of the small fraction which dissolved in the salt solution.

7. The fact that we were unable to obtain preparations of the globulin which consisted *entirely* of well formed crystals suggested that the extracts contained a trace of foreign material derived from the hulls present in the meal. Cantaloupe seeds of the Rocky Ford variety were secured. The hulls were entirely removed by hand, the meats ground and extracted with ether. The meats represented 60.4 per cent of the total weight of the seeds, and 31.13 per cent of the whole seed consisted of oil. Preparation XIV was obtained from 50 gm. of the meal thus prepared by extraction with 800 cc. of 2 per cent sodium chloride solution. The salt solution was first warmed to 60°C. and the meal added with stirring. The mixture was again warmed for a short time to bring the temperature back to 60°C., and then immediately and rapidly filtered by suction. The protein began to separate in the filter flask almost at once. The filtrate together with the precipitate was warmed to 50°C., at which temperature nearly all of the precipitate redissolved. The warm solution was again filtered rapidly. The separated globulin was again redissolved by warming the mixture to 50°C. The warm solution was placed in a warm water bath and allowed to cool slowly at room temperature. The globulin, which separated on cooling and standing over night, constituted 28.21 per cent of the meal used, and was wholly composed of regular microscopic octahedra.

The average results of analyses of the various globulin preparations are given in Table VIII.

Preparation of the Glutelin.—The residues remaining after exhaustively extracting the cantaloupe seed meal with sodium chloride in order to remove all traces of globulin were extracted

TABLE VIII.
Average Results of Duplicate Analyses of the Cantaloupe Seed Globulin.*

	Preparation.													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII†	XIII†	XIV†
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
C.....	52.71	52.53	52.53	52.57	52.74	52.77	52.69	52.84	52.61	52.46	52.67	52.63	52.56	52.74
H.....	6.67	6.62	6.66	6.70	6.64	6.64	6.83	6.35	6.66	6.71	6.69	6.70	6.76	6.72
N.....	18.45	18.47	18.48	18.56	18.28	18.38	18.40	18.36	18.25	18.43	18.34	18.42	18.55	18.42
S.....	1.13	1.12	1.19	1.18	1.13	1.16	1.10	1.16	1.03	1.13	1.04	1.19	1.13	1.11
O.....	21.04	21.26	21.14	20.99	21.21	21.05	20.98	21.29	21.45	21.27	21.26	21.06	21.00	21.01
Moisture.....	7.95	7.19	7.67	8.41	6.47	7.80	5.81	6.85	5.24	8.04	7.48	6.49	6.71	10.30
Ash.....	0.46	0.26	0.51	5.05	4.27	3.89	4.94	3.40	6.99	3.36	0.66	4.25	9.77	0.03

* Calculated on an ash- and moisture-free basis.

† Crystallized.

for about 1 hour with 0.5 per cent aqueous sodium hydroxide. The clear alkaline solutions obtained after filtering the extraction mixture through paper pulp by suction were slightly acidulated with acetic acid. The resulting precipitate was washed with distilled water, redissolved by 0.2 per cent sodium hydroxide solution, and reprecipitated by acetic acid. This process was repeated several times, and the glutelin finally dried in the usual way. The average yield was 5.1 per cent of the meal used. Analyses of these preparations are given in Table IX.

Properties of the Globulin and Glutelin.—One of the most striking properties of the cantaloupe seed globulin is the ease with which it can be obtained in the form of well defined crystals by the

TABLE IX.

*Average Results of Duplicate Analyses of the Cantaloupe Seed Glutelin.**

	Preparation.						
	I	II	III	IV	V	VI	Average.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
C.....	55.23	55.06	55.26	55.26	55.27	55.09	55.20
H.....	6.99	7.08	7.08	7.03	6.97	6.96	7.02
N.....	16.13	16.17	16.37	16.37	16.61	16.01	16.28
S.....	0.87	0.94	0.89	0.92	0.90	0.88	0.90
O.....	20.78	20.75	20.40	20.42	20.25	21.06	20.60
Moisture.....	7.28	6.93	6.92	7.75	6.84	5.67	
Ash.....	0.43	0.91	0.91	0.97	0.08	0.72	

* Calculated on an ash- and moisture-free basis.

method described on page 90. The crystals consisted of well formed octahedra with $N_D^{20} =$ approximately 1.545. Photomicrographs of these crystals are shown in Figs. 1 and 2.

The globulin has a narrow range of precipitability with ammonium sulfate, ranging in 5 per cent sodium chloride solution from 0.2 to 0.3-of saturation.

In aqueous sodium chloride, slightly acidified with acetic acid, the globulin started to flocculate at 73°C. and was completely coagulated at 75°C.

The glutelin was obtained in granular and powdery form, the color ranging from pale, creamy buff to deep buff. It is extremely soluble in dilute alkalis and mineral acids.

Both proteins gave strong positive tests for tyrosine with Millon's reagent.

Analyses of the Proteins by the Van Slyke Method.—Duplicate samples of 3 gm. each of the globulin and glutelin were hydrolyzed by boiling for about 30 hours with 100 cc. of 20 per cent hydrochloric acid. The phosphotungstates of the bases were decomposed with a mixture of ether and amyl alcohol in the usual way. The results of the analyses are given in Tables II, III, V, and X.

TABLE X.

*Distribution of Nitrogen in the Cantaloupe Seed Glutelin as Determined by the Van Slyke Method.**

Sample I, ash- and moisture-free, 2.7651 gm. protein, 0.4527 gm. nitrogen.†
 " II, " " " 2.7651 " " 0.4527 " " " †

	I	II	I	II	Average.
	gm.	gm.	per cent	per cent	per cent
Amide N.....	0.0339	0.0341	7.49	7.53	7.51
Humin N adsorbed by lime.....	0.0109	0.0112	2.41	2.47	2.44
Humin N in ether-amyl alcohol extract.....	0.0004	0.0003	0.09	0.07	0.08
Cystine N.....	0.0035	0.0034	0.77	0.75	0.76
Arginine N.....	0.1103	0.1106	24.37	24.43	24.40
Histidine N.....	0.0212	0.0195	4.68	4.31	4.50
Lysine N.....	0.0239	0.0247	5.28	5.46	5.37
Amino N of filtrate.....	0.2422	0.2432	53.51	53.73	53.62
Non-amino N of filtrate.....	0.0066	0.0062	1.45	1.37	1.41
Total N regained.....	0.4529	0.4532	100.05	100.12	100.09

* Nitrogen figures corrected for the solubilities of the phosphotungstates of the bases.

† Nitrogen content of the protein, 16.37 per cent.

Determination of Tryptophane.—Determination of the tryptophane content of the globulin and glutelin was carried out for the most part according to the colorimetric method described by May and Rose (3). Samples, equivalent to 0.1 gm. of the ash- and moisture-free proteins, were incubated for 24 hours at 35°C. with 100 cc. of 20 per cent hydrochloric acid containing 1 cc. of a 5 per cent solution of *p*-dimethylaminobenzaldehyde in 10 per cent sulfuric acid. After standing for 40 hours at room temperature, the intensity of the color developed was matched in a Bock-

Benedict colorimeter against a standard solution prepared by similarly treating 0.1 gm. of zein and 10 cc. of a 1.5 per cent solution of pure tryptophane.

A standard solution containing only tryptophane developed with the aldehyde reagent a shade of color different from that obtained with the hydrolyzed proteins. Consequently, the colors could not be accurately matched. This difficulty was eliminated by the use of zein. Control experiments had shown that the zein used contained no trace of tryptophane detectable by this method.

It may be noted that May and Rose took the tryptophane content of casein as their standard, and calculated the tryptophane in the proteins they examined on the basis that casein contains 1.5 per cent of tryptophane.

The values obtained for the tryptophane content of the cantaloupe seed globulin and glutelin are given in Table III.

Determination of Cystine.—Cystine was determined in several preparations of the proteins of the cantaloupe seed according to the colorimetric method of Folin and Looney (2). Quantities of the protein equivalent to 1 gm. each of the ash- and moisture-free material were hydrolyzed by boiling for 12 hours with 25 cc. of 20 per cent sulfuric acid, and the cystine in the hydrolysates was determined by colorimetric comparisons with a standard containing 1 mg. of cystine in 100 cc. of solution. The values thus obtained are in close agreement with those found by the Van Slyke method (Table III).

Globulin of the Squash Seed.

For the purpose of comparing the properties and composition of the cantaloupe seed globulin with those of the crystalline globulin of the squash seed, several preparations of the latter were made. Seeds of Hubbard squash were hulled by hand, the meats ground, and the oil was removed by extraction with ether. The meal thus obtained contained 10.43 per cent of nitrogen. The crystallized globulin was prepared, with but few modifications, according to the method described by Osborne (1), and according to which the cantaloupe seed globulin was obtained. An average yield of 30 per cent of the meal used was obtained.

These preparations consisted wholly of well formed octahedra (Fig. 2) with $N_D^{20} =$ approximately 1.545. Optical and crystal-

lographic examination revealed no difference between these crystals and those similarly obtained from the seed of the cantaloupe.

Elementary analyses (Table I), determination of the nitrogen distribution and diamino-acids by the Van Slyke method (Tables III, IV, and V), and colorimetric determinations of tryptophane and cystine (Table III) were made in the manner already described in the case of the cantaloupe seed globulin.

Anaphylactogenic Properties of the Globulins from the Squash and Cantaloupe Seeds.

The globulin preparations used for the anaphylaxis tests were wholly composed of octahedral crystals. By intraperitoneal injections guinea pigs were sensitized with 1 mg. doses of the proteins, and after an interval of 3 weeks a second, or intoxicating, dose of 50 mg. was administered. The results obtained (Table VI) show that these globulins are identical as far as their immunological relation is concerned. The cantaloupe seed globulin was a little less soluble than the globulin of the squash seed in very weak alkalies, which probably accounts for the fact that slightly less severe reactions were obtained when the cantaloupe seed globulin was used for intoxicating than when the squash seed globulin was used. This difference in solubility is probably due to the smaller size of the crystals of the squash seed globulin (Fig. 2).

SUMMARY.

Two proteins from cantaloupe seed, a globulin and a glutelin, have been isolated and analyzed. From ether-extracted meal of the hulled seeds, 28.21 per cent of globulin was obtained by extraction with 2 per cent aqueous sodium chloride at 60°C. On cooling, the globulin separated from the saline solution wholly as octahedral crystals. Elementary analyses showed the globulin to have the following percentage composition: C 52.65, H 6.67, N 18.41, S 1.13. Analyses by the Van Slyke method and colorimetric determination of cystine and tryptophane gave the following results for the diamino-acids: arginine, 16.26; histidine, 4.22; lysine, 3.29; cystine, 1.27; tryptophane, 2.63 per cent.

A comparison of the chemical and physical properties of the cantaloupe seed globulin with those of the globulin of the squash

seed similarly prepared showed no point of difference between these two proteins. Anaphylaxis experiments on guinea pigs also showed that these two proteins could not be distinguished immunologically.

The cantaloupe seed glutelin was obtained in 5.78 per cent yield from an ether-extracted meal which contained the hulls (yield of globulin from the same meal was 12.07 per cent), by extraction with 0.5 per cent sodium hydroxide. Analyses of the glutelin gave the following results: C 55.20, H 7.02, N 16.28, S 0.90, arginine 12.42, histidine 2.72, lysine 4.59, cystine 1.09, tryptophane 3.03 per cent.

BIBLIOGRAPHY.

1. Osborne, T. B., *Am. Chem. J.*, 1892, xiv, 662.
2. Folin, O., and Looney, J. M., *J. Biol. Chem.*, 1922, li, 421.
3. May, C. E., and Rose, E. R., *J. Biol. Chem.*, 1922, liv, 213.
4. Wells, H. G., and Osborne, T. B., *J. Infect. Dis.*, 1913, xii, 343.