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Protein and non-protein (free and protein-bound) tryptophan in legume seeds

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Abstract

The contents of protein and non-protein (free and protein-bound) tryptophan and of proteins in the flours of nine legume seeds were determined. Lupins and soybeans showed the highest protein concentrations, followed by groundnuts, beans, broad beans, lentils, vetches, chick-peas, and peas. Protein tryptophan content is higher in soybeans and lower in peas (502 and 192 mg/100 g of dry flour, respectively) than in the other legumes, which also contain non-protein tryptophan. Chick-peas show the highest value of free tryptophan and groundnuts the lowest (58.2 and 2.24 mg/100 g of dry flour, respectively). Tryptophan appears to be bound to water-soluble proteins and to proteins soluble at pH 8.9. In particular, chick-peas contain a high amount of tryptophan bound to water-soluble proteins, followed by beans. The results are evaluated, considering the importance, not only of protein, but also non-protein tryptophan, for assessing the nutritive value of a protein in foods.

Keywords: Legumes; Protein tryptophan; Free tryptophan; Protein-bound tryptophan

1. Introduction

The major source of protein in the human diet is vegetable food. Legumes, together with cereals, are very satisfactory sources of proteins. Pulses, such as soy, play a great role in both human and animal feeding (Gueguen & Cerletti, 1994). Besides soy, other legumes, such as peas, beans, lupins, vetches, lentils, broad beans, groundnuts and chick-peas, are of great importance in human feeding. Legumes are widely consumed in the world and are a very important source of protein in the diets of African, Asian and Latin American populations. However, they are poor sources of the sulphur-containing amino acids, methionine and cysteine, but they have high lysine and tryptophan contents (Mossé & Huet, 1990; Müller, 1983). Therefore, when they are used in mixture with cereals, which are poor in lysine and tryptophan (FAO, 1968, 1970; Taira, 1968), the proteins of legumes complement one another, giving a protein of a better quality by supplying to each other the respective limiting amino acids in significant amounts. Legumes and cereals are, therefore, excellent sources of proteins in human diets as alternatives to animal proteins.

Although much has been done on the amino acid composition, there is no information on the presence of non-protein tryptophan, free and protein-bound, in legumes. In fact, tryptophan, an essential amino acid, is present in man, both as a constituent of proteins and in free form or bound to proteins of blood (McMenany & Oncley, 1958). These two fractions are of great functional importance, being easily absorbable at the stomach level and more available to enter the brain, thus influencing the synthesis of serotonin (Gessa & Tagliamonte, 1974; Knott & Curzon, 1972; Tagliamonte, Biggio, Vargiu, & Gessa, 1973).

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The aim of the present study is to investigate the presence of non-protein tryptophan (free and protein-bound) in legumes, such as soybeans, lentils, beans, groundnuts, peas, broad beans, lupins, vetches, chick-peas, and to assess the extent of variation among different legumes.

2. Materials and methods

2.1. Materials

All chemicals used were of analytical-reagent grade, obtained from Sigma (St. Louis, MO, USA).

2.2. Legumes

Seeds of soy (*Glycine max*), bean (*Phaseolus vulgaris*, L.), broad bean (*Vicia faba* L.), chick-pea (*Cicer arietinum* L.), lentil (*Lens culinaris*), lupin (*Lupinus* sp.), pea (*Pisum sativum* L.), vetch (*Vicia sativa* L.), and groundnut (*Arachis hypogaea*) were local from the market in Padova, (2005 harvest).

Dry seeds were ground to a flour in a coffee mill and the flour was passed through a 300 μm sieve. Only the groundnuts were defatted before sieving.

2.3. Extraction and analysis of non-protein tryptophan

This procedure was carried out as previously reported by Comai et al. (2007). In brief, 1 g of the dry flour was defatted by suspension in 10 ml of acetone and stirred for 30 min at 37 °C. The sample was centrifuged at 0 °C for 10 min and the organic layer was removed. An additional 10 ml of acetone were added to the remaining pellet. The sample was shaken for 10 min and centrifuged. The organic layer was removed and the dried sediment was extracted with 10 ml of distilled water for 30 min at 37 °C under shaking. After centrifugation, the clear supernatant, containing the free tryptophan and the water-soluble protein fraction, was collected. The residue was re-extracted with a further 10 ml of distilled water for 30 min and, after centrifugation, the supernatants were combined. An aliquot (5 ml) was ultrafiltered by an Amicon model 12 ultrafiltration cell with an XM-50 Diaflo membrane (Amicon, Oosterhout, Holland) collecting the first 500 μl of ultrafiltrate used to determine the free fraction of tryptophan on a combined HPLC-fluorescence system according to the method of Eccleston (1973), slightly modified by Costa, Bettero, and Allegri (1987), using 0.1 ml of the ultrafiltrate. The remaining part of the supernatant was analysed for the determination of total water-soluble non-protein tryptophan, using only 0.02 ml of solution.

The sediment obtained after extraction with water was resuspended in 5 ml of 0.1 M potassium phosphate buffer, pH 8.9, shaken for 30 min at 37 °C and then centrifuged. The supernatant was analysed by HPLC (Costa et al., 1987) to determine the non-protein tryptophan eventually bound to water-insoluble proteins.

All the samples of legumes were prepared in the same way. The determinations of tryptophan were carried out in triplicate.

2.4. Protein content

Nitrogen was determined in legume flours by the micro-Kjeldahl method and nitrogen percentage was converted to crude protein by multiplying by 6.25.

2.5. Analysis of protein tryptophan

The analysis of tryptophan was done in triplicate, by HPLC, using the method of Slump, Flissebaalje, and Haaksman (1991), based on the alkaline hydrolysis of flours in $Ba(OH)_2$. The hydrolysis in $Ba(OH)_2$ was performed at 130 °C for 8 h. After cooling at room temperature, the pH was adjusted to 4.5 with concentrated HCl. A solution of 5-methyl-tryptophan was added as an internal standard to correct tryptophan losses during hydrolysis. The volume was adjusted with water to 50 ml. The suspension was mixed and filtered. An aliquot of the filtrate was diluted at least threefold with buffer. The mixture was homogenized and filtered and the filtrate was analysed by HPLC. The liquid chromatographic equipment consisted of an automatic injector (AS3000), a pump (P400) and a fluorescence detector (FL 3000) (Spectra System, Thermo Finnigan). The column was a Zorbax extended C18 (3×250 mm) type. The eluting solvent was 0.1 Na acetate/0.1 M acetic acid/methanol.

The mean values were expressed either in mg of tryptophan per 100 g of crude protein or per 100 g of flour (on a dry matter basis).

3. Results

In Table 1, the means ± standard error of three separate analyses in common legumes are summarized and the data are expressed in g/100 g dry weight of flour for protein content and in mg/100 g dry weight of flour or g/100 g of protein for protein tryptophan. As shown in this table, the protein content of lupin and soybean flours is higher (39.4 ± 1.21, 38.3 ± 0.98 g/100 g dry wt flour, respectively) than that of the other legumes, followed by groundnuts (28.4 ± 1.12), beans (28.1 ± 0.71), broad beans (27.7 ± 0.82), lentils (27.6 ± 1.02), vetches (24.8 ± 0.63), chick-peas (23.9 ± 0.56), and peas (22.8 ± 0.78 g/100 dry wt flour).

Protein tryptophan concentrations, calculated on a dry matter basis, differ greatly in the proteins of the considered legumes (Table 1). Soybean flour had higher protein tryptophan value (502 ± 51.0 mg/100 g dry wt) and peas lower (192 ± 13.3) than those of the other legumes. Bean, groundnut, and lupin flours had similar values of protein tryptophan (319 ± 4.2, 287 ± 25.9, and 274 ± 7.7 mg/100 g of dry flour, respectively). Chick-pea, broad bean, lentil, and vetch flours showed values of protein trypto-

Table 1
Protein content (g/100 g dry wt) and protein tryptophan (mg/100 g dry wt and mg/100 g protein) in flours of legume seeds (mean values ± standard error)

Flours	Protein content[a]	Protein tryptophan[b]	
	g/100 g dry matter	mg/100 g dry wt	mg/100 g protein
Bean (*Phaseolus vulgaris* L.)	28.1 ± 0.71	319 ± 4.2	1134 ± 15
Broad bean (*Vicia faba* L.)	27.7 ± 0.82	240 ± 13.1	868 ± 47
Chick-pea (*Cicer arietinum*)	23.9 ± 0.56	257 ± 9.6	1075 ± 40
Lentil (*Lens culinaris*)	27.6 ± 1.02	213 ± 7.0	770 ± 25
Lupin (*Lupinus* sp.)	39.4 ± 1.21	274 ± 7.7	695 ± 20
Pea (*Pisum sativum*)	22.8 ± 0.78	192 ± 13.3	842 ± 58
Vetch (*Vicia sativa* L.)	24.8 ± 0.63	208 ± 19.3	840 ± 78
Soybean (*Glycine max*)	38.3 ± 0.98	502 ± 51.0	1311 ± 133
Groundnut (*Arachis hypogaea*)	28.4 ± 1.12	287 ± 25.9	1012 ± 91

[a] Values are averages from three separate determinations and were calculated using 6.25 as nitrogen: protein conversion factor.
[b] Determined in triplicate by HPLC analysis after alkaline hydrolysis of flours.

Table 2
Values (means ± SE) of free and total (free + protein-bound) tryptophan (Trp) in the flours of legume seeds

Flours	Free Trp (mg/100 g flour[a])	Total protein-bound Trp, H_2O-soluble fraction (mg/100 g flour[a])	Protein-bound Trp, buffer fraction, pH 8.9 (mg/100 g flour[a])
Bean (*Phaseolus vulgaris* L.)	23.2 ± 0.87	30.7 ± 1.45	6.48 ± 0.51
Broad bean (*Vicia faba* L.)	4.08 ± 0.26	4.73 ± 0.29	0.59 ± 0.04
Chick-pea (*Cicer arietinum*)	58.2 ± 1.25	75.2 ± 1.11	5.47 ± 0.19
Lentil (*Lens culinaris*)	3.92 ± 0.13	4.26 ± 0.41	0.88 ± 0.04
Lupin (*Lupinus* sp.)	7.50 ± 0.30	10.3 ± 0.18	2.07 ± 0.13
Pea (*Pisum sativum*)	5.75 ± 0.20	7.19 ± 0.13	1.08 ± 0.06
Vetch (*Vicia sativa* L.)	7.07 ± 0.57	9.79 ± 0.17	1.57 ± 0.08
Soybean (*Glycine max*)	17.0 ± 0.07	16.9 ± 1.29	1.85 ± 0.12
Groundnut (*Arachis hypogaea*)[b]	2.24 ± 0.17	3.18 ± 0.19	0.36 ± 0.03

[a] On dry matter.
[b] Defatted and dried before sieving.

phan of 257 ± 9.6, 240 ± 13.1, 213 ± 7.0, and 208 ± 19.3 mg/100 g of dry flour, respectively. Also, calculating the content of protein tryptophan as mg/100 g of protein (Table 1), soybeans had the highest value (1311 ± 133 mg), followed by beans (1134 ± 15 mg), chick-peas (1075 ± 40 mg), and groundnuts (1012 ± 91 mg); broad beans, peas, vetches, lentils, and lupins were lower (868 ± 47, 842 ± 58, 840 ± 78, 770 ± 25, and 695 ± 20 mg/100 g protein, respectively).

Table 2 shows the values of free and protein-bound tryptophan contents, expressed as mg/100 g dry wt flour. The highest content of free tryptophan was found in chick-peas (58.2 ± 1.25 mg/100 g dry wt flour), followed by beans and soybeans (23.2 ± 0.87 and 17.0 ± 0.07 mg/100 g flour, respectively). Instead, the free tryptophan concentrations were similar among lupins and vetches (7.50 ± 0.30 and 7.07 ± 0.57 mg/g flour, respectively). Peas, broad beans, lentils, and groundnuts had lower contents of free tryptophan (5.75 ± 0.20, 4.08 ± 0.26, 3.92 ± 0.13, and 2.24 ± 0.17 mg/100 g flour, respectively).

In Table 2, the values of total non-protein tryptophan, free + protein-bound water-soluble fraction, expressed as mg/100 g dry wt flour, are summarized. Chick-peas present the highest value (75.2 ± 1.11 mg/100 g flour), followed by beans (30.7 ± 1.45 mg/100 g flour) and soybeans (16.9 ± 1.29 mg/100 g flour). Lupins and vetches show similar values (10.3 ± 0.18 and 9.79 ± 0.17 mg/100 g dry wt flour, respectively). Peas contain 7.19 ± 0.13 mg/100 g flour of the water-soluble non-protein tryptophan; broad beans and lentils contain similar amounts (4.73 ± 0.29 and 4.26 ± 0.41 mg/100 g flour, respectively) and groundnuts show the lowest value with respect to the other legumes (3.18 ± 0.19 mg/100 g flour).

Table 2 shows that protein-bound tryptophan was present both in the water-soluble fraction and in the fraction obtained by extraction at pH 8.9. In comparison with the protein-bound water-soluble fraction, tryptophan bound to basic proteins was present in smaller amounts. Beans showed the highest value (6.48 ± 0.51 mg/100 g dry wt flour), followed by chick-peas (5.47 ± 0.19 mg/100 g flour). Lupins had a content of protein-bound tryptophan (pH 8.9 fraction) (2.07 ± 0.13 mg/100 g) similar to that of soy (1.85 ± 0.12 mg/100 g dry flour), but higher than those of vetches, peas and lentils (1.57 ± 0.08, 1.08 ± 0.06 and 0.88 ± 0.04 mg/100 g dry wt flour, respectively), while broad beans and groundnuts had the lowest values (0.59 ± 0.04 and 0.36 ± 0.03 mg/100 g flour, respectively).

4. Discussion

The proteins of legumes are lacking in the sulphur-containing amino acids, methionine and cysteine, but, had high contents of lysine and tryptophan; they can improve the protein quality of cereals, in which tryptophan is usually the second most deficient amino acid after lysine (FAO, 1970; Taira, 1968). Thus, it is important to know the content mainly of the essential amino acids, such as tryptophan, in foods for human nutrition.

Tryptophan is a versatile amino acid; it regulates numerous physiological mechanisms, being converted into many substances of biological significance (Musajo & Benassi, 1964; Wolf, 1974). It is necessary for protein synthesis and it is the precursor of serotonin, melatonin, and nicotinamide and therefore of NAD coenzymes.

Several studies have been carried out on the content of proteic tryptophan in food. However, we found that tryptophan, besides being present in proteins, is also present in non-protein forms in foods, such as milk (Allegri, Biasiolo, Costa, Bettero, & Bertazzo, 1993; Zanardo, Stocchero, Biasiolo, Costa, & Allegri, 1987) and cereals (Comai et al., 2007). Also, legumes contain non-protein tryptophan. Soy is the chief and richest legume in protein consumed in the world. It is used to prepare foods for adults and children besides for animal feed.

Our data (Table 1) on the content of protein and protein tryptophan in soybean flour are similar to those reported by Sato, Seino, Kobayashi, Murai, and Yugari (1984), Duke and Atchley (1986), Souci, Fachmann, and Kraut (1994), higher than the value found by Friedman and Cuq (1988) and lower than that reported by Pinter-Szakacs and Molnar-Perl (1990) and Landry and Delhaye (1992).

Lupin and bean seeds are also rich in protein like soybeans, but the protein tryptophan content is lower than that in soybeans.

The genus *Lupinus* comprises more than 400 different species (Ruiz & Sotelo, 2001), several of which are cultivated for their seeds (Cerletti, 1982). In Europe only 12 species occur and *Lupinus albus* and *luteus* have been especially selected and cultivated. All the species of lupin seeds showed higher lysine and tryptophan contents and a low content of sulphur amino acids, like other legumes. The proteins of lupin seeds have a good nutritive value, but most wild lupin species are considered toxic due to their high content of quinolizidine alkaloids (Keeler, 1989). Ruiz and Sotelo (2001) analysed chemical composition, nutritional and toxicological value of some species of Mexican wild lupins and found that the seeds were a good source of protein, as found by us and reported by Duke and Atchley (1986). However, the seeds, due to the high content of alkaloids, must be extracted by hot water before being used for animal feed. In fact, in South America, where lupin grain is of considerable importance in the provision of human food, the use is preceded by boiling water extraction to remove alkaloids (Ruiz & Sotelo, 2001). Our data on the content of protein tryptophan in lupins are comparable to values found by Mossé, Huet, and Baudet (1987).

Beans are another legume rich in protein, consumed widely in many areas of the world. Their proteins are complementary to the cereal proteins (Sgarbieri, Garruti, Moracs, & Hartman, 1978, 1979, 1989). The high contents of protein tryptophan and lysine in bean seeds contribute to elevate the nutritive value of proteins when mixed with cereals. There are no great differences among our data and those reported by Sgarbieri, Antunes, and Almeida (1979), Wu et al. (1996) and Mbithi-Mwikya et al. (2000).

Groundnuts show a protein content similar to those of beans, broad beans, lentils and lower than that found in soybeans, but higher than those in pea, chick-pea and vetch seeds.

The content of protein tryptophan, as mg/100 g of dry wt flour, in groundnuts is similar to those observed in beans and lupins, but higher than those found in peas, broad beans, vetches and lentils. Considering the content as mg/100 g of protein, the protein tryptophan is similar to that reported by FAO/WHO (1985) . In pea seeds, a constant value of protein tryptophan, close to 800 mg per 100 g of protein, was found by Mossé and Huet (1990), exactly our value, while Müller (1983) reported that protein content can change from 0.5 to 2 g per 16 g nitrogen. Instead, Landry and Delhaye (1992) found 1040 mg/100 g of protein in peas.

Vetch is a legume rarely cultivated in Italy (central Italy and islands), but widely in India and in Mediterranean regions, even though it is rich in a very volatile alkaloid (latirine) which seems to cause lathyrism. It has been reported that the content of protein in this legume varies from 25 to 28 g/100 g flour (Secchi, 1979). We found a value around 25 g/100 g of dry flour with a value of protein tryptophan similar to that of pea seeds.

As regards the non protein tryptophan, our data show that legumes contain both free and protein-bound tryptophan. As reported by Friedman and Cuq (1988), free or protein bound tryptophan is relatively stable during processing and storage of foods; only severe treatments cause a significant degradation of this amino acid. Our data show that the highest content of free tryptophan was found in chick-peas, followed by beans and soybeans, whereas the lowest value was observed for groundnuts. Free tryptophan appeared to be always present, while this is not so for protein-bound tryptophan or water-soluble fraction. In fact, this fraction appears absent in broad bean, lentil and soybean seeds. Instead, it is present in higher amounts in chick-peas than in other legumes.

Tryptophan is also linked to protein fraction extractable at pH 8.9. Bean seeds have the highest concentration of tryptophan linked to the buffered fraction of proteins, followed by chick-pea seeds, with groundnuts showing the lowest amount. Therefore, comparing the contents of non-protein tryptophan among the legumes examined, it appears that groundnut seeds present the lowest values, both as free form and as protein-bound fractions.

Information on the free or protein-bound tryptophan in foods is very limited. The importance of the determination of non-protein tryptophan is easily understandable, considering that tryptophan is one of the limiting amino acids of the biological value in vegetable proteins. The biological value of a food is commonly used to assess the nutritive quality of the protein. Legumes appear to have, not only a high protein content, but also a high concentration of tryptophan, both protein and non-protein. The non-protein fractions are easily absorbed and could increase the availability of this amino acid to the brain (Allegri et al., 1993; Zanardo et al., 1987), as only the free form is able to cross the blood-brain barrier.

In conclusion, the amino acid score and thus the nutritive value of vegetable proteins, must be corrected, considering non-protein tryptophan. This is the most valid way of assessing food quality.

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