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Nonprotein amino acids in edible lentil and garden pea seedlings*

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Summary. Commercial edible seedlings of garden pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) contain high concentration of nonprotein amino acids and trigonelline. Both seedlings grown in the laboratory or purchased in a supermarket were studied by HPLC. Samples from both origins contained trigonelline, α-aminoadipic acid, homoserine, β-(isoxazolin-5-on-2-yl)-alanine (BIA), and γ-glutamyl-BIA. Garden pea seedlings also contained a uracil-alanine derivative (isowillardiine) in substantial amount. Some of these compounds such as BIA and α-aminoadipic acid have neurotoxic activity.

Keywords: Amino acids – Nonprotein amino acids – Trigonelline – Inherent toxicant – *Leguminosae* – Edible seedlings

Introduction

Pisum sativum L. (garden pea) and *Lens culinaris* L. (lentil) are ancient legumes sold for human consumption. Seedlings appear as novel foods in increasing variety in European markets. They are praised as health food because after germination the level of starch, carbohydrates (Frias et al., 1996), lipids and antinutritional factors (Frias et al., 1995) decrease while vitamins and fibers highly increase (Prodanov et al., 1997, Sierra and Vidal-Valverde, 1999). Germination improves the digestibility and reduces the flatulence factors (Vidal-Valverde and Frias, 1992). These novel foods include fresh seedlings of many different species such as lentil, garden pea, mungbean, soybean, lucerne, fenugreek, sunflower, cress, rocket salad, radish, leek, etc. This list keeps growing. In oriental cuisine, a variety of seedlings are traditionally utilized by frying or boiling for a few minutes, while in Europe these seedlings are often blended in salads and consumed raw.

Legume seedlings contain different free nonprotein amino acids that could be used as chemotaxonomic markers. Their effects on human health are

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mostly unknown and potential toxicity is not to be ruled out at this stage. These nonprotein amino acid contents were never studied in seedlings. Some nonprotein amino acids have pharmacological effects such as 4-hydroxyisoleucine isolated from fenugreek seeds (*Trigonella foenum-Graecum* L.) (Fowden et al., 1973). This amino acid has an insulin stimulating activity, which is very important for non-insulin dependent diabetics (Sauvaire et al., 1998). For several other nonprotein amino acids toxic effects were described for different organisms.

Janzen and collaborators (1977) reported that isowillardiine at 1% concentration in the normal diet of larvae of bruchid beetle (*Callosobruchus maculatus* F.) significantly inhibits the development of larvae into beetles. BIA found in many species of *Lathyrus* seedlings is the precursor of ODAP (β-N-oxalyl-L-α,β-diaminopropionic acid) (Kuo et al., 1994), a neurotoxin which is suggested to be the cause of a human neurodegenerative crippling disease lathyrism (Spencer et al., 1986). The most recent epidemic of lathyrism occurred in Ethiopia in 1997 (Getahun et al., 1999) after a drought and famine. New cases of this disease appear in Bangladesh, Nepal and Afghanistan. Kusama-Eguchi et al. (1999) recently found that in neural receptors expressed in *Xenopus* oocytes, γ-glutamyl-BIA has a weak antagonistic effect against NMDA receptors and weak agonistic effects at AMPA receptors and glutamate transporter.

Some homologues of modified protein amino acids such as homoserine, O-oxalyl homoserine, γ-glutamyl-D-alanine and α-aminoadipic acid, a homologue of glutamate, are found in legume seedlings (Rozan et al., 1999, 2000). Bridges and collaborators (1991) found a gliotoxic activity of α-aminoadipic acid in cultures of neonatal rat astrocytes and cultures of dissociated postnatal mouse cerebellum. O-oxalyl homoserine was reported to be an antifeedant for larvae of *Spodoptera littoralis* Boisduval (Bell et al., 1996). Trigonelline (N-methyl nicotinic acid) which is abundant in fenugreek seeds is a multifunctional natural plant hormone, but its effect on human health is unknown. It is one of the secondary messengers in plant cells under oxidative stress caused by UV-B (Kalbin et al., 1997) and is also involved in preventing water loss (Tramontano and Jouve, 1997). The latter authors have also demonstrated that trigonelline is a cell cycle regulator during the early growth of many legume root meristems. The content of trigonelline in the seedlings was also determined in this study. The seedlings have a high variation in amino acid content and usually there are more nonprotein amino acids in the seedlings than in the seeds. Before the biological effects of those compounds are fully examined, the use of seedlings for human consumption might pose a potential risk.

Material and methods

To determine the free amino acid content, garden pea (*Pisum sativum* L. cv. Mechelse Krombek) and green lentil (*Lens culinaris* L.) were used. These seeds were either germinated for 4 days in vermiculite in the laboratory (LP) (semi sterile conditions, darkness, 20°C). The commercial seedling product (CP) was purchased in a Belgian market with

unknown conditions of germination. For both products, the amino acid extraction was done with 70% ethanol and analyzed by HPLC in a reverse-phase C18 column with precolumn phenyl isothiocyanate derivatization (Khan et al., 1994). DL-Allylglycine was used as the internal standard. The amino acids were detected by their absorption at 254 nm with photodiode array detector. The UV-spectrum of individual peaks was useful in identification of the compounds. The results were analyzed with a Millennium software.

Results

Lentil seedlings from both commercial (CP) and laboratory product (LP) showed similar nonprotein amino acid patterns except that γ-glutamyl-D-alanine was only found in LP (Table 1). The three major components are α-aminoadipic acid, homoserine and trigonelline for both products. In LP we found less α-aminoadipic acid and homoserine, but more trigonelline and γ-glutamyl-D-alanine. The contents of BIA and γ-glutamyl-BIA are considerable and similar in both commodities. The O-oxalyl-homoserine content is lower in CP. The same nonprotein amino acids (BIA, isowillardiine, α-aminoadipic acid, homoserine and also trigonelline) were found in garden pea seedlings of both CP and LP products (Table 1). The content of homoserine is extremely high in garden pea seedlings (10 times the amount found in lentil seedlings) especially during the first days of germination. BIA level was also much higher in pea seedlings than in lentil seedlings while γ-glutamyl-BIA and γ-glutamyl-D-alanine were not detected. The content of homoserine was higher in CP than in LP and the opposite was observed for BIA.

In lentil seedlings CP and LP contained the same amount of free nonprotein amino acids even though the free protein amino acid in LP was double of

Table 1. Nonprotein amino acids and trigonelline content in laboratory (LP) and commercial (CP) lentil and garden pea seedlings (mg/100 g of fresh weight)

	Lentil seedlings		Garden pea seedlings	
	CP	LP	CP	LP
BIA[a]	8.94	8.99	24.91	48.68
γ-glu-BIA[b]	7.02	7.29	–	–
γ-glu-D-Ala[c]	–[d]	6.02	–	–
Isowillardiine	–	–	8.92	7.87
α-aaa[e]	24.62	19.15	17.35	10.39
Hse[f]	19.09	16.14	188.53	163.89
O-oxalyl-Hse	2.69	4.07	–	–
Trigonelline	20.09	31.68	30.45	27.30

[a] *BIA*, β-(isoxazolin-5-on-2-yl)-alanine; [b] *γ-glu-BIA* gama-glutamyl-BIA; [c] *γ-glu-D-Ala* gama-glutamyl-D-alanine; [d] not detected; [e] *α-aaa*, alpha-aminoadipic acid; [f] *Hse* homoserine.

Table 2. Total free nonprotein and protein amino acid content in laboratory (LP) and commercial (CP) products of lentil and garden pea seedlings (mg/100 g of fresh weight)

	Lentil seedlings		Garden pea seedlings	
	CP	LP	CP	LP
Free nonprotein amino acids	62.36	61.66	239.71	230.83
Free protein amino acids	336.81	695.89	260.93	383.44

that in CP (Table 2). In garden pea seedlings CP and LP also showed similar amounts of free nonprotein amino acids, while the content of free protein amino acids was 50% higher in LP (Table 2).

Discussion

These results show the presence of nonprotein amino acids in different quantities in the seedlings studied, the content of which is very different from the composition of dry seeds and depends on the variety of seeds and conditions of germination. Variation between CP and LP in protein amino acids is much higher than in nonprotein amino acids.

Considerable amounts of BIA were found in lentil and especially in garden pea seedlings. Riepe and collaborators (1995) reported that BIA is slightly neurotoxic and at concentrations of 0.5 to 2 mM it produced a concentration-dependent neurodegeneration in mouse cortical explants.

The high levels of free nonprotein amino acids in garden pea seedlings are mainly due to its high homoserine content. In plants homoserine plays a role in the biosynthesis of threonine, isoleucine, methionine and homocysteine. It is found in very high concentrations in the subfamily *Vicieae* (*Pisum*, *Lens*, *Lathyrus*, *Vicia*). The glutamate homologue α-aminoadipic acid has a gliotoxic activity and is found at rather high level in both lentil and garden pea seedlings. The content of free protein amino acids depends apparently on the protein biosynthesis that varies according to stages of germination, while the concentration of nonprotein amino acids is not related to protein synthesis.

The content of trigonelline is in the same order of magnitude for both species. The many functions of trigonelline may explain its ubiquitous presence. The nutritional value of trigonelline has not been studied until now, but considering its high concentration in seedling axis and cotyledon and its multiple effects in plants, there is an obvious need to study its potential effect on human health.

The toxic effect of some nonprotein amino acids was mostly studied with microorganisms, while very little information concerning their toxicity to higher animals or humans is available. In general legume seedlings are in

fashion and people consume increasing amounts without knowledge of their composition and their effects on human health. This is especially important for vegetarians who depend on legumes for their protein nutrition. Aspects of public health and food safety need consideration.

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