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Amino Acid Composition of Germinating Cotton Seeds

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ABSTRACT

Total and free amino acid composition of germinating cotton seeds (*Gossypium hirsutum* L.) was determined. The germinating seeds were separated into cotyledon and developing axis fractions daily and the composition of each tissue was summed to get the whole seed composition. By separating the developing seeds into these two tissue fractions, and determining total and free amino acids, a balance sheet was developed for each amino acid. This technique allowed changes in distribution with time of each amino acid to be followed in each tissue. Data for total content and amount in protein of each amino acid are presented. Asparagine increased in the whole seed, and most of this increase was found in the free pool of the developing axis. Other amino acids (*e.g.* arginine, glutamic acid) increased in the free pool but showed an over-all decrease, indicating that they were being metabolized. Amino acid contents of storage and nonstorage protein isolates were determined.

The free amino acid composition of developing and germinating cotton seeds and of young cotton roots has been determined (6, 11, 17). The reports show that asparagine is very prominent in the developing embryo as well as in the germinating seedling. Aspartic acid, however, is not nearly as prevalent in the protein as the prevalence of the amide might suggest. In various species others have suggested that asparagine is the predominant transport form of reduced N and that metabolic transformations between asparagine and other forms commonly occur at source and sink sites (2, 14). We determined the amino acid composition of germinating cotton seeds and of storage and nonstorage protein isolates from them. By separating the developing axis from cotyledons, we were able to follow the course of amino acid distribution from the storage organs into the newly synthesized axial regions.

MATERIALS AND METHODS

Plant Material. Uniformity of germination was improved by selection of acid-delinted cotton (*Gossypium hirsutum* L., cv. Deltapine 16) seeds according to density and weight. Seeds that floated in 5% (w/v) sucrose or sank in 25% (w/v) sucrose were discarded. The remaining seeds were rinsed free of sucrose solution and air-dried at room temperature for 48 hr or longer. The dry seeds were weighed individually, and those weighing between 95 and 100 mg were selected for study.

Germination. Seeds were germinated in the dark at 31 C between two sheets (30 × 45 cm) of wet germination paper (Anchor Paper Co., St. Paul, Minn.[2] Twenty seeds were situated in line 2.5 cm from the longer edge of the paper and each pair of sheets was fastened to a sheet (30 × 46 cm) of acrylic plastic. Eight such assemblies were placed vertically with spacers between them in a pan of water so that the seeds were in line along the top with their micropylar ends pointing downward. This arrangement resulted in relatively straight roots, facilitating their measurement.

After 1, 2, 3, 4 or 5 days germination, the seed coats were removed and discarded, and the cotyledons were separated from the embryonic axis. Radicle lengths were measured, cotyledons and axes were weighed in separate lots and then frozen and lyophilized. Dry weights were obtained, and the material was ground in a knife mill for further analyses.

Nitrogen and Amino Acid Analyses. Nitrogen content of the lyophilized material was determined with a Coleman model 29 N analyzer. Amino acid analyses of hydrolysates were determined as described by Elmore and Leffler (11), with a Beckman model 121 automatic amino acid analyzer. The free amino acids were extracted with acid alcohol (7), deproteinized with picric acid (1), and analyzed on the amino acid analyzer with a two-column procedure using lithium citrate buffers to separate the acidic and neutral amino acids (5), and sodium citrate buffers to separate the basic amino acids (1). The lithium column worked well for standard solutions but biological samples presented some difficulty, because glutamic acid and glutamine appear as consecutive peaks in this system. When glutamic acid was present in much greater amounts than glutamine, the glutamine peak was smothered and appeared as a shoulder on the glutamic acid peak. That happened in this experiment so the results are presented as glutamic acid plus glutamine.

Storage and Nonstorage Proteins. Storage and nonstorage protein isolates were extracted from Deltapine 16 cotton seeds as described by King and Lamkin (13). The amino acid composition of hydrolysates of the separated isolates was then determined as previously described.

RESULTS

Weight and Size. Changes in weight and size of the developing seedlings are summarized in Table I. The axes increased 20-fold in fresh weight and length, and more than 4-fold in dry weight over the 4 days of measurement; during the same period the cotyledons exhibited only a modest increase in fresh weight, and lost dry weight. The gain in dry weight by the axes was matched by the dry weight loss of the cotyledons resulting in constant weight of the whole seedlings over the 4-day period.

Nitrogen. The changes in dry weight were paralleled by changes in distribution of N (Fig. 1). The axis increased in N content and the cotyledons declined by a compensating amount, leaving the whole seed with no significant net change in N content. Figure 1 shows the N content of the developing seeds separated into free and total amino N components for each tissue. The free pool represented a relatively constant small portion of the total N of the cotyledons, but an increasingly larger amount of the axis N. On a whole seed basis, then, there is a significant increase in free

[1] Mississippi Agriculture and Forestry Experiment Station cooperating.
[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

Table I. Weight and size changes of developing seedlings.[1]

Day	Fresh Weight (mg)		Dry Weight (mg)		Length (mm)
	Cotyledons	Axis	Cotyledons	Axis	Axis
1	99.2±2.4	6.5±0.6	55.7±0.7	3.5±0.2	5.0±0.9
2	105.0±1.2	23.6±8.7	54.8±0.8	4.4±0.6	14.4±1.4
3	112.8±2.4	65.9±7.3	51.7±0.9	7.0±0.4	34.1±0.7
4	119.8±2.1	92.6±2.7	49.9±0.3	10.3±0.8	54.5±1.8
5	137.7±6.3	130.0±7.6	45.3±0.4	14.8±0.3	100.4±5.7

[1] Means ± SD of 160 individuals except for length measurements, where means ± SD of 80 individuals are given. Conditions of germination are given in Materials and Methods.

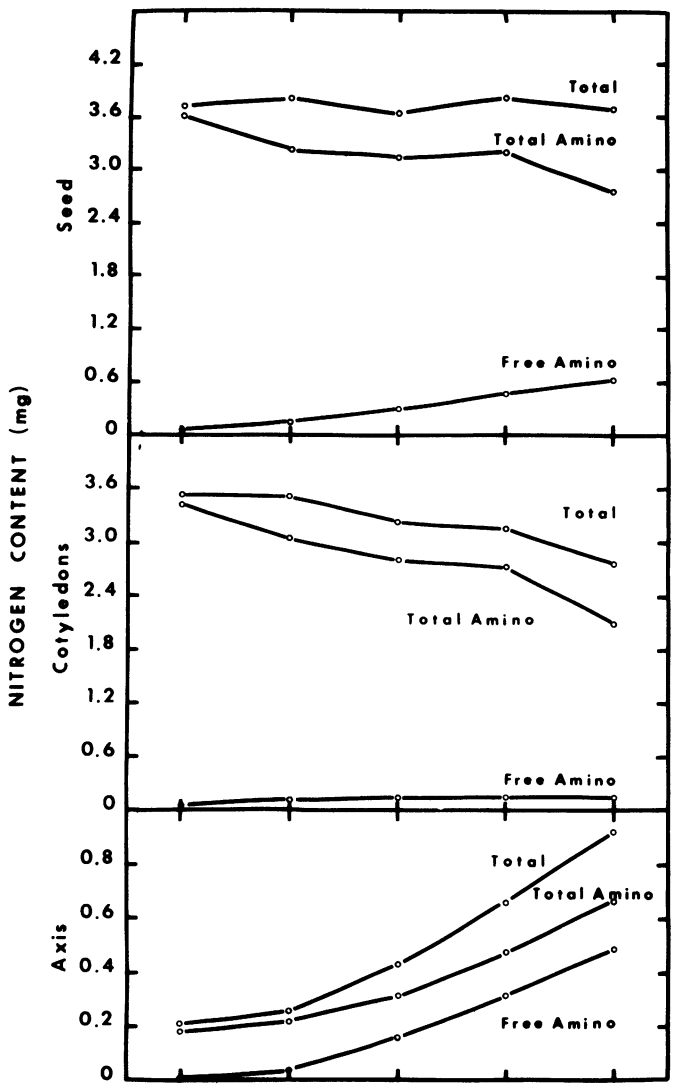

FIG. 1. N content of germinating cotton seeds separated into free and total amino plus amide N fractions for the whole seed (A), cotyledons (B), and developing axis (C). Total N content was determined by N analysis for each seed fraction and summed to get whole seed values. Total amino N was determined from the data in Table III as N recovery from amino acid analysis. Free amino N was obtained from Table II by summing the N in each amino acid in the free pool. Total amino N includes the free amino N.

amino acids which account for almost 17% of the total seed N. Total amino plus amide N, as determined from amino acid analyses which include free amino acids, accounted for 97% of the total seed N on day 1. Subsequently, the amount declined to about 75% by day 5. Combining the increases in free amino acids with the decreases in total amino plus amide N revealed that the seed protein content decreased significantly (3.5 mg of protein N/seed on day 1 to 2.2 mg of protein N/seed on day 5).

Amino Acids. Table II shows the distribution of free amino acids in the developing cotton seeds separated into axis and cotyledons. This table reemphasizes the data in Figure 1. In the cotyledons the free pool size was relatively constant after 3 days, and the major amino acids present at that time were glutamic acid plus glutamine, asparagine, aspartic acid, and serine. Along with arginine and threonine these amino acids represented the bulk of the free pool. The axis, however, had a larger free pool. One amino acid, asparagine, was very prominent, accounting for over half of the N in the axis free pool on the 5th day.

Table III gives the hydrolysate amino acid data. The axis differed from cotyledons with respect to relative amounts of each amino acid. This is not surprising since cotyledons contain reserve storage protein and the axis consists of newly developing and growing tissues importing free amino acids from the cotyledons.

We determined the content of each amino acid on a per seed basis for each date and we show the changes by the 5th day (glutamic and aspartic acids data include their respective amides) in Table IV. On a whole seed basis there was a net synthesis of aspartic acid. The cotyledons showed a net loss and the axis showed the most pronounced effect, with large increases. As was seen before (Table II) the asparagine content of the free pool was high in both cotyledons and axis, which accounts for much of the increase.

The pattern of change for asparagine and aspartic acid was markedly dissimilar to that of the other amino acids, due largely to the extraordinary increase of asparagine in the axis. Glutamic acid plus glutamine and arginine displayed a pattern that is more typical of the majority of amino acids in the germinating seed. Both acids declined on a whole seed basis and in the cotyledons but they increased in the axis, albeit arginine only slightly. The free pool content of each increased during germination, most notably in the axis.

The amino acid content of cotton seed protein was determined as the difference between total amino acid and free amino acid contents for each seed fraction. As shown in Table V, the protein amino acids decreased without exception in the whole seed and in the cotyledons. The axis showed a net increase for all but two, arginine and aspartic acid, again an indication of the dynamic metabolic role those two amino acids seem to play in germinating cotton seeds.

Storage and Nonstorage Protein. Storage and nonstorage protein isolates of cotton seeds were shown to have different amino acid compositions (Table VI). Yet the same three amino acids (glutamic acid, arginine, and aspartic acid) were predominant in each. The major differences between the two isolates were in their lysine, phenylalanine, and arginine content. Even though arginine was the second most abundant amino acid in each isolate, the storage protein isolate contained nearly a third more than the nonstorage isolate.

DISCUSSION

A procedure for the germination of cotton seeds was developed to decrease the wide variations in germination encountered among ungraded seeds when the common filter paper roll method was used. Selection of the seeds by density and weight resulted in a set

Table II. Amino acid composition of the free pool of amino acids in developing cotton seed tissues. Conditions of extraction and analyses are given in Materials and Methods.

	μmoles/g dry wt											
	Cotyledons						Axis					
	Days						Days					
	1	2	3	4	5	SE[1]	1	2	3	4	5	SE[1]
Asp	8.4	13.7	14.0	29.0	35.7	2.64**	11.3	44.4	104.9	101.0	189.5	ns
Thr	0.7	3.8	6.7	6.0	6.2	0.34**	1.0	13.8	19.7	26.8	27.7	0.63**
Ser	1.5	7.3	8.2	10.3	9.1	0.48**	1.6	25.5	48.4	73.2	71.4	2.33**
Asn	4.9	16.6	19.7	8.8	11.4	ns	4.7	95.4	442.6	702.4	723.7	30.06**
Glu+Gln	5.7	32.2	29.0	39.4	31.8	0.77**	7.4	49.5	71.2	65.9	66.1	9.02**
Pro	Tr	4.1	4.8	6.4	4.3	ns	Tr	5.6	6.7	7.7	2.3	0.39**
Gly	0.4	0.8	0.9	1.8	2.4	0.23**	0.6	5.1	19.8	33.0	34.2	1.33**
Ala	0.8	2.3	2.1	3.0	4.7	0.59**	1.6	16.6	55.6	88.1	63.0	4.13**
Cys	Tr	Tr	1.2	2.0	1.8	ns	Tr	4.6	1.4	0.7	Tr	ns
Met	0.4	1.0	1.6	2.8	1.6	ns	0.7	5.0	4.3	5.4	2.1	1.38**
Ile	0.2	1.0	1.7	2.0	1.5	0.16**	0.6	6.4	7.8	16.1	14.5	1.18**
Leu	0.3	1.5	2.3	3.8	2.9	0.16**	0.5	9.7	7.7	12.5	8.8	0.89**
Tyr	0.2	1.4	1.6	2.3	2.4	0.11**	0.4	5.2	4.4	3.5	1.8	0.16**
Phe	0.4	1.6	2.2	3.8	2.0	0.20**	0.4	9.5	6.2	9.1	4.6	0.22**
GABA	0.6	0.5	0.4	0.9	1.8	0.15**	0.6	4.2	4.3	6.8	4.8	0.17**
Etn	0.6	0.3	3.2	4.9	5.9	0.29**	0.4	6.5	5.1	5.2	5.4	0.54**
Lys	0.6	1.6	2.0	3.2	3.0	0.19**	0.5	5.1	3.1	9.3	15.0	0.26**
His	0.3	2.2	4.2	7.0	10.7	0.43**	0.8	7.4	13.5	18.3	22.6	0.72**
Arg	5.9	6.2	7.7	11.9	10.0	1.03**	14.5	31.8	23.2	19.3	17.5	0.73**
NH_3	3.6	13.9	34.5	(2)	(2)	4.20*	2.5	37.0	156.1	175.9	237.1	36.80*
mmole N	0.06	0.15	0.20	0.21	0.22		0.10	0.60	1.54	2.19	2.35	

[1] SE for the amino acid is presented whenever the analysis of variance indicates a significant change during development within the tissue. *, ** and ns indicate significance at the 0.05 or 0.01 level of probability or no significance, respectively.

[2] Not determined

[3] Abbreviations: GABA: γ-amino butyric acid; ETN: ethanolamine.

Table III. Amino acid percentage composition of hydrolysates of germinating cotton seed tissue.[1] Conditions of hydrolysis and analysis are given in Materials and Methods.

	Cotyledons					Axis					
	Days					Days					
	1	2	3	4	5	1	2	3	4	5	SE
Lys	5.02	5.44	5.79	5.93	6.54	5.42	5.18	4.33	3.57	3.48	0.03
His	3.14	3.27	3.32	3.34	3.56	2.86	2.67	2.36	2.20	2.44	0.02
NH_3	2.26	2.49	2.36	2.36	2.40	2.01	2.33	4.00	4.83	5.23	0.03
Arg	13.28	13.69	13.02	12.38	10.81	10.70	8.99	5.30	3.62	3.17	0.09
Asp	9.91	9.72	10.08	10.74	10.97	10.03	13.48	26.82	35.70	40.95	0.25
Thr	2.87	2.52	2.66	2.74	3.07	3.50	3.60	3.13	3.10	2.97	0.03
Ser	4.07	3.73	3.84	3.96	4.09	4.24	4.48	4.32	4.51	4.34	0.03
Glu	20.70	20.75	20.07	19.11	18.02	21.25	19.87	16.64	14.18	11.50	0.13
Pro	2.28	4.41	4.14	4.89	4.03	3.94	3.76	2.73	2.09	1.94	0.12
Gly	3.99	3.67	3.63	3.65	3.76	4.42	4.10	3.49	3.20	2.92	0.02
Ala	4.35	3.92	3.34	3.76	4.09	4.90	4.81	5.29	5.16	4.33	0.04
Cys	1.26	1.24	1.09	1.27	0.92	1.34	1.20	0.87	0.55	0.49	0.04
Val	4.88	4.70	4.77	4.91	5.18	5.17	5.20	4.26	3.93	3.71	0.02
Met	1.43	1.42	1.48	1.57	1.57	1.62	1.56	1.30	1.04	1.01	0.02
Ile	3.28	3.26	3.46	3.52	3.80	3.42	3.48	2.96	2.80	2.70	0.04
Leu	6.25	6.22	6.62	6.60	7.16	6.50	6.47	5.24	4.07	3.77	0.04
Tyr	3.35	3.10	3.63	3.45	3.66	3.35	3.62	3.19	2.55	2.44	0.06
Phe	6.24	6.46	6.73	6.41	6.36	5.36	5.19	3.76	2.88	2.63	0.04
gAN[2]	0.97	0.85	0.88	0.87	0.76	0.86	0.86	0.74	0.74	0.74	

[1] A percent computation based upon the amount of amino acids recovered from the column such that the total equaled 100%.

[2] g amino N recovered per g N added to column.

of seeds with increased uniformity and vigor. Classification by density selected for seed kernels that uniformly filled the hard seed coat of cotton (3, 18), and may have had other effects as well. The other effects may include high protein content with a higher specific gravity than oil. High seed protein content has been shown to affect seed germination and seedling performance beneficially (16). At any rate this grading, classification, and germination procedure gave superior seedlings and in our hands seemed to be quite satisfactory.

The changes in N distribution during the course of germination follow a quite predictable pattern, with the gain by the axis offsetting the loss by the cotyledons. There is, however, a selectivity about the N exchange that reflects the degradation of cotyledonary storage proteins and concomitant synthesis of metabolic proteins in the axis. It is reasonable to presume that axis proteins represent a major portion of the nonstorage protein isolate of mature seeds. As germination proceeds, the amino acid composition of the axis becomes less like that of the nonstorage isolate (*cf.* Tables III and VI). These changes in composition reflect the transition from maintenance metabolism to the activities associated with rapid synthesis, enlargement, and differentiation. The amino acid composition of the axis during the period of this experiment is therefore dynamic, reflecting at any one time the nature and requirements of the ongoing metabolism.

The decrease in N accounted for by amino and amide N suggests that a considerable amount of N was used for synthesis of other N-containing metabolic products including nucleic acids as well as amino sugars, alkaloids, etc. Since these seeds were germinated without an exogenous source of N, the seed reserve protein was the only source of N. The distribution and synthesis of these other N-containing compounds were not the subject of this study, but it is apparent that considerable N is needed for these metabolic products. They should not be ignored in a balance sheet approach to the study of the fate of seed proteins (amino

plus amide N) during germination.

Arginine is present in high amounts in the cotyledon tissue, primarily in the storage protein. A major cotton seed storage globulin has been reported to contain 13.2% arginine (12), and we show 12.5% in the storage isolate. This relative level of arginine is comparable to that found in storage proteins of such disparate plants as squash (8), hemp (10), coconut (10), and peanut (9).

Arginine probably represents a special case deserving further study in cotton seeds. As shown by the data of Elmore and Leffler (11) and emphasized by Capdevila and Dure (6), arginine is the most prevalent free amino acid in mature seeds. The reason for this high concentration of free arginine is not clear (6), but free arginine would be a rich source of readily available reduced N for the early stages of germination before appreciable protein hydrolysis occurs. With a C/N ratio of 6:4, arginine is the most conservative of carbon of all of the common amino acids, an important consideration for N storage efficiency.

During germination of cotton seeds both percentage composition and total content of arginine decrease (Tables III and IV), suggesting that arginine is being used preferentially to some other amino acids. This decrease is logical for an amino acid that is as well suited as arginine for storage of N. The slight increase of arginine in the free pools during the course of germination may indicate that degradation of storage protein proceeds at a faster rate than synthesis of new protein, or that most of the arginine from storage protein is not incorporated as such into new protein. It could also indicate that arginine is somewhat mobile itself.

Inasmuch as their storage protein is rich in glutamic acid, cotton seeds are similar to the seeds of many unrelated plant species (4). It is the predominant amino acid in both storage and nonstorage isolates of the mature seeds, comprising about 25% of both fractions (Table VI). Glutamic acid and its amide behave similarly to arginine during cotton seed germination. In most seeds, glutamine is the principal metabolic form of reduced N (4); it is reasonable to suspect that glutamine accounts for most of the increase of the glutamic acid-glutamine complex in the free pool of both cotyledons and axis (Table II).

The data on aspartic acid and asparagine (Tables II, III, and IV) imply that these acids, particularly asparagine, play an important transport role in the germination process. Available evidence indicates that asparagine is the chief N transport compound in most plants (14), and the dramatic increase of asparagine in the axis free pool of germinating cotton seeds is supportive of that concept. Ting and Zschoche (17) and Radin (15) have shown that asparagine is the predominant amino acid in cotton roots and that it is present in xylem exudate; corroborating evidence comes from the studies of Capdevila and Dure (6).

This study complements and extends the work of Elmore and Leffler (11), showing that asparagine is apparently a key to cotton seed metabolism both during development and germination, as Atkins *et al.* (2) reported for lupine. We also show implicitly that storage proteins were preferentially consumed during cotton seed germination. Future work should deal with phloem mobile forms of reduced N in cotton and with the physiology of the storage proteins in cotton seeds.

Table IV. Changes in total amino plus amide nitrogen content of cotton seed during germination.[1]

Amino Acid	Total Content on Day 1			Change by Day 5		
	Axis	Cotyledon	Whole Seed	Axis	Cotyledons	Whole Seed
	μmoles·seed^{-1}					
Lys	0.45	6.95	7.40	0.59	-1.01	-0.42
His	0.22	4.10	4.32	0.47	-1.06	-0.59
Arg	0.74	15.41	16.15	0.04	-7.19	-7.15
Asp	0.91	15.09	16.00	12.51	-4.15	8.36
Thr	0.35	4.90	5.25	0.74	-1.49	-0.75
Ser	0.48	7.88	8.36	1.32	-2.70	-1.38
Glu	1.74	28.45	30.19	1.68	-12.19	-10.51
Pro	0.41	6.54	6.95	0.32	-1.87	-1.55
Gly	0.71	10.82	11.53	0.98	-4.15	-3.17
Ala	0.66	9.89	10.55	1.47	-3.77	-2.30
Cys	0.13	2.14	2.27	0.05	-1.13	-1.08
Val	0.53	8.46	8.99	0.85	-2.59	-1.74
Met	0.13	1.96	2.09	0.17	-0.55	-0.38
Ile	0.31	5.08	5.39	0.59	-1.24	-0.65
Leu	0.60	9.71	10.31	0.65	-2.47	-1.82
Tyr	0.22	3.74	3.96	0.37	-1.06	-0.69
Phe	0.39	7.66	8.05	0.31	-2.55	-2.24
NH_3	1.42	26.81	28.23	11.97	-8.12	3.85

[1] Negative signs indicate a net decrease. Total content on Day 1 was determined from the actual recovered amino acid contents as reported in Table III. The change by Day 5 was obtained similarly and is shown as the net increase or decrease for each amino acid.

Table V. The change in content of amino acids in cotton seed protein during germination.[1]

Amino Acid	Total Content on Day 1			Change by Day 5		
	Axis	Cotyledons	Whole Seed	Axis	Cotyledons	Whole Seed
	μmoles·seed^{-1}					
Lys	0.45	6.92	7.37	0.37	-1.12	-0.75
His	0.22	4.08	4.30	0.14	-1.52	-1.38
Arg	0.69	15.08	15.77	-0.16	-7.31	-7.47
Asp	0.86	12.95	13.81	-0.86	-4.15	-5.01
Thr	0.35	4.62	4.97	0.33	-1.49	-1.16
Ser	0.47	7.47	7.94	0.27	-2.70	-2.43
Glu	1.71	27.01	28.72	0.73	-12.19	-11.46
Pro	0.41	6.35	6.76	0.29	-1.87	-1.58
Gly	0.71	10.71	11.42	0.47	-4.15	-3.68
Ala	0.65	9.68	10.33	0.55	-3.77	-3.22
Cys	0.13	2.06	2.19	0.05	-1.13	-1.08
Val	0.53	8.46	8.99	0.85	-2.59	-1.74
Met	0.13	1.89	2.02	0.14	-0.55	-0.41
Ile	0.31	5.01	5.32	0.38	-1.24	-0.86
Leu	0.60	9.58	10.18	0.52	-2.47	-1.95
Tyr	0.22	3.63	3.85	0.34	-1.06	-0.72
Phe	0.39	7.57	7.96	0.24	-2.55	-2.31

[1] Content of amino acids in protein calculated by difference between total amino acids and free amino acids for each fraction.

Table VI. Percentage amino acid composition of protein isolates.

Amino Acid	Storage	Non-storage
	%	%
Lys	2.81	7.27
His	2.81	2.33
NH_3	6.57	1.92
Arg	12.52	9.69
Asp	9.82	9.31
Thr	2.79	3.79
Ser	5.33	4.39
Glu	23.95	25.72
Pro	3.97	4.34
Gly	3.92	4.22
Ala	3.66	4.82
Cys	Trace	1.50
Val	3.72	3.59
Met	1.00	2.01
Ile	2.25	2.60
Leu	5.60	5.75
Tyr	2.81	3.55
Phe	6.46	3.18

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