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Chemically determined total and available methionine in beans (*Phaseolus vulgaris* L.) and isolated protein fractions

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The total and non-oxidized methionine contents in *Phaseolus vulgaris* L. whole beans and in isolated protein fractions from seven bean varieties were studied. The total methionine contents determined by classical ion-exchange chromatography (IEC) after performic acid oxidation and HCl hydrolysis, ranged in bean meals from 1.30 to 1.53% with an average of 1.42%. Total methionine in bean protein extracts, which accounted for both water- and salt-soluble proteins was similar (1.45%); water-soluble albumins showed an average methionine content of 1.20%. The non-oxidized (considered available) methionine contents in whole beans measured by reaction with BrCN and gas chromatography of methylthiocyanate formed, were unexpectedly higher than total methionine. These results might indicate that interfering compounds, probably S-methylcysteine or its dipeptide with glutamic acid, present in variable amounts in leguminous seeds, over-estimated the potentially available methionine in whole beans. The protein extracts and isolated albumin fractions were devoid of these interfering substances and, as expected, significantly lower available methionine contents were observed. The protein extracts showed values of methionine equivalents, determined as methylthiocyanate, between 0.70 and 0.90% in protein, 37% lower than total methionine determined by IEC, corresponding to an available methionine content of 63%. The albumins showed methylthiocyanate values varying between 0.65 and 0.85% in protein, on average 32% lower than values obtained by IEC, corresponding to 68% available methionine.

INTRODUCTION

True protein quality measurement, carried out by growth and/or metabolic balance studies, cannot be done on a routine basis without great difficulties, and efforts are being made to develop *in vitro* assay techniques as suitable indicators to express protein quality. The nutritional value of bean proteins cannot be predicted only on the basis of their amino acid composition and true protein digestibility, determined by the rat balance method. The low bioavailability of methionine limits the nutritive value of beans and increases the relative deficiency of sulphur amino acids in beans (Nielsen, 1991; Deshpandhe & Damodaran, 1989; Rayas-Duarte *et al.*, 1988). The digestibility of bean methionine was found to be 43% lower than total protein digestibility (Sarwar *et al.*, 1989). Consequently, bean protein digestibility was not a good predictor of bioavailability of methionine from grain legumes. Most studies attribute no more than 50% to the bioavailability of methionine in beans (Evans *et al.*, 1974; Evans & Bauer, 1978; Eggum *et al.*, 1989; Sarwar *et al.*, 1989; Bodwell *et al.*, 1989; McDonough *et al.*, 1989), while bean protein digestibility was estimated to be about 70% (Sarwar & Peace, 1986; Lanfer Marquez & Lajolo, 1990).

Chemical assays for measuring nutritionally available forms of methionine are important for rapid screening purposes, and, in view of the current interest in adopting amino acid scores and/or availability, are corrected by true protein digestibility as the preferred approach for assessing protein quality (Pellet & Young, 1980; Sarwar & McDonough, 1990; Young & Pellet, 1991; FAO/WHO, 1991).

Identification of cultivars or protein fractions with higher amounts of total methionine and/or available methionine could contribute to an improvement in the nutritional level of bean proteins. One strategy is to

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identify the primary structure of seed proteins and to apply, in the future, different molecular approaches to alter amino acid composition and/or bioavailability (De Lumen, 1990).

Development of analytical techniques (even 20 years ago), with powerful resolution, has rendered automatized ion-exchange chromatography after performic acid oxidation and HCl hydrolysis, the method of choice for sulphur amino acid analysis. By this technique the methionine level in bean seeds ranges from 1.1 to 1.7% of total protein (Tobin & Carpenter, 1978). Values reaching 1.3%, on average, are the most frequent (Koehler & Burke, 1988; Rayas-Duarte *et al.*, 1988; Peace *et al.*, 1988). Numerous studies have compared the methionine content in Brazilian bean varieties. Classical ion-exchange chromatography, preceded by performic acid oxidation and acid hydrolysis, indicated that Brazilian beans also contained about 1.3% methionine in protein (Sgarbieri *et al.*, 1979; Sgarbieri, 1989).

This method, however, does not distinguish between methionine, methionine sulphoxide or pre-existing methionine. Chemical tests for measuring nutritionally available forms of methionine have been adopted by some authors in view of the need for further research into the effects of methionine oxidation on the nutritional value of proteins.

Methionine reaction with nitroprusside, as an approach for assessing available methionine, has been utilized and modified, in order to eliminate some coloured interferences. When reduced methionine was determined by enzymatic hydrolysis and colorimetric determination after reaction with nitroprusside, the values varied widely, between 0.85% (Antunes & Sgarbieri, 1979), 1.3% (Antunes & Sgarbieri, 1980), 1.36% (Costa de Oliveira *et al.*, 1987) and 1.42% (Tezoto & Sgarbieri, 1990). These authors carried out the determinations without intent to screen the *Phaseolus* varieties with the exception of Sgarbieri & Martins Galeazzi (1990). They reported a range of 0.40–1.73% methionine in 55 Brazilian varieties of common dry beans quantified by the nitroprusside reactant. Varietal versus analytical differences, as the basis for different methionine values, are difficult to distinguish and the efficiency of removing the interferences may be further improved.

A further approach might be to estimate intact methionine. The procedure for methionine analysis by reaction of unoxidized methylthio groups with cyanogen bromide is specific for methionine in its intact, reduced form, and may be of value for determining the potential nutritionally available methionine. Durigan *et al.* (1987) analysed (comparatively) 12 bean varieties by reaction of cyanogen bromide with methionyl residues. They reported an available methionine content between 0.96 and 1.99% in the bean proteins with an average value of 1.52%. However, the same seeds analysed by ion-exchange chromatography revealed lower methionine contents with an average value of only 1.10%. Methionine levels did not show the expected differences likely to be attributable to the proper specificity of each analytical method used.

It remains to be determined how the 'available' methionine determined by the reaction with nitroprussiate or BrCN is unexpectly higher than total methionine by ion-exchange chromatography, or what reasons can justify the unpredicatable relationship between the methods used.

The experiments reported here had the objective of determining, in different bean varieties and isolated bean fractions, the total methionine content by ion-exchange chromatography, and the content of methionine (in its bioavailable form), by gas chromatography, of methylthiocyanate formed by the reaction of methionine with BrCN. Although analytical information about total methionine is available, inconsistent data have been published on the relationship between total and non-oxidized methionine in beans.

Additional basic information is needed on the methionine content and its availability in isolated bean protein fractions.

MATERIALS AND METHODS

Dry bean seeds of *Phaseolus vulgaris* L. varieties were obtained from the Agronomic Institute of Campinas. Carioca Iapar was a gift from the Agronomic Institute of Paraná, PR. All seeds were harvested and frozen at −20°C until required.

Whole bean flours were obtained by grinding beans in a Janke and Kunkel laboratory mill and passing them through a 0.250 mm sieve.

Extraction and fractionation of proteins

Soluble bean proteins were extracted from 10 g of meal with 100 ml buffer (0.1-M phosphate, 0.5-M NaCl, pH 7.6) using a magnetic stirrer. The suspension was stirred for 1 h and centrifuged at 12 100×*g* for 30 min using a SS-34 (Sorvall) rotor. The resulting clear supernatant was decanted, dialysed against distilled water, lyophilized and stored at −20°C. All procedures were performed at 4°C.

The albumin fraction was obtained by extraction of 10 g of bean meal with 100 ml distilled water during 1 h at 4°C. The homogenate was filtered through layers of cheesecloth and centrifuged at 12 100×*g* for 30 min at 4°C. The supernatant was dialysed against distilled water, lyophilized and stored at −20°C until use.

Analytical techniques

Total N was determined by the micro-Kjeldahl method (Association of Official Analytical Chemists, AOAC, 1984) and a conversion factor of 6.25 was used to convert N to protein content.

Methionine was determined as methionine sulphone after performic acid oxidation and amino acid analysis was carried out on a Beckman 7300 autoanalyser after hydrolysis with 6-N HCl at 110°C for 22 h in vacuum-sealed tubes, essentially as described by Moore (1963).

The chemically available methionine content of the meals and protein fractions was determined by measuring the methylthiocyanate produced from the reaction of methionine with BrCN by gas liquid chromatography according to the procedure described by Finlayson & Mackenzie (1976), with some minor modifications. Samples containing 5–8 mg protein were mixed with 400 μl of BrCN (10% w/v in 98% formic acid) and 100 μl of ethylthiocyanate as an internal standard (3 nanomoles/μl in 40% aqueous formic acid). The reaction was performed in 1-ml screw-cap Pierce Reactivials maintained at 95°C for 1 h in a Pierce Reactitherm digestion block. Aliquots of 1.0 μl of the clear supernantant were injected in a Varian Series 2800 Moduline gas chromatograph equipped with a flame ionization detector and a Spectra Physics SP4100 automatic data integrator. The analysis was done in duplicate, injecting five replications of each duplicate in the column. A glass column (1.8 m×2 mm i.d.) previously treated with dimethyldichlorosilane according to Ellinger & Duncan (1976) packed with Chromosorb 101 as suggested by Duncan *et al.* (1984*a*) was used with nitrogen at a flow rate of 30 ml/min, hydrogen at 30 ml/min and air at approx. 500 ml/min. Column and detector temperatures were kept at 160 and 200°C, respectively. The detector output was attenuated at 2×10^{-11} A.

Statistical analysis

Analysis of variance was performed on the data using one-way ANOVA from the STATGRAPHICS statistical package. When the F value was statistically significant at the $P<0.05$ level, Tukey's significant difference test was used to compare the means (Neter *et al.*, 1985).

RESULTS AND DISCUSSION

Methionine content

Methionine and protein contents of the eight *Phaseolus vulgaris* seed varieties analysed are listed in Table 1. The methionine contents of the varieties, determined by the classical IEC (automated ion-exchange chromatography) preceded by performic acid oxidation, were statistically similar ($P<0.05$) and it was not possible to show any bean variety with a higher methionine content in the bean protein.

Among the varieties studied, the mean methionine content was 1.42% in the protein, ranging from 1.30 to 1.53%. In the present study, data on total methionine from bean varieties, grown in Brazil, are as expected, in agreement with those found by other researchers (Koehler & Burke, 1988; Rayas-Duarte *et al.*, 1988; Peace *et al.*, 1988; Marletta *et al.*, 1992) and even similar to previously published reports of other legumes. Sosulski & McCurdy (1987) reported methionine contents of 1.5% in protein for soybean isolates.

Despite the lack of significant differences among bean varieties, the *Moruna*, *Goiano Precoce* and *Aeté-3* varieties deserve special emphasis because these species had more protein and therefore a higher methionine content in the seed. Although the relationship between protein and methionine concentration is still unclear, a higher level of phaseolin which represents an important part of the total protein (~50%), has been suggested as being associated with increased available methionine contents in bean seeds. (Kelly & Bliss, 1975; Gepts & Bliss, 1984).

Table 1. Protein and methionine contents in bean flours[1]

Variety	Protein %	Methionine[2,3]
Moruna	27.4	1.42 ± 0.05^{a}
Goiano Precoce	26.5	1.30 ± 0.08^{a}
Aeté-3	23.6	1.48 ± 0.05^{a}
Aysó	22.1	1.40 ± 0.12^{a}
Carioca IAC	21.8	1.53 ± 0.13^{a}
Catu	21.8	1.37 ± 0.04^{a}
Carioca 80	20.8	1.38 ± 0.05^{a}
Carioca Iapar	18.8	1.45 ± 0.09^{a}
Mean		1.42 ± 0.07^{a}

[1]Data expressed on a weight basis (10% moisture); mean of triplicate determinations.
[2]Methionine values by ion-exchange chromatography after performic acid oxidation and HCl hydrolysis, expressed in g per 100 g protein.
[3]Mean values in the same column with different letter designations are significantly different ($P<0.05$).

Data on methionine contents of isolated protein extracts and albumin fractions from different bean varieties are given in Table 2. The protein extract represents 57.0–67.0% of the total bean proteins, and comprises the combined extractable water- and salt-soluble proteins. The methionine contents in protein extracts showed an average value of 1.45%. It should be noted that values are in close agreement with those exhibited by bean flours (1.42%). This fact implies that the phaseolin greatly contributes to the methionine levels in beans, while methionine contents of

Table 2. Protein and methionine contents in protein extracts and isolated albumin fractions of beans[1,2]

	Protein Extract		Albumin	
Variety	Protein %	Methionine[3] %	Protein	Methionine[3]
Moruna	82.3	1.55 ± 0.08^{cd}	75.7	1.25 ± 0.04^{b}
GoianoPrecoce	83.2	1.17 ± 0.03^{a}	53.0	1.33 ± 0.09^{b}
Aeté-3	84.2	1.48 ± 0.05^{bcd}	71.2	1.28 ± 0.05^{b}
Aysó	78.1	1.48 ± 0.02^{bcd}	68.8	0.95 ± 0.01^{a}
Carioca IAC	79.2	1.54 ± 0.05^{cd}	68.1	1.16 ± 0.01^{ab}
Catu	77.2	1.41 ± 0.04^{bc}	68.0	1.13 ± 0.09^{ab}
Carioca 80	78.6	1.38 ± 0.05^{b}	72.3	1.27 ± 0.17^{b}
Carioca Iapar	74.7	1.62 ± 0.07^{d}	ND	ND
Mean		1.45 ± 0.05		1.20 ± 0.13

[1,2]Mean of triplicate determinations. Methionine values by ion-exchange chromatography after performic acid oxidation and HCl hydrolysis expressed in g per 100 g protein.
[3]Mean values in the same column with different letter designations are significantly different ($P<0.05$).

water-soluble proteins (albumins) which comprised about 10–15% of total protein content, ranged from 0.95 to 1.33% with a mean value of 1.20%. Since the albumins showed low methionine contents, and their total sulphur amino acid contents (methionine + cysteine) were high (mean 1.95% in protein; unpublished), this can be attributed to the high trypsin inhibitor activity and therefore high levels of cysteine exhibited by this protein fraction. The major storage protein of the beans, the phaseolin or G1, unlike the albumins, showed a very low (< 0.3%) cysteine content (Lanfer Marquez & Lajolo, 1981), but discrepancies from these values have been observed and seem to be due to cross-contamination of the water- and salt-soluble proteins (Bhatty, 1982).

Methionine availability

The procedure for methionine analysis by reaction of unoxidized methylthio groups with cyanogen bromide was applied to the whole bean flours, bean protein extracts and albumins. The available methionine content of the bean flours from seven varieties is presented in Fig. 1, in comparison with data on total methionine content determined by ion-exchange chromatography. Among the varieties studied, the average available methionine was similar to, or slightly higher than, total methionine determined by IEC, varying between 1.4 and 1.8% in protein. These results are difficult to explain but, in other studies, similar high values have been observed (Durigan *et al.*, 1987).

High non-oxidized (available) methionine contents were also observed by other researchers. Duncan *et al.* (1984*b*) found an average value of 1.10% methionine in the protein of seven *Phaseolus* varieties. A similar value was reported by McIntosh & Ellinger (1976). Kohnhorst *et al.* (1987) reported 1.4 and 1.2% methionine in navy and kidney beans. Their findings refer to analysis of methylthiocyanate by gas chromatography as a result of reaction of reduced methionine with BrCN.

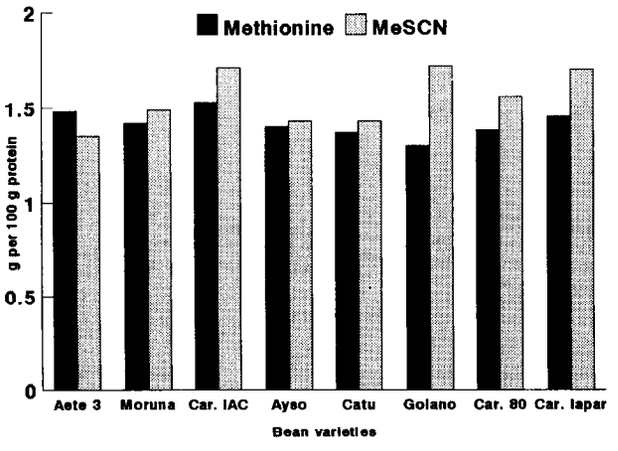

Fig. 1. Mean level of total and available methionine in whole bean flours of seven varieties. Total methionine by ion-exchange chromatography and available methionine by MeSCN after reaction with BrCN and gas chromatography.

Nevertheless, the methionine bioavailability data obtained by feeding trials with laboratory animals has been very low in beans. Bean protein digestibility was estimated to be about 70% (Sarwar & Peace, 1986; Lanfer Marquez & Lajolo, 1990). According to a review (Sarwar, 1987), digestibilities of protein and most individual amino acids differ by less than 10% for animal protein sources, low fibre cereals and oilseeds but true digestibilities of limiting amino acids from beans were 43% lower than those of the protein, respectively, in contrast to other protein sources (Sarwar *et al.*, 1989). Therefore, low digestibility does not seem to be the unique explanation for the observed reduced methionine bioavailability (Sarwar & McDonough, 1990). The presence of residual levels of antinutritional factors such as trypsin inhibitors, lectins, polyphenols, tannins and perhaps the limiting amino acids in a non-available form (oxidized), seems to be implicated with their low bioavailability.

Although the cyanogen bromide reaction is considered a very specific method for methionine analysis, there are non-methionine compounds in legume seeds that could possibly release methylthiocyanate by reaction with BrCN and thereby contribute falsely to the apparent high available methionine content in the whole bean flours. One of these compounds, *S*-methylcysteine, generally present as a dipeptide with glutamic acid, γ-glutamyl-*S*-methylcysteine, has a restricted occurrence in plants. They are characteristic constituents of Allium species, whereas some works describe their presence in variable quantities in legumes (Kasai & Larsen, 1980). In beans, their content was estimated at 0.87% in protein (Evans & Boulter, 1975). Zacharius (1970) estimated in 11.5 μmol of γ-glutamyl cysteine per g of bean seed, which corresponds to 1.28% in the protein, assuming a protein content of 25% in the bean. However, Apostolatos & Hoff (1981) suggested that the contribution of non-protein sulphur compounds such as γ-glutamyl-*S*-methylcysteine, isolated from an acidic ethanolic bean extract may be considered negligible to the determination of methionine by the BrCN method. Durigan *et al.* (1987) reported the BrCN-determined methionine contents of seven common bean varieties, after previously removing interfering compounds having a methylthio side chain. The results showed methionine contents (0.96–1.99% in proteins) up to two-fold higher than when determined by IEC (1.10%). It is quite likely that the previous ethanolic extraction of γ-glutamyl derivatives had not been successful.

Methionine reaction with nitroprusside, after enzymic digestion of the protein as an approach for assessing available methionine, has been utilized and modified by some authors as mentioned before, but in our laboratory we were not able to eliminate the interference of coloured byproducts and we cannot recommend this method for intact methionine contents in whole beans; in addition, interference of sulphur γ-glutamyl peptides as observed by the BrCN reaction can also be observed by reaction with nitroprusside (not published). Marletta *et al.* (1992) reported (following colorimetric reaction

with sodium nitroprusside), over 90% of total methionine in white and brown varieties, but not two red varieties (55 and 74%). Further studies on usefulness of this method for bean methionine availability are needed. For whole beans, additional studies are in progress and preliminary results in our laboratory showed a considerable content of the previously-mentioned sulphur dipeptide in the ethanolic extract of beans, which will be an object of further investigations.

The protein extracts, derived from the seven bean varieties studied, showed values between 0.70 and 0.90% methionine, determined as MeSCN equivalents. On average, these values are 37% lower than methionine determined by IEC, as illustrated in Fig. 2. These results provide information about the real methionine availability which could be conveniently used as an additional correction factor in evaluation of procedures based on the protein digestibility-corrected amino acid score method.

The available methionine contents in the albumin fractions are also lower than total methionine, varying between 0.65 and 0.85% in the protein and, as observed

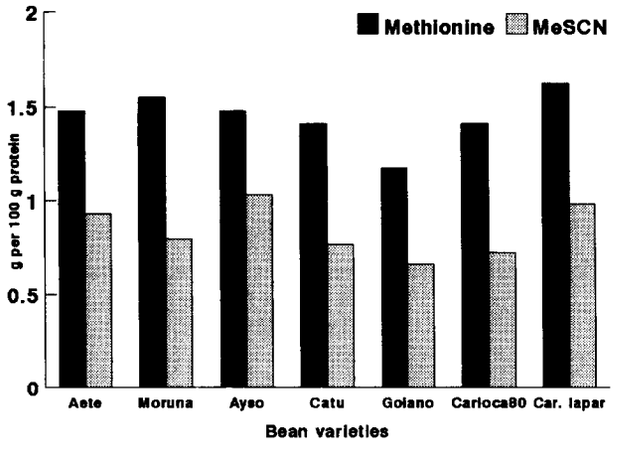

Fig. 2. Mean level of total and available methionine in bean protein extracts of seven bean varieties. Total methionine by ion-exchange chromatography and available methionine by MeSCN after reaction with BrCN and gas chromatography.

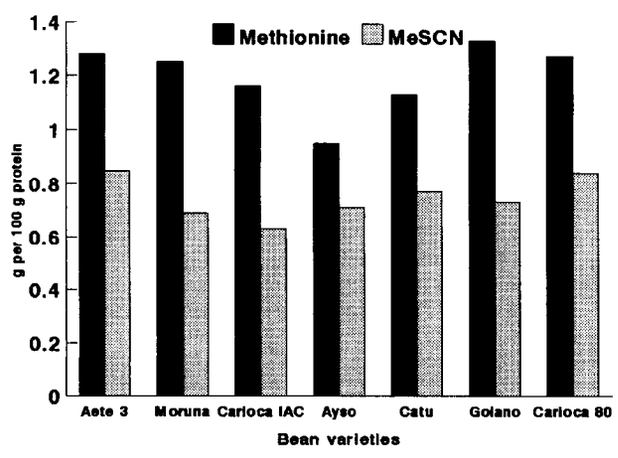

Fig. 3. Mean level of total and available methionine in albumin fractions of seven bean varieties. Total methionine by ion-exchange chromatography and available methionine by MeSCN after reaction with BrCN and gas chromatography.

for protein extracts, are on average 32% lower than by the IEC method (Fig. 3). The method presently used seems to accurately reflect the methionine availability and the data would provide a realistic amino acid score.

The calculated available methionine from protein extracts and albumins corresponds, respectively, to 63 and 68% of total methionine and this value can be recommended to be adopted for calculation of the protein digestibility-corrected amino acid score.

Therefore, true whole bean protein digestibility (70–80%) (Sarwar & Peace, 1986; Lanfer Marquez & Lajolo, 1990), when corrected by the highest available methionine in bean protein fractions found in this study, results in digestibility-corrected methionine scores between 52 and 54%. In conclusion, this study appears to indicate that the non-oxidized methionine content from individual bean protein fractions can be extrapolated to whole bean proteins and can be used to predict the bioavailability of bean methionine. Values of methionine bioavailability in whole beans, determined by rat balance and microbiological assays, seem to be actually lower than 50% (Evans *et al.*, 1974; Evans & Bauer, 1978; Eggum *et al.*, 1989; Sarwar *et al.*, 1989; Bodwell *et al.*, 1989; McDonough *et al.*, 1989).

On the other hand, the available methionine analysis applied to whole bean flours produced significantly more MeSCN than the isolated protein fractions and, according to our hypothesis, these differences are due to to the presence of some interfering compounds present in whole beans, which needs more research to substantiate. Studies on the interfering sulphur-containing non-protein amino acids and peptides are in progress.

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