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RELATIONSHIPS BETWEEN NITROGEN, AMINO ACIDS AND STORAGE PROTEINS IN *LUPINUS ALBUS* SEEDS

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Key Word Index—*Lupinus albus*; Leguminosae; seeds; nitrogen; amino acid composition; storage proteins; accumulation.

Abstract—*Lupinus albus* seeds (20 samples from 10 different lines or cultivars) with protein contents distributed from 23.8 to 48.4% were analysed for their amino acid composition with great accuracy (from 6 hydrolysates per sample). Amino acid levels in seeds increased as linear functions of nitrogen content with correlation coefficients close to unit in all the lupin genotypes and phenotypes. Hence the composition of any lupin seed sample can be predicted from its nitrogen content. Amino acid in total seed protein varied as hyperbolic functions of nitrogen content. The same was true for nitrogen-to-protein conversion factors. By controst, the composition of storage proteins accumulated in seeds remained constant and independent of seed protein content.

INTRODUCTION

The genus *Lupinus* comprises *ca* 100 different species [1], several of which are cultivated for their seeds [2, 3]. The species *L. mutabilis* is of particular importance in the U.S.A., and *L. angustifolius* is most studied in Oceania [4–8]. In Europe, *L. luteus* and especially *L.* albus have recently been selected and cultivated. From the literature there appear to be no important differences among the amino acid compositions of these four species. The FAO [9] lists only one composition without specifying whether one or several species are involved. For *L. albus*, each of the previously published studies of composition [8, 10–14] treats one sample as the object of a single analysis. None of them is complete; either one or several amino acids were not determined or the nitrogen content, N (g per 100 g seed dry wt) in the sample studied was not indicated, which excludes all possibility of comparison with other analyses. The absence of results for N shows to what extent it is still not widely known that the amino acid composition of the seeds of a given species varies as a function of N. For example the protein of maize seeds with $N = 1$ compared with that of seeds three times richer in protein ($N = 3$) contains 50% more Gly and Trp and 60% more Lys [15]. Similarly, when N is doubled from three to six in pea seed, the His content in total seed protein is unchanged, but Arg increases by 53% while Tyr decreases by 20 and Cys by 33% [16]. The study of such variation in amino acid composition has shown, in several species of cereal and legumes [15–18], the existence of linear relationships between N and the levels of each amino acid in seed. These relationships appear to be independent of genotype as well as of culture conditions and environmental factors. Their linearity seems to be general to all species. A similar investigation was thus performed on the white lupin. This was made possible by the availability of seed samples covering a vast N range as well as by amino acid analyses performed under experimental conditions exceeding recent recommendations [19] and of the greatest possible accuracy.

RESULTS AND DISCUSSION

Nitrogen content and amino acid composition in lupin seed

The nitrogen content N of the seeds analysed varied between 3.78 and 7.75% (Table 1). The amino acid composition can be expressed in a number of different ways. Several of them have been used in the present study: in g (A_i), in number of moles of residues (B_i), in g of nitrogen (D_i) or in g of residues (E_i), of the ith amino acid, related in each case to 100 g of seed dry weight. Other expressions, much more prevalent in nutritional comparisons, correspond to the amino acid content of total seed protein, either in g amino acid per 16 g of total seed nitrogen (C_i) or in mg amino acid per g of seed nitrogen (G_i). The experimental data first obtained in the present work were the A_i values from which the others can be calculated, particularly the C_i values given in Table 1.

It is revealing to plot A_i against N. As an example, Fig. 1 shows the distribution of experimental points for Asx, Leu and Pro from the different lines or cultivars studied (circled points). For each amino acid, the representative points from a given cultivar are on a straight line that coincides with those from the other cultivars or lines. The relationships between A_i and N are thus described by linear equations such as: $A_i = a_i N + b_i$ in which a_i is the slope of the line relative to the ith amino acid and b_i its intercept with A_i axis. The coefficients a_i and b_i for regression lines (drawn in Fig. 1 for Asx, Leu and Pro) and the correlation coefficients r_i for each of the 18 amino acids analysed and for the ammonium from the amide groups are indicated in Table 2. The correlation coefficients equal 0.987 ± 0.007 for amide nitrogen and for all amino acids except four: Thr (0.942), Trp (0.9), Met (0.35) and Cys (0.6) which are precisely those for which exper-

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Table 1. Amino acid composition of lupin seeds (C_i in g/16 g nitrogen)

Variety*	Kie	Lub	Luc	Kal	Kal	Kal	Kal	TR2	C-8	Luc
N†	3.8	3.9	4.35	4.4	4.45	4.5	4.95	5.65	5.7	5.85
Gly	4.5	4.6	4.4	4.4	4.5	4.25	4.15	4.0	3.95	4.0
Ala	4.15	4.15	3.9	3.85	3.95	3.75	3.6	3.25	3.25	3.45
Val	4.95	5.05	4.85	4.65	4.95	4.65	4.5	4.3	4.15	4.45
Leu	7.8	7.8	7.9	7.65	7.85	7.5	7.45	7.2	7.4	7.3
Ile	4.9	4.85	4.85	4.65	4.8	4.55	4.6	4.55	4.65	4.7
Ser	5.45	5.35	5.45	5.7	5.35	5.7	5.65	5.45	5.15	5.25
Thr	4.35	4.45	4.15	4.5	4.5	4.35	4.25	3.65	3.55	3.75
Tyr	4.25	4.55	4.6	4.65	4.5	4.5	4.7	4.35	4.55	4.6
Phe	4.15	4.1	4.15	4.15	4.0	4.05	4.05	3.8	3.85	3.9
Trp	0.9	0.95	0.8	0.8	0.9	0.75	0.75	0.75	0.65	0.7
Pro	4.3	4.3	4.35	4.05	4.35	3.95	4.0	4.45	4.35	4.35
Met	1.0	1.1	1.05	1.15	1.1	1.05	1.0	0.75	0.8	0.75
1/2 Cys	2.15	2.15	2.25	1.9	2.15	1.85	1.65	1.8	2.0	1.7
Lys	5.6	5.65	5.45	5.3	5.6	5.3	5.2	4.7	4.9	4.9
His	2.45	2.5	2.45	2.4	2.5	2.4	2.25	2.2	2.25	2.2
Arg	7.85	7.75	8.7	8.55	8.4	8.2	8.85	9.4	10.3	10.3
Asx‡	10.4	10.1	10.7	10.9	10.5	10.5	10.5	10.3	10.6	10.8
Glx‡	18.8	18.8	20.0	20.2	19.3	19.8	20.2	21.4	21.1	20.6
Recovery %	93.5	93.7	96.3	95.4	95.2	93.0	93.9	93.7	95.9	95.9

Variety*	C-9	Kal	Kal	C-32	Lub	C-13	C-14	C-14	C-14	C-14
N†	5.9	6.2	6.3	6.45	6.45	7.05	7.3	7.4	7.6	7.75
Gly	4.0	3.9	3.9	3.95	3.85	3.95	3.6	3.75	3.85	3.85
Ala	3.45	3.25	3.3	3.3	3.3	3.3	3.1	3.2	3.25	3.2
Val	4.2	4.2	4.3	4.2	4.2	4.3	3.95	4.15	4.3	4.15
Leu	7.5	7.3	7.05	7.05	7.05	7.35	7.3	6.95	7.0	7.05
Ile	4.65	4.4	4.5	4.6	4.5	4.75	4.5	4.7	4.7	4.65
Ser	5.35	5.3	5.5	5.15	5.35	5.35	5.45	5.2	5.25	5.25
Thr	3.6	3.85	3.95	3.6	3.5	3.6	3.2	3.4	3.55	3.5
Tyr	4.65	4.6	4.85	4.95	4.25	4.6	4.6	4.95	4.9	4.75
Phe	3.85	3.9	3.9	3.9	3.7	3.9	3.6	3.9	3.95	3.85
Trp	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.65	0.65
Pro	4.3	4.35	4.1	4.25	4.1	4.25	4.0	4.2	4.3	4.2
Met	0.85	0.75	0.9	0.75	0.75	0.7	0.6	0.6	0.7	0.65
1/2 Cys	1.9	1.5	1.45	1.5	1.6	1.7	1.9	1.25	1.3	1.35
Lys	4.8	4.85	4.8	4.95	4.8	4.8	4.35	4.65	4.8	4.75
His	2.25	2.2	2.2	2.15	2.1	2.25	2.05	1.95	2.1	2.05
Arg	10.3	10.7	10.9	11.0	10.8	11.5	12.2	12.3	12.5	12.1
Asx‡	10.4	10.4	11.0	10.6	10.4	10.4	10.4	11.1	11.0	10.8
Glx‡	20.9	21.4	21.4	20.3	20.3	20.9	22.6	20.8	20.5	20.6
Recovery %	96.1	96.0	96.9	96.0	94.0	98.1	98.8	98.2	99.5	97.6

*Kiev = Kievski; Lub = Lublanc; Luc = Lucky; Kal = Kali.
†N: seed nitrogen content (g/100 g dry matter).
‡Asx and Glx were calculated as Asp and Glu.

imental determinations are the most difficult. These results show that for all the samples studied, any modification of N, whether entirely phenotypic and thus due to the environment or cultivation conditions, or genotypic and thus attributable to varietal factors, always entails (or always results from) the same variation in amino acid composition. Since this kind of result has already been discussed for some other species [15–18], it seems unnecessary to review it here: it is plausible that these results are general and that they also apply for N greater than those investigated. Only biochemical mutants not yet found for the lupin which might introduce significant genotypic variation could be the source of modifications in the values of the coefficients a_i and b_i in Table 2. As far as phenotypic variations are concerned, only severe sulphur deficiencies, already studied in lupin [5, 20], could be the basis of a break in the correlations between A_i and N, as was shown in the case of maize [21]. These linear relationships thus permit the prediction of the amino acid composition of any white lupin simply from the measurement of the nitrogen content of the seed sample.

Total seed protein composition

The level G_i of each amino acid i in total seed protein is related to A_i by $G_i = 10^3 A_i/N = 10^3 a_i + 10^3 b_i/N$.

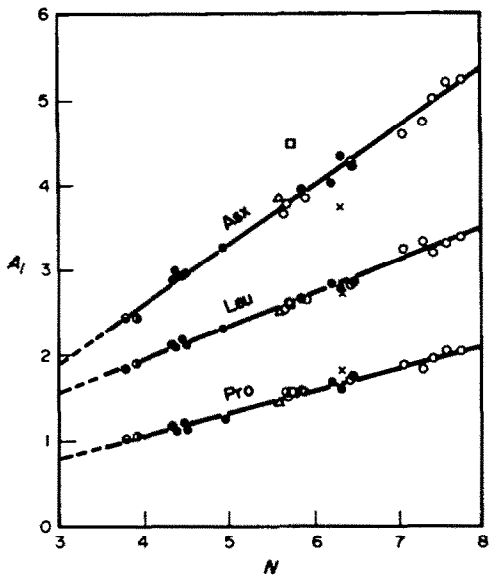

Fig. 1. Variation of amino acid content A_i in seed (g amino acid/100 g seed dry matter) as a function of total seed nitrogen percentage N (on a dry wt basis) for Asx, Leu and Pro. Solid or half solid circles correspond to cultivars: ●——● Kali; ◒——◒ Kievski; ◑——◑ Lublanc; ◐——◐ Lucky. Open circles: O—— O Lines; the three others correspond to data from literature: Δ—— Δ FAO (1970) [9]; □——□ Oomah and Bushuk (1983) [14]; X Ballester *et al.* (1980) [13].

Therefore, G_i varies with N according to a second degree equation represented by a segment of equilateral hyperbola having as asymptotes the G_i axis and the line $G_i = 10^3 a_i$. Such segments are represented in Fig. 2 for Pro, Lys, Ala, and Arg, along with the points corresponding to the experimental values. These four amino acids illustrate the three possible cases of variation of G_i. The importance of the variations of G_i depends on the ratio b_i/a_i, while the orientation of the concavity of the hyperbola toward the positive or negative ordinates depends on the sign of this ratio, *i.e.* on that of b_i (a_i always being positive).

Table 2 shows that $b_{Pro} \sim 0$. Hence, the Pro level in lupin proteins: $G_{Pro} = 10^3 a_{Pro} = 263$ (mg amino acid per g seed nitrogen) is constant and does not depend on N: the segment of the hyperbola is reduced to a horizontal line (Fig. 2). Ile and Asx are also characterized by ratios b_i/a_i low enough that their levels in protein are considered as constant.

For Lys and Ala, b_i/a_i ratios are positive (respectively, 2.1 and 3.2 g nitrogen per 100 g seed dry wt): the concavity of the corresponding segments of hyperbola is turned towards the positive ordinates (Fig. 2). Hence the content G_{Lys} in total protein decreases significantly, as does G_{Ala} when N increases. Table 2 shows that most amino acids other than those for which b_i is zero or negligible, correspond to this case. The relative decreases of G_i are similar or even more significant for Gly, Val, Thr, Trp, Met, Cys and His. They are less important for Leu, Ser and Phe.

In contrast, Arg is characterized by a negative and highly significant ratio $b_{Arg}/a_{Arg} = -1.71$: its content G_{Arg} in protein shows a marked increase and is almost doubled (Fig. 2) in the range of N covered by the samples

Table 2. Slope (a_i ± s.d.), intercept (b_i ± s.d.) and correlation coefficient (r_i) of regression lines representing amino acid content (A_i) as a function of nitrogen content, both expressed in g/100 g of seed dry matter

Amino acid	a_i ± s.d. × 10^3	b_i ± s.d. × 10^3	r_i* × 10^3
Gly	185 ± 6	379 ± 36	990
Ala	138 ± 6	443 ± 38	981
Val	204 ± 9	398 ± 52	984
Leu	389 ± 10	393 ± 59	994
Ile	278 ± 8	69 ± 45	993
Ser	311 ± 9	138 ± 53	993
Thr	142 ± 12	553 ± 71	942
Tyr	319 ± 13	−168 ± 75	986
Phe	219 ± 7	148 ± 40	992
Trp	23 ± 3	124 ± 16	900
Pro	263 ± 8	5 ± 49	991
Met	7 ± 4	256 ± 26	354
1/2 Cys	41 ± 13	378 ± 77	603
Lys	227 ± 10	472 ± 59	983
His	99 ± 5	229 ± 28	980
Arg	1070 ± 27	−2419 ± 160	994
Asx†	693 ± 17	−174 ± 102	994
Glx*	1442 ± 44	−887 ± 263	992
NH3‡	136 ± 5	−86 ± 27	990

*Significance level of r_i: 0.68 ($P = 0.001$); 0.56 ($P = 0.01$)
†Calculated as Asp and Glu, respectively.
‡Amide nitrogen calculated as NH_3.

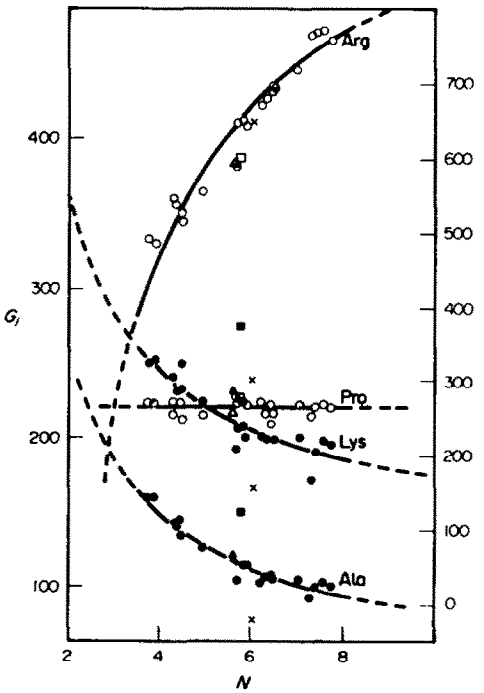

Fig. 2. Variation of amino acid content G_i in total seed protein (mg amino acid/g nitrogen as a function of total seed nitrogen percentage N for Arg, Pro (open points), Lys and Als (solid point). Circles correspond to the present results. For the other shapes, see Fig. 1.

studied. Tyr and Glx also show negative values of b_i, but their b_i/a_i ratios are rather low and the corresponding variations of G_i are weak.

The present results have been compared, with equal protein contents, with those in the literature on the amino acid composition of white lupin seeds [9, 13, 14]. For the six amino acids concerned in Figs 1 and 2, the data from these three publications have been represented as non-circled points. For the 14 amino acids common to the analyses of these three publications, the relative discrepancies are less than 10% for only Pro and Arg. However, for most of these amino acids the discrepancies are systematically distributed around the predicted values, as can be seen in Figs 1 and 2. With a few exceptions (Tyr, Trp, and Cys) the results of the FAO [9], for $N = 5.6$ are very close to the present predicted values.

Recovery of the analyses and non-protein nitrogen of lupin seed

The nitrogen recovery R of the analyses is indicated on the bottom line of Table 1 (as a percentage). As already explained elsewhere [17] R depends on N. It can be calculated from the amino acid level $D_i = 14\, n_i A_i/M_i$ in the seed, n_i being the number of nitrogen atoms in the ith amino acid and M_i its M. Like A_i, D_i is a linear function of N (as well as B_i and E_i). The same is true for the sum $\Sigma D_i = pN + q$ extended to all the amino acids and to the amide nitrogen. A regression calculation gives: $p = 1.025$ and $q = -0.375$ (with $r = 0.996$). Hence $R = (\Sigma D_i)/N = p + q/N$ increases as a hyperbolic function of N from 92.5 to 97.6% when N increases from 3.78 to 7.75. It must be noted that R cannot reach 100%, because a small amount of total seed nitrogen is not aminated and cannot be detected by amino acid analysis. Still the level D_{NPN} in seed of this NPN (non-protein nitrogen) can be estimated: $D_{NPN} = N - \Sigma D_i = (1 - p)N - q$. Since $1 - p = 0.029$ is negligible, $D_{NPN} \sim -q$ and can be considered as constant and equal to 0.39 g nitrogen per 100 g seed dry wt. But as a percentage of seed nitrogen ($100\, D_{NPN}/N$) the NPN concentration decreases by half, from 10 to 5.1% as N increases from 3.78 to 7.75. This shows that the storage proteins accumulated in the lupin seed are not accompained by a proportional amount of NPN, in fact quite the opposite is true.

Degree of amidation and nitrogen-to-protein conversion factor

The degree of amidation of protein, equal to the molar ratio (Asn + Gln)/(Asx + Glx) can be deduced from the data in Table 2 [17]. The amount of ammonia due to the amide groups of Asn and Gln and the amounts of Asx and Glx expressed as number of moles ($B_i = A_i/M_i$) are: $B_{NH_3} = 10^{-5}(799\, N - 505)$ and $B_{Asx} + B_{Glx} = 10^{-5}(1501\, N - 734)$. As the number B_{NH_3} of the amide groups of Asn and Gln is equal to the mole number of these two amino acids, the degree of amidation is equal to the ratio of the two preceding equations. Even though it increases slightly as a hyperbolic function of N, it is virtually constant and close to 52%.

The nitrogen-to-protein conversion factors and their variations can also be determined from the data in Table 2. Three different conversion factors can be distinguished [17]: $k_p = (\Sigma E_i)/N$ gives the amount of true proteins (*i.e.* polypeptide chains) from N (in this case, the summation ΣE_i does not include the ammonia of the amide groups); $k_A = (\Sigma E_i)/\Sigma D_i$ previously measured in other species [22, 23] gives the ratio of true protein to seed amino-nitrogen ΣD_i obtained from amino acid analyses; $k_N = (\Sigma E_i) + 6.25\, D_{NPN}/N$ provides an evaluation of crude proteins (true proteins + NPN compounds). The calculation shows that $k_p = 5.25 + 0.128/N$; $k_A = (5.25\, N + 0.128)/(1.03N - 0.392)$; $k_N = 5.07 + 2.58/N$. These are decreasing functions of N (Fig. 3), k_A and k_N being very close to one another and decreasing from about 5.75 to 5.4 in the N range studied, while k_p can be considered constant and close to 5.27. In other words, true proteins of lupin seed have a nitrogen content ($100/k_p$) nearly constant and close to 19%, whereas that of crude proteins ($100/k_A$) increases from 17.5 to 18.5% in the range investigated.

Composition of lupin seed storage proteins

Knowledge of the relationships between Ai and N enables the determination of the composition of storage proteins that accumulate in the lupin seed as their content increases. The preceding results show that two different samples of seed with nitrogen contents N_1 and $N_2 > N_1$, have amino acid levels equal to $A_i(N_1)$ and $A_i(N_2) > A_i(N_1)$, respectively. The supplementary proteins that characterize the 2nd sample with respect to the 1st have a composition, in g amino acid per 100 g nitrogen, equal to $100[A_i(N_2) - A_i(N_1)]/(N_2 - N_1) = 100\, a_i$. These additional proteins have therefore a constant composition, regardless of N_1 and N_2 i.e. of N. Table 3 shows that it is indeed storage proteins that are at issue because their composition is virtually identical to that of storage globulins studied by several authors [2, 12, 20] and which, in turn, constitute the majority of storage proteins. If (half)cystine for which determinations are always dubious, is discarded the only amino acid presenting significant deviations is Arg which is clearly more abundant in the supplementary proteins than in the globulins. Meanwhile, Arg corresponds to the amino acid found in

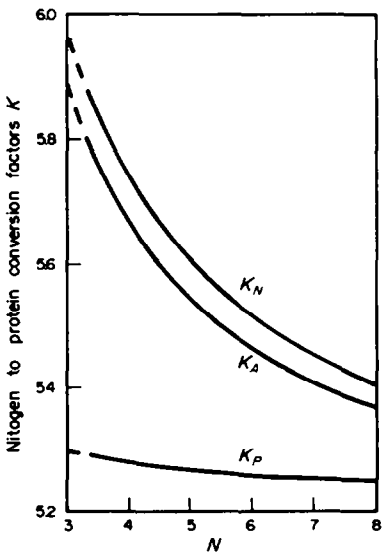

Fig. 3. Variations of the nitrogen to protein conversion factors of lupin seed as a function of their nitrogen content N.

Table 3. Amino acid composition of lupin globulins as compared with the predicted composition of lupin seed storage proteins (values are expressed as residues per 1000 residues)

	Globulins *	Globulins †	Storage proteins
Gly	70	65	56
Ala	43	40	35
Val	39	36	39
Leu	73	74	67
Ile	46	46	48
Ser	67	66	67
Thr	36	34	27
Tyr	31	41	40
Phe	35	31	30
Trp	nd	3	3
Pro	50	51	52
Met	3	2	1
Cys	13	16	8
Lys	38	35	35
His	22	15	14
Arg	96	90	139
Asx	113	105	118
Glx	228	247	222

*Average values for globulins extracted from four different species: *L. angustifolius*, *L. consentinii*, *L. elegans* and *L. luteus*. from ref. [20].

†Calculated from total globulins of *L. albus* from ref. [12].

nd: not determined.

the free state in significant amounts in various legume seeds [24]. In short, if amino acid composition of lupin seeds does depend upon their protein content, the composition of their storage proteins does not.

EXPERIMENTAL

Material. Among many lupin samples of winter or spring lines or of different field-grown cultivars cultivated in various conditions and locations, the seed samples analysed were chosen for their distribution over a wide range of *N*. Seed sampling and milling for analysis were performed in the conditions already described [17].

Analytical methods. Dry wt percentage was determined in triplicate, as well as *N* (by micro-Kjeldahl). Amide nitrogen amount corresponding to half of Asn plus Gln nitrogen was measured as free NH_3 after a separate hydrolysis (3 hr in 2M HCl at 115°) [25]. Amino acids were analysed by single CC from five different hydrolysates [26]. Four of these were acid (6M HCl) in order to account for losses due either to partial degradation or to incomplete release: 15, 24 and 48 hr plus an 18 hr hydrolysis of a sample previously oxidized by performic acid. These four hydrolysates were analysed and compared for the stable amino acids (i.e. Gly, Ala, Leu, Phe, Pro, His, Arg, Asp, Glu). The results were used provided the overall variation was $< 3\%$. Otherwise, either aliquots of hydrolysates were re-analysed or new hydrolyses were performed. With such consistent values of stable amino acids, the 15 hr results were used for Ser, Thr and Tyr and the 48 hr ones for Val and Ile. For Met and Cys, duplicate analysis of the 18 hr hydrolysate was performed. Moreover, an alkaline hydrolysis in $Ba(OH)_2$ was performed in order to determine Trp in triplicate. The overall variation accepted was less than 4% for Trp [27]. Two reference proteins (commercial egg-white lysozyme and human serum albumin) were used to check the accuracy of the amino acid determinations, while a laboratory defatted soybean preparation was used as a standard for each ninhydrin preparation to monitor the reproducibility. All the methods used have been already described [17]. Regression analysis was made according to ref. [28].

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