Tryptophan Contents of Pea and Broad Bean Seeds as a Function of Nitrogen Content

Jacques Landry* and Sonia Delhaye

Laboratoire de Chimie Biologique, CBAI, INRA, Centre de Grignon, F-78850 Thiverval-Grignon, France

The tryptophan contents of pea and broad bean seeds were determined for samples originating from commercial and experimental cultivars, using a procedure that ensured the true quantity of this amino acid to be evaluated. For both species a relationship was found indicating a strict proportionality between the levels of tryptophan (Trp) and nitrogen (N) of the seed. The values available in the literature for tryptophan (159 for pea and 53 for broad bean) were compared with those predicted from the nitrogen of samples through the relationship between Trp and N. They ranged from 68 to 153% with a weighted mean of 100% for pea and from 98 to 114% with a mean of 101% for broad bean when they were expressed as percentage of predicted value. The lowest percentages were related to underestimation owing to degradation of tryptophan by alkali or its incomplete release by enzyme during hydrolysis preparatory to analysis, and the highest to overestimation attributed to lysinoalanine generated during alkaline hydrolysis and coeluted with tryptophan when ion exchange chromatography is used for analysis. Some linear relationships previously reported appeared to be close from those determined in the present study, suggesting the absence of tryptophan degradation with some hydrolytic conditions and limited influence of genetic factors upon tryptophan content of pea and broad bean seeds.

Keywords: *Tryptophan; nitrogen; pea seed; broad bean seed*

INTRODUCTION

It is obvious that levels of proteins and every amino acid incorporated into proteins in the dry matter of any biological sample are correlated closely. The same holds for nitrogen (or crude proteins) and every free and protein-incorporated amino acid as far as free amino acids represent a minor part of nitrogen (Landry and Moureaux, 1984). Linear relationships have been found to exist between levels of nitrogen and any amino acid for mature seeds of cereals and legumes, enabling the calculation of amino acid composition from nitrogen content through regression equations [see Mossé and Huet (1990) and references cited therein]. However, data available for tryptophan can be questionable, considering that they are the result of assays involving a preparatory hydrolysis, leading to a underestimation of this amino acid. Tryptophan analysis of 127 samples of wheat, maize, and barley grains using a procedure that has proved to be quantitative (Delhaye and Landry, 1986; Landry et al., 1988; Landry and Delhaye, 1996) has confirmed the existence of a linear relationship between tryptophan and nitrogen for every cereal, revealing a 10% average underestimation of tryptophan content from regression equations available in the literature and great variability in grain tryptophan content (Landry and Delhaye, 1993). The present paper reports data relative to a similar investigation performed on tryptophan of pea and broad bean seeds with the purpose of determining whether the previous observations made with cereals can be extended to legumes.

MATERIALS AND METHODS

The pea and broad bean seed samples represented a variety of experimental and commercial cultivars having wide variations in protein content.

* Author to whom correspondence should be addressed (fax 33 1 30 81 53 73).

Tryptophan was assayed using the simplified procedure described by Landry and Delhaye (1992) with the following modification: α-methyltryptophan, because of its stability, was substituted for 5-methyltryptophan as internal standard and was added to medium after, instead of before, hydrolysis.

Nitrogen was assayed using the Kjeldahl method (N × 6.25).

RESULTS

As shown below, data from this study (laboratory L1) will be compared with data reported by two other laboratories, namely L2 and L3, for broad bean and from three other laboratories, namely L2, L3, and L4, for pea. For instance, the samples (or resulting tryptophan data) in this work were denoted S1 and were subdivided into two subsets, namely SS1p and SS1bb, relative to pea and broad bean, respectively.

Table 1 sums up characteristics relating to nitrogen and tryptophan contents of samples reported by different laboratories. Subsets SS2p and SS2bb contained the most and the fewest samples, respectively, but nitrogen and tryptophan contents varied less than in the other subsets. Subset SS4p was divided into subsubsets: one, α, contained 16 samples originating from the same line; the second, *β*, was made of 16 samples originating from seven different lines. For pea, mean tryptophan content was the same for subsets SS1 and SS2, lower for subset SS3, and higher for subsubset SS4α for identical or very close nitrogen contents. For broad bean, no significant differences were seen between tryptophan and nitrogen for the three subsets.

Detailed data are depicted in Figures 1 and 2 for pea and broad bean, respectively. In each figure the tryptophan values, when available, were plotted against nitrogen values, and the corresponding lines were graphed. When individual values were not reported, only regression lines were depicted. Figure 1 highlights the marked scattering of individual values and the different regression lines for each subset or subsubset taken into consideration. With respect to the lines

S0021-8561(97)00107-6 CCC: $14.00

Table 1. Nitrogen and Tryptophan Contents of Sample Subsets

		nitrogen (g/100 g of DM[a])				tryptophan (g/100 g of DM)			
	n^b	mean	CV%[c]	min	max	mean	CV%	min	max
pea									
SS1[d]	23	3.83	12.1	3.10	4.37	0.221	11.3	0.187	0.260
SS2	62 (51)	3.83	5.9	3.00	4.24	0.216	7.0	0.170	0.239
SS3	33	3.93	13.4	2.84	5.15	0.199	14.1	0.142	0.268
SS4α	16	3.68	19.2	2.50	4.75	0.257	13.9		
SS4β	16	4.61	12.0	3.76	5.53	0.297	9.5	0.256	0.343
broad bean									
SS1	83	4.72	11.2	3.76	5.81	0.250	10.7	0.195	0.311
SS2	27 (17)	4.62	3.8	4.40	5.05	0.250	4.4	0.227	0.272
SS3	24	4.88[e]		3.68	6.08	0.234[e]		0.200	0.268

[a] Dry matter. [b] Sample number: the figures in parentheses indicate the number of samples considered for tryptophan assay. [c] Coefficient of variation. [d] SS1 (present study), SS2 from Heimbeck and Balschukat (1990), SS3 pea from Mossé *et al.* (1987), SS3 broad bean from Mossé (1990), SS4 from Holt and Sosulski (1979). [e] Value estimated (within 2%) by averaging minimum and maximum values.

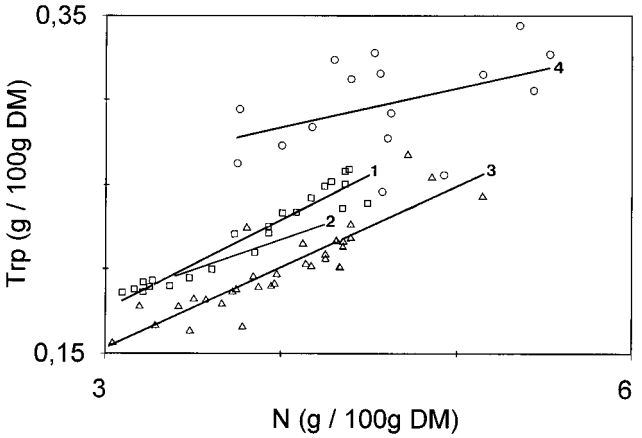

Figure 1. Relationships between tryptophan (Trp) and nitrogen (N) contents of pea seeds:(□, 1) experimental points and regression line from laboratory L1 (present study); (□, 2) regression line from laboratory L2 (Heimbeck and Balschukat, 1990); (△, 3) experimental points and regression line from laboratory L3 (Mossé *et al.*, 1987); (○, 4) experimental points and regression line from laboratory L4 (Holt and Sosulski, 1979).

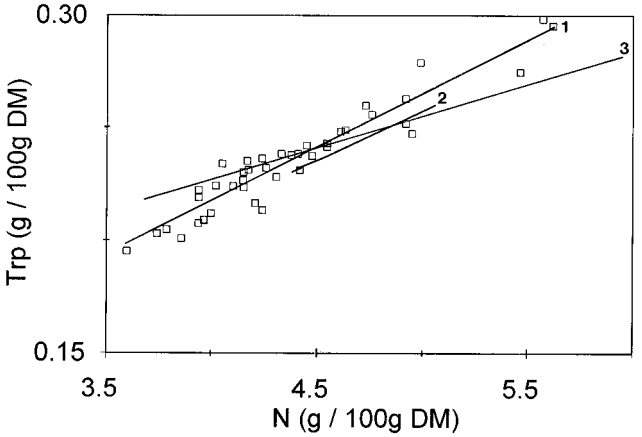

Figure 2. Relationships between tryptophan (Trp) and nitrogen (N) contents of broad bean seed: (□, 1) experimental points and regression line from laboratory L1 (present study); (□, 2) regression line from laboratory L2 (Heimbeck and Balschukat, 1990); (□, 3) regression line from laboratory L3 (Mossé, 1990).

relative to subsets SS1 and SS2, which were very close, those relative to subset SS3 and subsubset SS4β appeared lower and higher, respectively. In contrast, Figure 2 shows very close lines for the three subsets.

Statistical parameters for regression lines depicted in Figures 1 and 2 are presented in Table 2, giving some quantitative aspects of previous observations. The coefficients of determination (r^2) of lines from subsets

Table 2. Statistical Parameters Concerning the Relationship $[Trp]_{DM} = a[N]_{DM} + b$ or $[Trp]_{DM} = a[N]_{DM}$[a]

		$[Trp]_{DM} = a[N]_{DM} + b$			$[Trp]_{DM} = a[N]_{DM}$
	n^b	a (SD)	b (SD)	r^2	a(SD)
pea					
SS1[c]	23	0.053 (0.003)	0.018 (0.014)	0.927	0.058 (0.002)
SS2	51	0.036 (nc[d])	0.074 (nc)	0.325	0.056 (nc)
SS3	33	0.048 (0.004)	0.010 (0.015)	0.830	0.051 (0.003)
SS4α	16	nc	nc	nc	0.070 (nc)
SS4β	16	0.025 (0.011)	0.194 (0.057)	0.192	0.064 (0.010)
broad bean					
SS1	83	0.047 (0.002)	0.029 (0.034)	0.855	0.053 (0.002)
SS2	17	0.028 (nc)	0.115 (nc)	0.240	0.053 (0.002)
SS3	24	0.046 (0.004)	0.027 (0.020)	0.895	0.052 (nc)

[a] Results are expressed as g/100 g (dry matter). [b] Sample number. [c] Subsets as indicated in Table 1. [d] Not calculated due to the absence of individual data.

SS1 and SS 3 were close together and deviated weakly from 1, indicating a regular increase of tryptophan with increasing nitrogen. They deviated greatly from 1 for lines concerning subsets SS2 and SS4β, probably related to the greater amount of data for a narrow range of nitrogen content (SS2) or to a variability of analytical origin (SS4β). The parameter b (y-intercept) for lines corresponding to sets S1 and S3 was not significantly different from 0, indicating a nearly simultaneous and regular accumulation of all proteins present in the seed since they do not have the same content of tryptophan. This was supposed to be true for other sets. Therefore, an average gradient (which corresponds to average concentration of tryptophan in protein) for every subset was determined by calculating the average of individual values of concentrations of tryptophan in protein, which enables standard deviation to be known, or by taking the quotient of average concentration of tryptophan by that of nitrogen in dry matter (see in Table 1). This parameter enables one to establish the same ranking between subsets as that observed when the respective positions of regression lines toward the x-axis were considered. However, it is noteworthy that the regression line concerning the subsubset SS4β has a gradient not significantly different from 0, indicating an accumulation of tryptophan-free proteins in the seed. This incoherence has to be attributed to analytical errors.

DISCUSSION

In the present study all data were obtained using a simplified assay which differs slightly from that reported previously (Landry and Delhaye, 1992). It consisted of spiking the hydrolysate with an internal standard, α-methyltryptophan. The addition of 5-methyltryptophan as an internal standard prior to hydrolysis, although it was shown to give reliable results for

Table 3. Tryptophan in Pea and Broad Bean Seeds: Comparison of Mean Contents Reported in the Literature with Those Predicted from Nitrogen Content of Samples and the Linear Relationship between Tryptophan and Nitrogen

			tryptophan (as % of predicted value)[a]				
	subset	n[b]	mean	CV[c]	min	max	ref[d]
pea							
	1	23	100[e]	3.2	93	104	1
	2	51	97	5.8	68	104	2
		18	97	3.4	92	103	3
	2β	20	100	2.8	96	104	4
	3	33	88	5.9	77	103	5
	4α	16	122	13.9	90	153	6
	4β	16	111	16.1	75	149	7
	5	2	90	4.3	87	92	8
	6	1	87				9
	7	1	127				10
	8	1	120				11
	Σ_{2-8}[f]	159	100		68	153	
broad bean							
	1	83	100[g]	4.1	92	109	12
	2	16	100	4.4	94	107	13
	3	24	98				14
	5	1	100				15
	9	5	104	12.5	78	113	16
	10	7	114				17
	Σ_{2-10}	53	101		78	114	

[a] To the tryptophan content $[Trp]_i$ of a sample i having a nitrogen content $[N]_i$ is assigned a percentage defined by $([Trp]_i: 0.058[N]_i) \times 100$ (pea) or $([Trp]_i: 0.053[N]_i) \times 100$ (broad bean), $0.058[N]_i$ or $0.053[N]_i$ being the tryptophan content predicted through equations given in Table 2 for SS1. [b] Number of samples. [c] Coefficient of variation (%). [d] References are as follows: 1, 12, present study; 2, 3, 4, 13, Heimbeck and Balschukat (1990); 5, Mossé *et al*. (1987); 6, 7, Holt and Sosulski (1979); 8, 15, Mendes Pereira and Pion (1977); 9, 10, 11, IAEA (1978); 14, Mossé (1990); 16, Wilson (1977); 17, Sosulski and Holt (1980). [e] 100 per definition. [f] Data available in the literature.

tryptophan of wheat, maize, and barley grains and other complex samples (Landry and Delhaye, 1992, 1993), was found to overestimate tryptophan content of pea and broad bean seeds. Furthermore, the hydrolysis conditions used in the present study were supposed to not induce appreciable losses since the recovery of tryptophan from feedstuff samples, pea included, spiked with lysozyme ranged from 98.8 to 100.9% (Landry *et al*., 1988). The reproducibility of our procedure has been estimated as being 4% (Landry and Delhaye, 1993), similar to that reported for the procedure used by laboratory L3 (Mossé *et al*., 1987).

The high variability of the tryptophan content of pea seed, as evidenced from data shown in Figure 1 and Table 2, may come from the natural variability among cultivars or from uncertainty surrounding the use of various assays. An answer to the first alternative is to evaluate the reliability of the diverse procedures used for assaying tryptophan to access the status of the results available in the literature. For this purpose the data of Table 2 together with others reported in the literature were transformed by expressing the tryptophan content in the protein of each subset as a percentage of the corresponding content, as it can be deduced from the nitrogen content and the equations developed in the present study. Resulting percentages, given in Table 3, ranged from 68 to 153% with a weighted mean of 100% for pea and from 98 to 114% with a weighted mean of 101% for broad bean. As for pea, the mean percentages relative to subsets fell into three categories depending on whether they are close to, far below, or above 100. The first group comprised percentages running about 100%, as is the case for subset SS2p. The presence of SS2p in this category was unexpected since tryptophan was assayed after hydrolysis in the presence of 4 M LiOH, a procedure shown to underestimate tryptophan of cereal grains by 10% (Landry and Delhaye, 1993). Percentages relative to subsets SS3p, SS5p, and SS6p, and ranging from 87 to 90% with a weighted mean of 88%, made up the second category. Tryptophan was assayed after barytic hydrolysis and gel filtration chromatography for samples from subsets SS3p and SS5p and after enzymatic hydrolysis for samples from SS6p. These procedures, when applied in the absence of careful conditions, were found to underestimate tryptophan by about 10%, due to its partial degradation during alkaline hydrolysis or its incomplete release from proteins by enzyme (Landry and Delhaye, 1993; Delhaye and Landry, 1992). The third category included percentages corresponding to subsets SS4p, SS7p, and SS8p and ranging from 111% to 127% with a weighted mean of 117%. In that case tryptophan was assayed using ninhydrin colorimetry after barytic hydrolysis and ion exchange chromatography.

The difference recorded between the weighted means concerning the second and third categories must be assumed to be mainly from an analytical origin for the following reasons:

(1) A discrepancy of the same order of magnitude was seen when the percentage relative to the subset SS6p (assay after enzymic hydrolysis) was compared with those of subsets SS7p and SS8p (assay after ion exchange chromatography), although they pertained to the same sample analyzed by three laboratories as a part of collaborative study (IAEA, 1978).

(2) Tkachuk and Irvine (1969) found that ion exchange chromatography procedure led to a tryptophan recovery 139% higher when compared with the colorimetry procedure of Miller (1967). Since the latter led to underestimation averaging 13%, as calculated from the free tryptophan added to extra samples (Miller, 1967; Williams *et al*., 1982; Nielsen and Hurrell, 1985), the tryptophan yield using ion exchange chromatography, as employed by Holt and Sosulski, (1979) has to average $87 \times 1.39 = 121\%$, a percentage close to the weighted mean relative to the third category (117%).

Such an overestimation could be due to formation of lysinoalanine during alkaline hydrolysis and to its coelution with tryptophan on ion exchanger under conditions used by the authors. Such an interference has been evidenced by Hugli and Moore (1972) and by Wilkinson *et al*. (1975). It could also explain the high coefficient of variation (13.9%) found for the tryptophan contents in protein of 16 pea samples originating from the same variety (subset SS4α) (Table 3). It is of interest to note that Delhaye and Landry (1992), using the Hugli and Moore procedure and chromatography conditions allowing tryptophan to be separated from lysinoalanine and expressing results the same way as stated above, have reported a yield of 95.8 ($\pm$ 2.3)% for seven samples of plant material. This is to compare with a tryptophan recovery of 94.6%, calculated from the data reported for the tryptophan content of a pea sample assayed by six laboratories using the Hugli and Moore technique (Sarwar *et al*., 1983).

A similar picture, although different in detail, is seen from the comparison of data relative to tryptophan of broad bean seeds and obtained using diverse assay techniques. No significant differences of mean recovery were observed for the subsets SS1, SS2, SS3, and SS5 (Table 3), suggesting the lack of tryptophan degradation by alkali, irrespective of the conditions used for hydroly-

sis. On the other hand, the high recovery associated with the high coefficients of variation found for the subset SS9 emphasized that the tryptophan determination based on enzymatic digestion followed by colorimetry, as developed by Holz (1972), gave variable results due to incomplete release of protein-bound tryptophan and interferences with the final colorimetric assay. Finally, the high recovery found with the subset SS10 resulted from the use of the Tkachuk and Irvine procedure and was consistent with the observations made with pea.

This comparative study points out that the tryptophan contents assigned to some pea and broad bean seed samples must be viewed with proper caution. It shows that some recipes used for assaying tryptophan, which were found to underestimate it when they were applied to cereal grains, seem to give accurate results with broad bean and pea seeds eventually. This could be related to insolubilization of proteins of these seeds in alkali when hydrolysis starts, isolating them from oxygen present in the medium, as it was assumed for the conditions used in our own assay procedure (Landry and Delhaye, 1996). However, the reverse could be considered: the absence of protein insolubilization when our procedure was applied to some samples used in the present study would lead to an underestimation of tryptophan. Such an assumption is to be excluded since it would involve quantitative recovery of tryptophan for alkaline hydrolyses performed under standard conditions by Tkachuk and Irvine (1969) or by Holt and Sosulski (1979).

The strict linear relationship between tryptophan and nitrogen is in agreement with the fact that in legume seeds the cotyledons constitute the bulk of the seed and synthesize most of the proteins. It cannot be perfect since seeds accumulate storage proteins, namely vicilin and legumin, the respective tryptophan contents of which are 0 and 1.2 g/100 g of protein; the ratio varies with cultivar (Gueguen and Barbot, 1988). However, the low standard deviations recorded for the average gradients concerning the subsets SS1, SS2, and SS3 suggest a limited influence of genetic factors upon tryptophan content of pea and broad bean seeds. It is worth recalling that tryptophan and nitrogen contents of cereal grains were found to be not strictly proportional, due to the synthesis of proteins in germ and endosperm. In addition, a high variability was observed (Landry and Delhaye, 1993).

ACKNOWLEDGMENT

We are grateful to P. Maupetit from Institut Technique des Céréales et Fourrages and to G. Duc from INRA for providing pea and broad bean samples, respectively.

LITERATURE CITED

Delhaye, S.; Landry, J. HPLC and UV spectrophometry for quantification of tryptophan in barytic hydrolysates. *Anal. Biochem*. **1986**, *159*, 175−178.

Delhaye, S.; Landry, J. Determination of tryptophan in pure proteins and plant material by three methods. *Analyst* **1992**, *117*, 1875−1877.

Gueguen, J.; Barbot, J. Quantitative and qualitative variability of pea (*Pisum sativum* L.) protein composition. *J. Sci. Food Agric.* **1988**, *42*, 209−224.

Heimbeck, W.; Balschukat, D. In *The Amino Acid Composition of Feedstuffs*; Degussa: Hanau, Germany, 1990.

Holt, N. W.; Sosulski, F. W. Amino acid composition and protein quality of field peas. *Can. J. Plant Sci.* **1979**, *59*, 653−660.

Holz, F. Automatische bestimmung von tryptophan in proteinen und proteinhaltigen pflanzenprodukten mit dimethylaminozimtaldehyd (Automated procedure for tryptophan determination of pure proteins and of plant products using *p*-dimethylaminocinnamaldehyde). *Landwirtsch. Forsch.* **1972**, *27*, 96−109.

Hugli, T. E.; Moore, S. Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolysates. *J. Biol. Chem*. **1972**, *247*, 2828−2834.

IAEA. Interlaboratory comparison of amino acid analyses. In *Seed Protein Improvment by Nuclear Techniques*; IAEA: Vienna, 1978; pp 393−402.

Landry, J.; Delhaye, S. Simplified procedure for the determination of tryptophan of foods and feeedstuffs from barytic hydrolysis. *J. Agric. Food Chem*. **1992**, *40*, 776−779.

Landry, J.; Delhaye, S. The tryptophan contents of wheat, maize and barley grains as a function of nitrogen content. *J. Cereal Sci.* **1993**, *18*, 259−266.

Landry, J.; Delhaye, S. Tryptophan content of foods and feeds: conditions for a reliable quantitation. In *Recent Advances in Tryptophan Research. Tryptophan and Serotonin Pathways*; Allegri Filippini G., Costa, C. V. L., Bertazzo, A., Eds.; Plenum Publishing: New York, 1996; pp 703−709.

Landry, J.; Moureaux, T. A quantitative analysis of amino acid accumulation in developing grain of normal and *opaque-2* maizes. *J. Sci. Food Agric.* **1984**, *35*, 1051−52.

Landry, J.; Delhaye, S.; Viroben, G. Tryptophan content of Feedstuffs as determined from three procedures using chromatography of barytic hydrolysates. *J. Agric. Food Chem*. **1988**, *36*, 51−52.

Mendes Pereira, E.; Pion, R. Metabolic and digestive utilisation of amino acids in some proteaginous seeds. In *Protein Quality from Leguminous Crops*; CEC: Luxembourg, 1977; pp 198−216.

Miller, E. L. Determination of the tryptophan content of feedingstuffs with particular reference to cereals. *J. Sci. Food Agric.* **1967**, *18*, 381−386.

Mossé, J. Amino acids of cereals, legumes and oilseeds: variation and prediction of composition as a function of seed nitrogen content in 16 crop species. Nutritional consequences. *INRA Prod. Anim*. **1990**, *3*, 103−119.

Mossé, J.; Huet, J. C.; Baudet, J. Changements de la composition en acides aminés des graines de pois en fonction de leur taux d'azote (Variations in the amino acid composition of pea seeds in relation to their nitrogen content). *Sci. Aliments* **1987**, *7*, 301−324.

Nielsen, H. K.; Hurrell, R. F. Tryptophan determination of food proteins by HPLC after alkaline hydrolysis. *J. Sci. Food Agric.* **1985**, *36*, 893−907.

Sarwar, G.; Christensen, D. A.; Finlayson, A. J.; Friedman, M.; Hackler, L. R.; Pellet, S. L.; Mackenzie, P. L.; Tkachuk, R. Inter- and intra-laboratory variation in amino acid analysis of food proteins. *J. Food Sci.* **1983**, *48*, 526−531.

Sosulski, F. W.; Holt, N. W. Amino acid composition and nitrogen-to-protein factors for grain legumes. *Can. J. Plant Sci*. **1980**, *60* , 1327−1331.

Tkachuk, R.; Irvine, G. N. Amino acid composition of cereals and oilseed meals. *Cereal Chem*. **1969**, *46*, 206−218.

Wilkinson, M.; Iacobucci, G. A.; Myers, D. V. 5-methyltryptophan: an internal standard for tryptophan determination by ion-exchange chromatography. *Anal. Biochem*. **1976**, *70*, 470−478.

Williams, A. P.; Hewitt, D.; Buttery, P. J. A collaborative study on the determination of tryptophan in feedingstuffs. *J. Sci. Food Agric.* **1982**, *33*, 860−865.

Wilson, B. J. The composition of faba beans (*Vicia faba* L.). In *Protein Quality from Leguminous Crops*; CEC: Luxembourg, 1977; pp 58−63.

Received for review February 3, 1997. Revised manuscript received June 9, 1997. Accepted July 21, 1997.[⊗]

JF970107E

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.