

# Free Amino Acids Present in Commercially Available Seedlings Sold for Human Consumption. A Potential Hazard for Consumers

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The importance of fresh seedlings for human consumption on European markets continues to increase. Although the contents of free amino acids and potentially toxic free nonprotein amino acids in these fresh and supposedly healthy seedlings is very different from those of the seeds, the crude composition is never mentioned on commercial packages. A commercial product containing seven different kinds of fresh seedlings including kamut, adzuki bean, chickpea, mungbean, pinto bean, garden pea, and lentil has been analyzed by HPLC. Per 100 g of fresh product, 548.2 mg of total free amino acids was found, of which 56.7 mg is free nonprotein amino acids including  $\beta$ -(isoxazolin-5-on-2-yl)alanine, homoserine, and isowillardiine and the plant hormone trigonelline (*N*-methylnicotinic acid). The highest amounts of free nonprotein amino acids and trigonelline are found in garden pea (28.3 mg/100 g), mungbean (9.59 mg/100 g), and lentil (7.50 mg/100 g) seedlings. Trigonelline is present in all legume seedlings examined.

**Keywords:** *Nonprotein amino acids; trigonelline; inherent toxicant; Leguminosae; edible sprouts*

## INTRODUCTION

Besides the ever-popular cress seedlings (*Lepidium sativum* L., Cruciferae), a number of germinated seeds, mainly of leguminous and cruciferous plant species, are becoming a fashionable component of the human diet in Europe. Recently, the selection of fresh seedlings available in food shops has increased and now contains soybean, mungbean, lentil, pea, lucerne, fenugreek, sunflower, cress, rocket salad, radish, leek, etc. The reason for this rising popularity may be dietetics, the image of exotic health food, or the phenomenon of fashion. In oriental cuisine, a variety of seedlings are traditionally utilized; these ingredients are, however, not consumed in the raw state but are fried or boiled for a few minutes, whereas in Europe these seedlings are often blended in salads and consumed raw.

Apart from the 20 amino acids that are normal protein constituents and some 25–30 amino acids that are known to be metabolic intermediates in amino acid biosynthesis (e.g., ornithine, homoserine) or in other primary metabolic pathways (e.g., *S*-adenosylmethionine in methylation reactions) (Kuo, 1983), a much larger number of amino acids are found as secondary metabolites in nature. Over 700 of such compounds have been listed (Hunt, 1985).

During the germination of leguminous seeds, some nonprotein amino acids decrease while others increase or new compounds appear. In pea and lentil, high concentrations of homoserine appear during germination; this remains a prominent metabolite during the vegetative life cycle, but it is absent from the dry seeds. A number of heterocyclic compounds, containing a uracil or an isoxazolinone ring, also appear during the germination of pea and lentil.

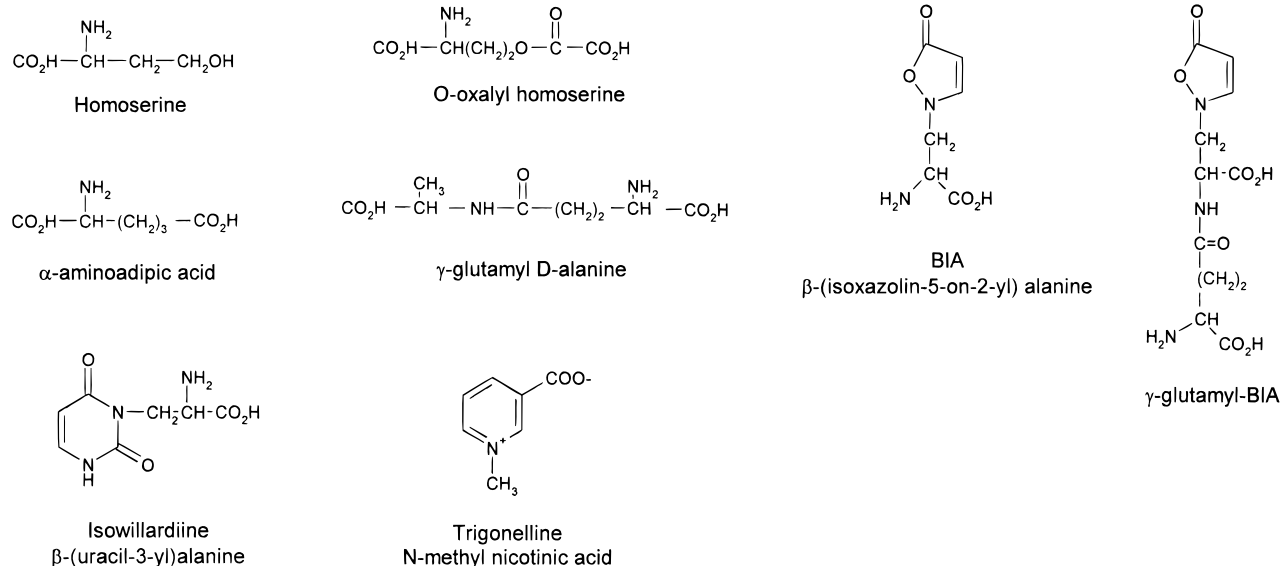
The compounds in this study have different chemical structures and different biochemical origins: homologues or derivatives of protein amino acids ( $\alpha$ -amino-adipic acid, homoserine, *O*-oxalylhomoserine,  $\gamma$ -glutamyl-D-alanine); a uracil derivative (isowillardiine); isoxazolinones [ $\beta$ -(isoxazolin-5-on-2-yl)alanine (BIA) and  $\gamma$ -glutamyl-BIA], and a derivative of nicotinic acid (trigonelline). The structures of these compounds are presented in Figure 1.

Some of these nonprotein amino acids are potentially toxic for humans. One compound common to all pea and lentil species is BIA. This compound is also present in many *Lathyrus* species, where it is the metabolic precursor of a neurotoxic amino acid  $\beta$ -*N*-oxalyl-L- $\alpha$ , $\beta$ -diaminopropionic acid ( $\beta$ -ODAP) (Kuo et al., 1994), which is thought to be responsible for a crippling human disease, lathyrism (Spencer et al., 1986). This irreversible neurodegenerative disease seems to occur only after prolonged and almost exclusive consumption of the seeds of grasspea (*Lathyrus sativus* L.), locally named khesari in India and guaya in Ethiopia. The most recent epidemic of lathyrism has occurred in Ethiopia since 1997 after a drought and famine (Getahun et al., 1999) and is still going on. New cases of this disease have appeared in Bangladesh, Nepal, and Afghanistan. BIA itself is slightly neurotoxic; at concentrations of 0.5–2.0 mM it produced a concentration-dependent neurodegeneration in mouse cortical explants (Riepe et al., 1995).

Besides BIA, several other heterocyclic amino acids containing the five-membered isoxazolinone ring have been found in legume seedlings belonging to the genera *Lathyrus*, *Pisum*, and *Lens*, where the metabolism of BIA is different (Kuo et al., 1998).

The  $\gamma$ -glutamyl derivative of BIA was found in *Lens culinaris* L. (Lambein et al., 1992). The physiological role of this compound in the plant is not known. Recently, Kusama-Eguchi et al. (1999) found in cultured

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**Figure 1.** Some nonprotein amino acid and trigonelline structures present in legume seedlings.

**Table 1. Total Free Protein and Nonprotein Amino Acids Including Trigonelline, in Seedling Axes and Cotyledons of the Seven Studied Seedlings (Percent of Fresh Weight)**

	adzuki	kamut	chickpea	lentils	mungbean	garden pea	pinto bean	total
	Seedling Axes							
TFA <sup>a</sup> (%)	1.17	1.16	0.71	0.92	0.46	1.71	0.37	6.50
TFNA <sup>b</sup> (%)	0.07	— <sup>c</sup>	0.07	0.18	0.02	1.0	0.08	1.42
fresh wt (g)	1.0	0.6	3.6	5.3	27.8	2.4	0.4	41.1
	Cotyledons							
TFA (%)	0.38	0.16	0.35	0.57	0.88	0.66	0.63	3.64
TFNA (%)	0.03	—	0.02	0.06	0.06	0.16	0.07	0.40
fresh wt (g)	28.0	11.6	24.2	24.8	22.0	15.6	4.8	131.0

<sup>a</sup> TFA, total free amino acids. <sup>b</sup> TFNA, total free nonprotein amino acids. <sup>c</sup> Not detected.

cortical neurons of rat that  $\gamma$ -glutamyl-BIA showed weak antagonistic effects against NMDA receptors and weak agonistic effects at AMPA receptors and cloned glutamate transporter.

The leguminosae (peas, beans) are a rich hunting ground for nonprotein amino acids. Especially for vegetarians and for those who cannot afford animal protein, legumes are the main source of dietary protein. The garden pea, lentil, and mungbean are the most studied seeds, but the new foods include a large diversity of seedlings for which there is insufficient nutritional, toxicological, or pharmacological information.

Isowillardine was identified in our laboratory as a uracil-alanine compound (Lambein and Van Parijs, 1968). The quantity of isowillardine in pea seedlings can reach 0.2% of the dry weight in the whole seedling (Mettrie, 1974). Janzen et al. (1977) studied the toxicity of nonprotein amino acids to the seed-eating larvae of the bruchid beetle *Callosobruchus maculatus* F. and reported that isowillardine at 1% concentration in the normal diet significantly inhibits the development of larvae into beetles.

Trigonelline is not an amino acid, but its multiple functions in plant growth and development are very important (Tramontano and Jouve, 1997), and there is little or no information on its nutritional properties. Trigonelline was first isolated in 1895 from *Trigonella foenum-graecum* L. (fenugreek), and it is also an important constituent in green coffee beans (Stennert and Maier, 1996; Wu et al., 1997). Trigonelline is a polyvalent metabolite in the physiology of plants. In certain growth conditions, trigonelline is one of the secondary

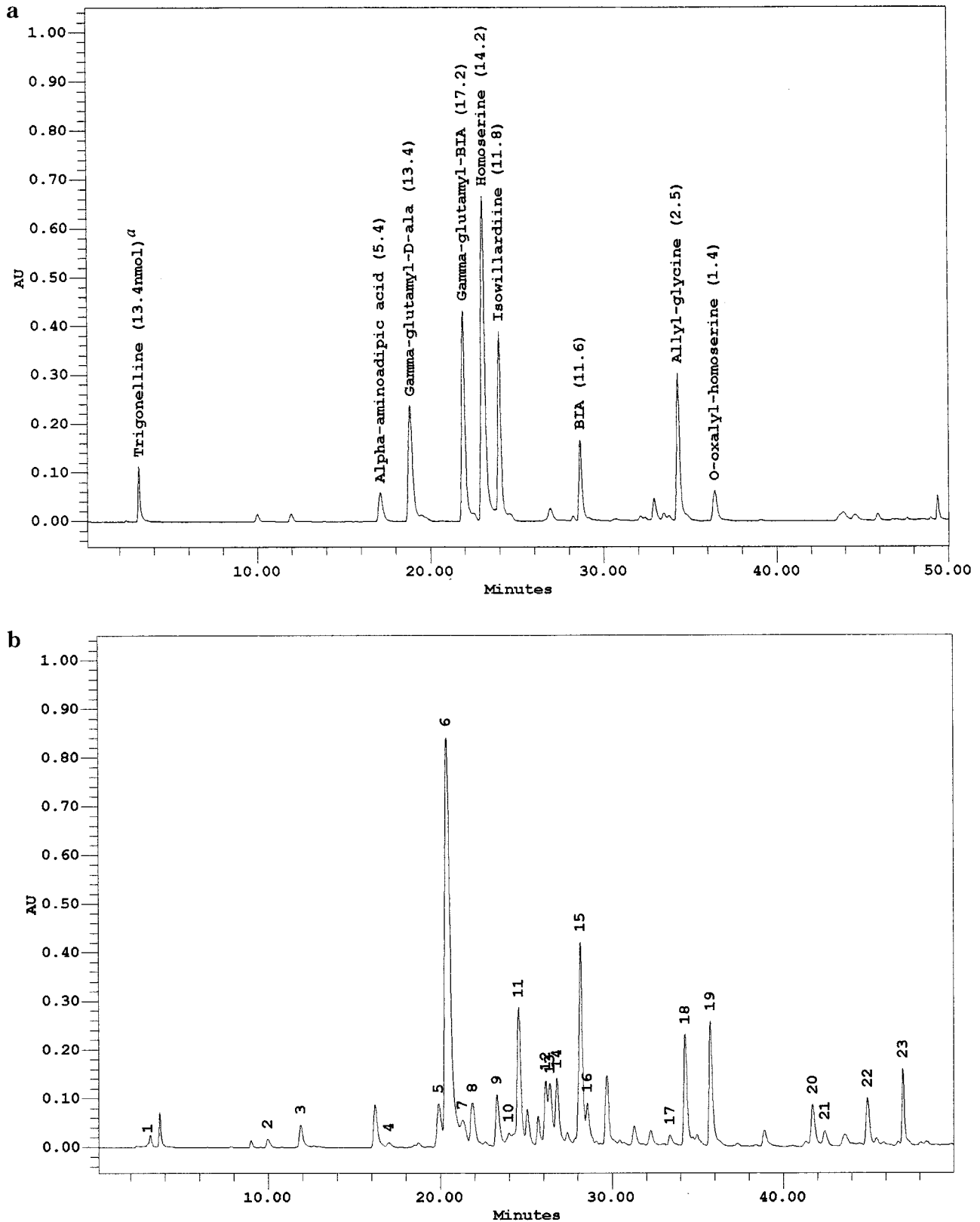
messengers in plant cells under stress, preventing oxidative stress caused by UV-B light (Kalbin et al., 1997a,b) or preventing water loss (Tramontano and Jouve, 1997). Trigonelline can be considered to be a multifunctional natural plant hormone: it also has a function as a cell cycle regulator during the early growth of many legume root meristems (Tramontano and Jouve, 1997).

For consumer's safety, it is imperative to determine the presence and concentration of those compounds in these new foods and to evaluate the potential hazard for human health. A previous study (Rozan et al., 1999) comparing seedlings of garden peas and lentils grown in semisterile conditions in the laboratory and commercial products showed no important differences in free nonprotein amino acid between these two growth conditions. Most of the work on seedlings studied the presence of antinutritional factors such as trypsin inhibitor (Frias et al., 1995), carbohydrates (Vidal-Valverde and Frias, 1992; Frias et al., 1996), thiamin, riboflavin, and niacin (Prodanov et al., 1997).

The aim of this study is to determine any inherent toxic amino acid in the seedlings presently available in European markets and to expose eventual hazards to the consumer.

## MATERIALS AND METHODS

**Plant Material.** Fresh commercial seed sprouts were bought in a local supermarket. The seeds were already germinated with unknown conditions. The extracts were made at the last day of the recommended consumption period. The



**Figure 2.** Chromatograms of nonprotein amino acid standards (a) and total amino acids in lentil seedlings (b) by HPLC. Peaks: 1, trigonelline; 2, Asp; 3, Glu; 4,  $\alpha$ -aminoadipic acid; 5, Ser; 6, Asn; 7, Gly; 8,  $\gamma$ -glutamyl-BIA; 9, Hse; 10, isowillardiine; 11, His; 12, Arg; 13, Thr; 14, Ala; 15, Pro; 16, BIA; 17, Tyr; 18, allylglycine (internal standard); 19, Val; 20, Ile; 21, Leu; 22, Phe; 23, Lys. Peaks without numbers are unidentified compounds. <sup>a</sup>The nanomoles injected are shown in parentheses.

germinated seeds used in this product consisted mainly of a variety of wheat already used in Pharaonic Egypt, *Triticum polonicum* L. (kamut), *Vigna angularis* L. (adzuki bean), *Cicer arietinum* L. (chickpea), *Lens culinaris* L. (lentil), *Pisum sativum* L. (garden pea), *Vigna radiata* L. (mungbean), and

*Phaseolus vulgaris* L. (pinto bean). Kamut is the only cereal in this study. The cotyledons and seedling axes were separated and frozen.

**High-Performance Liquid Chromatography (HPLC) Analysis of Free Amino Acids.** Extraction was done by

**Table 2. Composition of Free Nonprotein Amino Acids and Trigonelline in Seedling Axes (Milligrams per Gram of Fresh Weight  $\pm$  SD<sup>a</sup>)**

	adzuki	chickpea	lentils	mungbean	garden pea	pinto bean
trigonelline	0.64 $\pm$ 0.02	0.68 $\pm$ 0.04	0.37 $\pm$ 0.04	0.13 $\pm$ 0.00	0.86 $\pm$ 0.00	0.73 $\pm$ 0.05
$\alpha$ -aaa <sup>b</sup>	0.06 $\pm$ 0.01	- <sup>c</sup>	0.62 $\pm$ 0.07	0.02 $\pm$ 0.00	0.13 $\pm$ 0.00	0.03 $\pm$ 0.00
$\gamma$ -glu-D-Ala <sup>d</sup>	—	—	—	—	—	0.05 $\pm$ 0.00
BIA <sup>e</sup>	—	—	0.17 $\pm$ 0.01	—	1.31 $\pm$ 0.01	—
$\gamma$ -glu-BIA <sup>f</sup>	—	—	0.40 $\pm$ 0.09	—	—	—
Hse <sup>g</sup>	—	—	0.16 $\pm$ 0.02	—	6.97 $\pm$ 0.08	—
<i>O</i> -oxalyl-Hse	—	—	0.04 $\pm$ 0.00	—	—	—
isowillardine	—	—	—	—	0.67 $\pm$ 0.01	—

<sup>a</sup>  $\pm$ SD, standard deviation (four replicates). <sup>b</sup>  $\alpha$ -aaa,  $\alpha$ -amino adipic acid. <sup>c</sup> Not detected. <sup>d</sup>  $\gamma$ -glu-D-Ala,  $\gamma$ -glutamyl-D-alanine. <sup>e</sup> BIA,  $\beta$ -(isoxazolin-5-on-2-yl)alanine. <sup>f</sup>  $\gamma$ -glu-BIA,  $\gamma$ -glutamyl-BIA. <sup>g</sup> Hse, homoserine.

crushing the frozen seedling axes or cotyledons with a mortar and pestle and adding 4 volumes of cold 70% ethanol. After storage overnight at 4 °C, the samples were centrifuged (34800g, 20 min), and the pellets were washed twice with 70% ethanol. The supernatants were pooled and concentrated under vacuum and finally stored in the deep freezer at  $-20$  °C.

The free amino acid and trigonelline contents of the extracts were analyzed by an HPLC gradient system with precolumn phenylisothiocyanate (PITC) derivatization (Khan et al., 1994). Buffer A (0.1 M ammonium acetate, pH 6.5) and buffer B (0.1 M ammonium acetate containing acetonitrile and methanol, 44:46:10, v/v, pH 6.5) were used.

For sample preparation, a 50  $\mu$ L aliquot of extract was removed and dried under vacuum (37 °C, 20 mmHg). Then 20  $\mu$ L of a first coupling reagent [methanol, water, triethylamine (TEA) (2:2:1; v/v)] was added. After mixing, the sample was directly dried under vacuum during 10 min and was then reacted with 30  $\mu$ L of PITC reagent [methanol, PITC, TEA, water (7:1:1:1; v/v)] at room temperature for 20 min before drying under vacuum to remove PITC.

The derivatized samples were then redissolved in 500  $\mu$ L of buffer A that is used as mobile phase for HPLC and filtered through a Millipore membrane (0.22  $\mu$ m). A 20  $\mu$ L sample was injected into an HPLC system (Waters model 991 equipped with a photodiode array detector) using a gradient system of buffer A (100–0% after 50 min) and buffer B (0–100% after 50 min). The operating temperature was 43 °C. A C18 reversed-phase column from Alltech (Alltima C18 5U, 250  $\times$  4.6 mm) was used. The absorbance at 254 nm was used for calculations. The UV absorption spectrum was useful for the identification. A standard protein amino acid mixture (food hydrolysate A 9656, Sigma) was prepared as above. Some nonprotein amino acids were purified from plant extracts as standards. DL-Allylglycine (Sigma) was used as the internal standard with a recovery of 91.6%. The results were analyzed by Millennium software (Waters, version 1.10).

## RESULTS

Table 1 shows the total free amino acids (TFA) and the total free nonprotein amino acids plus trigonelline (TFNA) in percent of total fresh weight of seedling axes or cotyledons of the varieties of sprouts. The total free amino acids include free protein amino acids, free nonprotein amino acids, and trigonelline.

The content of total free amino acids is very different in the various species of seedling axes. The garden pea seedling axes produced the highest contents in total free amino acids (1.71% of fresh weight) and in total free nonprotein amino acids (1.00% of fresh weight): 58% of the free amino acids are nonprotein, including trigonelline. The lentil seedling axes contained 0.92% of fresh weight of total free amino acids and 0.18% of fresh weight of total free nonprotein amino acids (19% of total free amino acids). Figure 2 represents the chromatograms of nonprotein amino acid standards (Figure 2a) and total free amino acids found in lentil seedling axes (Figure 2b) by HPLC. The other seedling axes contained

lower amounts of total free nonprotein amino acids, and in the cereal kamut no free nonprotein amino acids were found.

Considering the weight of seedling axes of each variety, the legumes mungbean, lentil, and garden pea seedling axes contribute 82.6% of the total free amino acids of this food and almost 100% of the total free nonprotein amino acids.

The difference in content of total free amino acids in cotyledons of each species was much less than in seedling axes. The cotyledons contained between 0.16% of fresh weight (for kamut) and 0.9% of fresh weight (for mungbean) of total free amino acids. In general, cotyledons contained less total free amino acids and total free nonprotein amino acids than seedling axes except for pinto bean and mungbean, for which the contents of both total free amino acids and total free nonprotein amino acids were higher in the cotyledons than in the seedling axes.

Considering that the total weight of cotyledons was higher than the total weight of seedling axes (131.0 and 41.1 g, respectively), the contribution by cotyledons to the total free nonprotein amino acids in this food is considerable.

In the commercialized product (seedling axes and cotyledons) the garden pea contributes 50% of the total free nonprotein amino acids in this food, mungbean 16.9%, lentil 13.2%, and adzuki 9.7%. The free protein amino acids are present in higher quantities (8.6 times more) than the free nonprotein amino acids and trigonelline.

About 56.66 mg in 100 g of fresh weight (0.057%) of this food is found to consist of total free nonprotein amino acids.

In seedling axes the major free nonprotein amino acids found were  $\alpha$ -amino adipic acid,  $\gamma$ -glutamyl-D-alanine, BIA,  $\gamma$ -glutamyl-BIA, isowillardine, homoserine, *O*-oxalylhomoserine, and trigonelline or *N*-methylnicotinic acid (Table 2; Figure 1). No free nonprotein amino acids were found in kamut; the results are not given in the table. The major compounds were homoserine (0.16 and 6.97 mg/g of fresh weight of lentil and garden pea seedlings, respectively), BIA (0.17 and 1.31 mg/g in lentil and garden pea seedlings, respectively), trigonelline (from 0.13 to 0.86 mg/g),  $\alpha$ -amino adipic acid (from 0.02 to 0.62 mg/g), isowillardine (0.67 mg/g in garden pea seedling axes), and finally  $\gamma$ -glutamyl-BIA (0.40 mg/g in lentil seedling axes) (Table 2).

In cotyledons the major free nonprotein amino acids found were homoserine (0.20 mg/g in lentil and 1.10 mg/g in garden pea),  $\alpha$ -amino adipic acid (from 0.10 to 0.29 mg/g), and trigonelline (from 0.15 to 0.41 mg/g) (Table 3). No free nonprotein amino acids were found in kamut; the results are not reported in the table.

**Table 3. Composition of Free Nonprotein Amino Acids and Trigonelline in Cotyledons (Milligrams per Gram of Fresh Weight  $\pm$  SD<sup>a</sup>)**

	adzuki	chickpea	lentils	mungbean	garden pea	pinto bean
trigonelline	0.22 $\pm$ 0.03	0.15 $\pm$ 0.02	0.17 $\pm$ 0.04	0.26 $\pm$ 0.03	0.22 $\pm$ 0.01	0.41 $\pm$ 0.01
$\alpha$ -aaa <sup>b</sup>	0.10 $\pm$ 0.01	— <sup>c</sup>	0.17 $\pm$ 0.02	0.29 $\pm$ 0.00	0.18 $\pm$ 0.00	0.23 $\pm$ 0.01
BIA <sup>d</sup>	—	—	0.07 $\pm$ 0.01	—	0.09 $\pm$ 0.00	—
$\gamma$ -glu-D-Ala <sup>e</sup>	—	—	—	—	—	0.07 $\pm$ 0.00
Hse <sup>f</sup>	—	—	0.20 $\pm$ 0.02	—	1.10 $\pm$ 0.00	—
O-oxalyl-Hse	—	—	0.03 $\pm$ 0.00	—	—	—

<sup>a</sup>  $\pm$ SD, standard deviation (four replicates). <sup>b</sup>  $\alpha$ -aaa,  $\alpha$ -amino adipic acid. <sup>c</sup> Not detected. <sup>d</sup> BIA,  $\beta$ -(isoxazolin-5-on-2-yl)alanine. <sup>e</sup>  $\gamma$ -glu-D-Ala,  $\gamma$ -glutamyl-D-alanine. <sup>f</sup> Hse, homoserine.

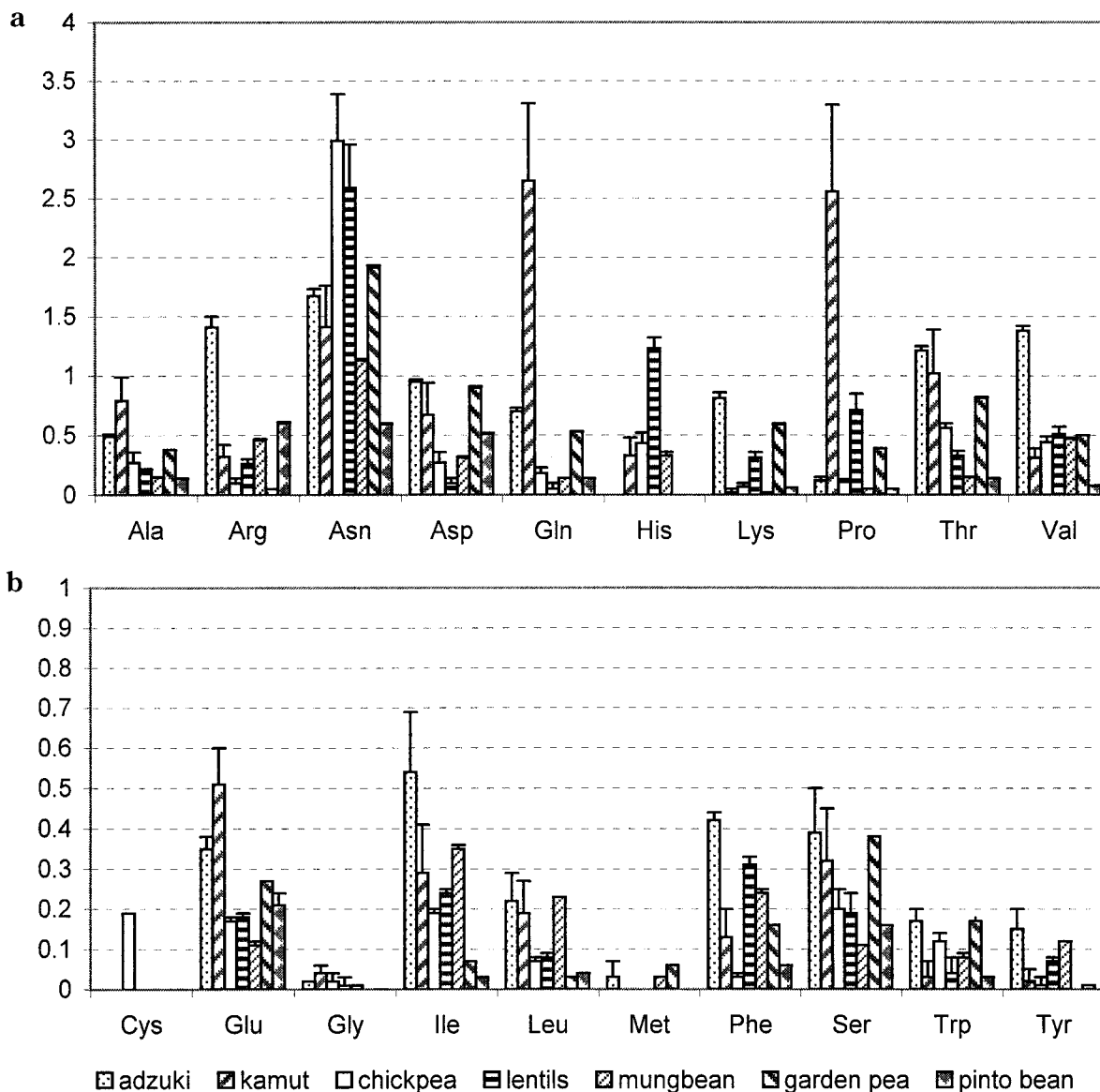
**Figure 3.** Composition of free protein amino acids in seedling axes (milligrams per gram of fresh weight  $\pm$  standard deviation, four replicates).

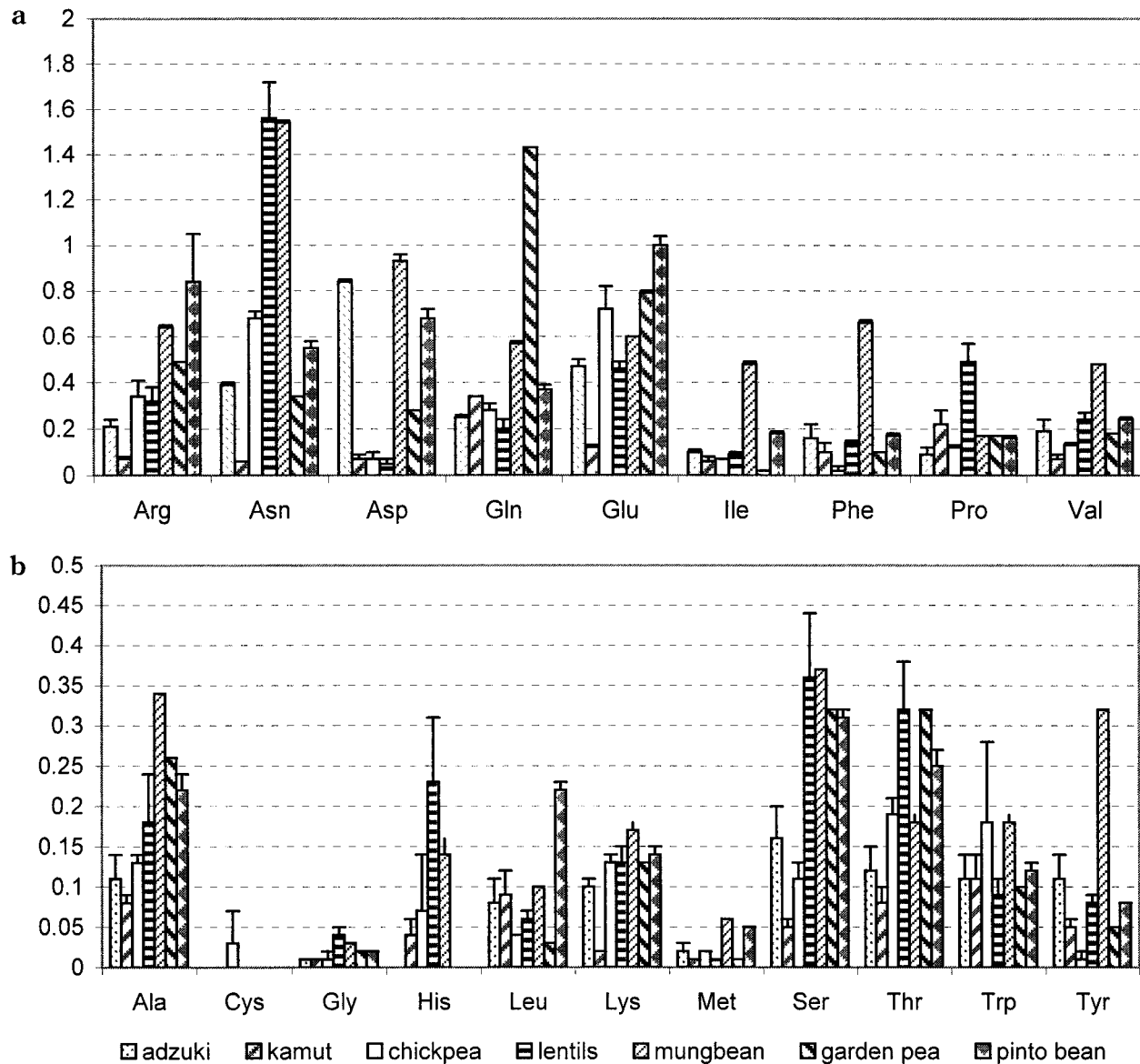
Figure 3 shows the composition of free protein amino acids in the seven seedling axes studied. Asparagine is quantitatively the most important amino acid (from 0.6 mg/g for pinto bean to 3.0 mg/g for chickpea) in all of the seedling axes examined (Figure 3). High variations were found in total free amino acid amounts for the different kinds of seedling axes.

Figure 4 shows the composition of free protein amino acids in the seven cotyledons studied. The asparagine amount is also the highest except for kamut, garden pea, and adzuki. The kamut cotyledons contain very small

amounts of amino acids. The highest concentrations of glutamine ( $0.79 \pm 0.01$  mg/g of fresh weight) and aspartic acid ( $0.84 \pm 0.01$  mg/g) were found in garden pea and in mungbean cotyledons, respectively. The mungbean cotyledons contain a higher amount of phenylalanine ( $0.66 \pm 0.01$  mg/g) than the other species.

#### DISCUSSION

The cotyledons of seedlings have a storage function, and the stored nutrients promote the growth of the



**Figure 4.** Composition of free protein amino acids in cotyledons (milligrams per gram of fresh weight  $\pm$  standard deviation, four replicates).

seedling axis. There is a high catabolic and a low biosynthetic activity in cotyledons, and metabolites are transported to the axes. The major storage materials in garden pea cotyledons are starch, protein, and phytin, together with small amounts of lipid (Sutcliffe and Bryant, 1977). However, the weight of cotyledons for each kind of seed after germination is higher than the weight of the seedling axes; it is thus relevant to also determine the contents of total free amino acids and total free nonprotein amino acids in cotyledons.

The seedlings are the sites of high amino acid biosynthetic activity resulting in high contents of free protein amino acids (Sutcliffe and Bryant, 1977). This supports the synthesis of proteins and the development of the plant.

In plants homoserine plays a role in the biosynthesis of threonine, isoleucine, methionine, and homocysteine. Only in the subfamily Viciae (*Pisum*, *Lens*, *Lathyrus*, and *Vicia*) is it found in very high concentrations. Homoserine is not a constituent of the ripe pea seeds, but it increases dramatically during the first week of growth (Pate, 1977). It can constitute 70% of the soluble

nitrogen content and 12% of the dry seedling weight. Pate (1977) reports that the accumulation of homoserine in root and shoot of pea is usually within a range of 50–75  $\mu\text{mol/g}$  of fresh weight. In our study we find 6.97 and 1.10 mg/g of fresh weight in seedling axes and cotyledons, respectively, corresponding to 58.5 and 9.2  $\mu\text{mol/g}$  in seedling axes and cotyledons, respectively. The non-protein amino acids of plants are usually found in the free state or as simple condensation products such as the  $\gamma$ -glutamyl, acetyl, and oxalyl derivatives (Kuo, 1983). We find a small amount of *O*-oxalylhomoserine in cotyledons and seedling axes of lentils. *O*-Oxalylhomoserine was reported to be an antifeedant for larvae of *Spodoptera littoralis* Boisduval (Bell et al., 1996). *O*-Acetylhomoserine is an important metabolite of pea pericarps (Pate, 1977).

The unusual peptide  $\gamma$ -glutamyl-D-alanine is found in garden pea together with other alanine derivatives (Ogawa et al., 1973; Fukuda et al., 1973). These authors used seedling axes of garden peas after 6 days of germination, but in the pea seedlings included in the commercial package we analyzed, there was no detect-

able amount of  $\gamma$ -glutamyl-D-alanine. Low levels of  $\gamma$ -glutamyl-D-alanine were detected in pinto bean seedling axes and cotyledons.

The higher homologue of glutamate,  $\alpha$ -amino adipic acid, represents 6.2 and 24.0% of the total free nonprotein amino acids in seedling axes and cotyledons, respectively. It was previously detected in peas (Pate, 1977). The gliotoxic activity of  $\alpha$ -amino adipic acid has been studied on cultures of neonatal rat astrocytes and cultures of dissociated postnatal mouse cerebellum (Bridges et al., 1991; Huck et al., 1984). We find  $\alpha$ -amino adipic acid in almost all of the seedlings studied except in kamut and chickpea. In this food commodity,  $\alpha$ -amino adipic acid is present in relatively high quantities, and the medical consequence of its presence should be evaluated.

Trigonelline (*N*-methylnicotinic acid) was present in virtually all seedlings studied. The many functions of trigonelline may explain its ubiquitous presence. Trigonelline represents 24.0 and 36.2% of the total unusual free metabolites of seedling axes and cotyledons, respectively, determined in this study (Tables 2 and 3). The nutritional effects of trigonelline have not been studied until now, but considering its high concentration in the seedling axis and cotyledon and its multiple effects in plants, there is an obvious need to study its potential effect on human health. Recently several researchers (Al-Habori and Raman, 1998; Sauvaire et al., 1998) found that fenugreek seeds (*Trigonella foenum-graecum* L.), in which trigonelline was first isolated, have an insulin-stimulating activity because of the presence of another free nonprotein amino acid, 4-hydroxyisoleucine. Trigonelline seems not to be involved in this activity.

Most of the isoxazolinone compounds were not found in ungerminated seeds but were present in high concentrations during the early stages of development in seedlings belonging to the Viciaeae (Lambein et al., 1976). The seedling axes of garden pea and lentil contain up to 2% (of the dry weight) of the weak neurotoxic amino acid BIA (Riepe et al., 1995), which is absent from the seed. In our study BIA represents 9.3 and 1.2% of total free nonprotein amino acids in seedling axes and 2.3 and 1.8% in cotyledons of pea and lentil, respectively. This compound is one of the predominant free nonprotein amino acids in this commercial food, mainly in garden pea.

Some difficulties were noted in separating the cotyledons from the seedlings, especially for small seedlings; hence, an overestimation of amino acid content in cotyledons could occur (pinto bean, kamut, adzuki), but these seedlings contained very low amounts of amino acids. The cotyledon of kamut consists almost only of the cuticles (containing primarily fibers).

Besides the thermostable nonprotein amino acids, other antinutritional factors such as trypsin inhibitors and the recently discovered anorexigens (proteoglycans) (Upreti et al., 1994) may be destroyed by cooking but can be hazardous when consumed raw. Garden pea contains different toxicants such as saponins (1.1–1.8 g/kg), trypsin inhibitor (60–360 mg of trypsin/kg), and traces of lectins (Holm et al., 1998). Cerioli et al. (1998) found that raw peas have a good nutritional value and can partially substitute for soybean meal in livestock diets. Lentil seeds contain also high levels of saponins (1.1–5.1 g/kg) (Holm et al., 1998). Chickpea contains lower levels of trypsin and amylase inhibitors (Savage

and Frokioer, 1998), proteases, lectins, polyphenols, phytic acid, and undigestible carbohydrates (Sánchez-Vioque et al., 1999) than raw soybean, but its protein quality is equivalent (Cerioli et al., 1998). Pinto beans combine the presence of soluble or bound condensed tannins, toxic lectins (Weder et al., 1997), and trypsin inhibitors with a storage protein that has low digestibility in the native state (Beumer et al., 1998).

The content of free protein amino acids in seedlings is not well studied, and the producers of this commodity for raw consumption seem not to be aware of its importance. No information concerning the nutritional value was indicated.

The industrial practices for the germination of the seeds are unknown and perhaps pesticides, bactericidal products, and artificial growth promoters may have been used, so the consequences of such compounds on human health cannot be estimated. People using a very restricted diet because of philosophic convictions (vegetarians or religious fasting), chronic disease (allergic people), or a fear of contamination (for example, BSE or dioxine) may be at risk. The effect of a high consumption of fresh seedlings on health is completely unknown. The marketing of these foods as "healthy" foods is steadily increasing, and our results indicate the need for a more realistic approach to determine their correct nutritional quality.

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