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Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes

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Abstract

Seeds of beans, lentils and peas were germinated for 2, 4 and 6 days, either under continuous light or continuous dark, and the free amino acids analysed by HPLC. The effects of germination on the free protein amino acids (FPA) and non-protein amino acids (FNPA) depended on the type of legumes and on the processing conditions. After germination of beans, histidine, glutamate, glycine, arginine, tyrosine and tryptophan contents decreased while, in lentils and peas, FPA increased after germination. Light germination gave the highest amounts of FPA in beans and lentils, but the lowest in peas. The FNPA changed markedly with germination. In beans, germination produced a reduction of α-amino adipic acid and an increase of GABA (γ-aminobutyric acid). All FNPA increased in lentils and peas. Light germination resulted in the highest α-amino adipic acid contents in beans, and the highest value of taurine in lentils. The highest FNPA content was found in peas after dark germination.

Keywords: *Phaseolus vulgaris*; *Lens culinaris*; *Pisum sativum*; Legumes; Germination; Seedlings; Free amino acids; Light effect

1. Introduction

Legume seeds have made a significant contribution to the human diet since ancient times. They are a good and inexpensive source of dietary proteins, carbohydrates, vitamins and minerals. However, legumes contain large amounts of antinutritional factors (i.e. trypsin inhibitors, alpha-galactosides, inositol phosphates) in the raw seeds that need to be reduced by processing before consumption (Augustin & Klein, 1989).

Germination of legume seeds for human consumption has been a common practice in the Orient for centuries and appears to be a simple and effective processing method for achieving desirable changes in nutritional quality. At present, germinated legumes are becoming an increasing proportion of the total consumption of food legumes in the world (Ghorpade & Kadam, 1989), and they are also used to produce flours of high nutritional value (Doughty & Walker, 1982). Sprouts have been used in culinary preparations for generations, but the selection of seedlings available for consumers has rapidly increased in recent years. Besides the traditional watercress (*Lepidium sativum* L.), many other species including legumes, are now offered in the markets and in health food shops. These include alfalfa (*Medicago sativa* L.), lentil (*Lens culinaris* L.), mung bean (*Vigna radiata* L.), soybean (*Glycine max* (L.) Merril), pea (*Pisum sativum* L.), adzuki bean (*Vigna angularis* Ohwi et Ohashi), pinto bean (*Phaseolus vulgaris* cv pinto) and chickpea (*Cicer arietinum* L.).

Germination causes important changes in the biochemical, nutritional and sensory characteristics of legume seeds. Extensive breakdown of seed-storage compounds and synthesis of structural proteins and other cell components take place during this process. Fats and carbohydrates, that are often at surplus levels in Western diets, are broken down while dietary fibre, that is mostly at a sub-optimal level, increases. Vitamins

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and secondary compounds, many of which are considered beneficial as antioxidants, often change dramatically during germination. Phytic acid and dietary fibre both affect the uptake of micro-nutrients in the digestive tract and these compounds change differently during the germination process. Other antinutrient factors, such as the flatulence-producing α-galactosides, trypsin and chymotrypsin inhibitors, which affect the digestion of proteins, are also reduced after germination (Frías, Díaz-Pollán, Hedley, & Vidal-Valverde, 1995; Prodanov, Sierra, & Vidal-Valverde, 1997; Sierra & Vidal-Valverde, 1999; Urbano et al., 1995; Vidal-Valverde & Frías, 1992; Vidal-Valverde et al., 1994). The occurrence of some potentially toxic non-protein amino acids in commercially available seedlings has been reported (Rozan, Kuo, & Lambein, 2000). Changes in nutrients and in antinutrient factors occurring during germination depend on the type of legume and on the sprouting conditions (i.e. time, temperature, light cycle) (Frías et al., 1995; Prodanov et al., 1997; Sierra & Vidal-Valverde, 1999). This clearly indicates potential for optimisation.

Because seedlings are often consumed raw in salads or as decorative appetisers, the nutritional quality of seedlings needs to be considered separately different from the seed. The growth conditions during the germination process can have important effects on the composition of secondary metabolites of nutritional importance.

There are some reports about the effect of germination on the nutrient and antinutrient contents of some legumes, such as soybeans, mung beans or lentils, but very little information is available for peas and beans. Besides this, most studies have been conducted using a single set of germination conditions, and reports on the effect of modifying the processing conditions are scarce.

The purpose of this work was to study the effect of different germination conditions on the content of free amino acids, and of beneficial or potentially toxic free non-protein amino acids in bean, lentil and pea. Trigonelline, a versatile plant hormone, is also determined as an ubiquitous metabolite in legumes (Rozan et al., 2000).

2. Materials and methods

2.1. Legumes

Beans (*P. vulgaris* L, var. La Granja), lentils (*L. culinaris* L, var. Castellana) and peas (*P. sativum* L, var. Esla) were purchased in Spain and used for the germination experiments.

2.2. Germination

The process was carried out on a semi-pilot scale as previously (Vidal-Valverde et al., 2002). 500 g of legume seeds were soaked in 2500 ml of 0.07% sodium hypochlorite solution for 30 min at room temperature. Seeds were then drained and washed to neutral pH, and then soaked in distilled water for $5\frac{1}{2}$ h. Finally, imbibed seeds were germinated, on a pilot scale, by layering them over a moist filter paper, continuously watered by capillary in a seed germinator (G-120 Snijders, Holland), for 2, 4 and 6 days with continuous light (10,000 lumen daylight) (abbreviated, as 2DL, 4DL and 6DL, respectively) and in the dark (abbreviated, as 2DD, 4DD, 6DD, respectively), at 20 °C, with 99% relative humidity. Sprouted seeds were freeze-dried and ground to pass of 0.18 mm sieve for analysis.

2.3. Determination of free protein and non-protein amino acids

The extraction and analyses by HPLC of free protein (FPA) and non-protein amino acids (FNPA) were determined as described in Rozan et al. (2000). The aliquots of 70% ethanol extract were derivatised with PITC (Phenylisothiocyanate 99%, Aldrich). A Waters 625 LC system and Waters 991 Photodiode Array detector were used for analysis. The linear gradient system with buffer A (0.1 M NH_4OAc) and buffer B (0.1 M $NH_4OAc/ACN/MeOH$; 44/46/10; v/v/v) at pH 6.5, respectively, allowed separation of the amino acids in 50 min using a C_{18} reversed phase column of 250 mm length at a constant temperature of 43 °C (Rozan et al., 2000).

3. Results

3.1. Overall contents

Figs. 1–4 show the FPA and FNPA contents and Table 1 shows the total free protein and non-protein amino acid contents of raw and germinated bean, lentil and pea seeds.

3.2. Free protein amino acids

In raw beans, lentils and peas, the major FPAs found were aspartic acid, glutamic acid, asparagine and arginine (Figs. 1–3). The sulphur amino acids, methionine and cysteine, were not detected as free amino acids in the raw legumes under study.

Germination of beans gives a high increase of alanine and lysine (from 2.93 and 0.106 mg/g d.m., respectively, in raw beans, to 4.40 and 0.530 mg/g d.m., respectively, in germinated beans). Histidine, that was present in raw beans (0.446 mg/g d.m.), disappeared after germination, while glutamic acid, glycine, arginine, tyrosine and tryptophan decreased markedly (from 11.2, 0.504, 13.2, 4.0 and 0.68 mg/g d.m., respectively, in raw beans to 4.09,

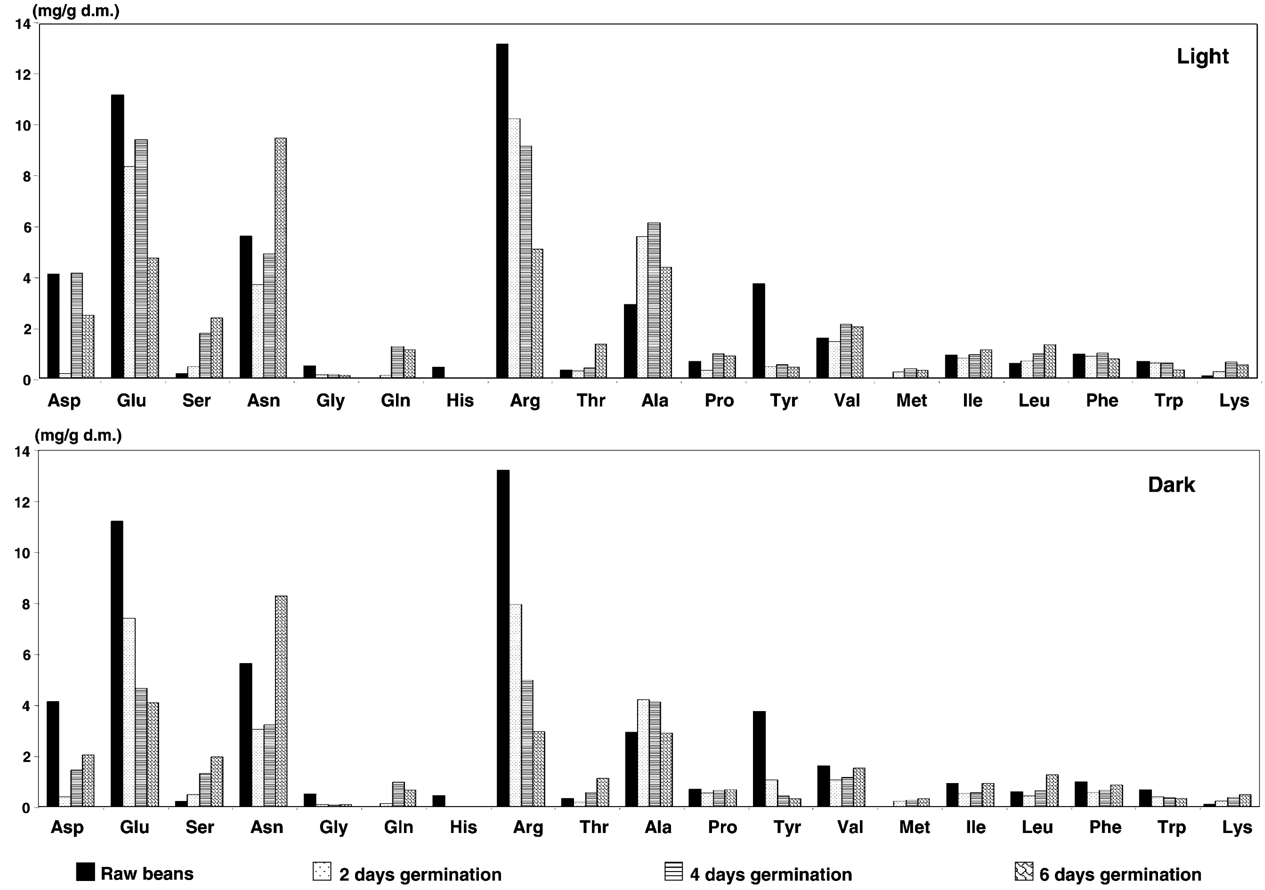

Fig. 1. Effect of germination on the free protein amino acid contents of beans.

0.089, 2.95, 0.325 and 0.334 mg/g d.m., respectively, in germinated beans). Glutamine and methionine, which were not detected in the raw beans, appeared after germination in quite high amounts (up to 1.27 and 0.382 mg/g d.m., respectively). The other FPAs present in the raw beans increased or decreased, depending on the germination conditions (Fig. 1).

The germination of lentils produced a very high increase of all FPAs, except aspartic acid (0.704 mg/g d.m. in raw lentils) which increased up to 1.27 mg/g d.m. or decreased to 0.472 mg/g d.m., depending on the germination conditions (Fig. 2). Dramatic increases of 2.6–50-fold were seen for glutamic acid, asparagine, glycine, histidine, threonine, proline and valine (from 1.34, 0.881, 0.077, 0.304, 0.042, 0.228 and 0.107 mg/g d.m., respectively, in raw lentils to 3.93, 28.7, 0.346, 0.802, 2.12, 2.91 and 2.42 mg/g d.m., respectively, in germinated lentils). Eight FPAs were not detected in raw lentils but appeared after germination: serine (up to 2.43 mg/g d.m.), glutamine (up to 1.06 mg/g d.m.), tyrosine (up to 0.639 mg/g d.m.), isoleucine (up to 0.990 mg/g d.m.), leucine (up to 0.605 mg/g d.m), phenylalanine (up to 1.04 mg/g d.m.), tryptophan (up to 0.270 mg/g d.m.), and lysine (up to 1.05 mg/g d.m.) (Fig. 2).

The contents, in raw peas, of serine (0.02 mg/g d.m.), glycine (0.066 mg/g d.m.), alanine (0.252 mg/g d.m.), proline (0.533 mg/g d.m.), tyrosine (0.057 mg/g d.m.), leucine (0.01 mg/g d.m.), phenylalanine (0.15 mg/g d.m.) and lysine (0.081 mg/g d.m.) increased drastically after germination (up to 1.51, 0.301, 2.58, 2.23, 0.518, 0.518, 1.16 and 0.745 mg/g d.m., respectively). The histidine present in raw peas (0.138 mg/g d.m.) disappeared after germination, as it did in beans. Glutamine, threonine, valine and isoleucine, which were not detected in the raw peas, appeared after germination (up to 2.38, 0.356, 1.78, and 0.580 mg/g d.m., respectively). The contents of other FPAs present in the raw peas changed in a variable manner, depending on the germination conditions.

The presence of light during germination produces, with only few exceptions, higher amounts of FPA in beans and lentils than with dark germination, while the opposite effect was observed in peas (Figs. 1–3).

The germination time affected the FPA content in different ways. In beans (Fig. 1); with increasing germination time, the contents of serine, asparagine, threonine and leucine consistently increased while the increases of methionine, lysine and glutamine were irregular. The contents of arginine, tyrosine and tryptophan decreased when the time of germination increased. In lentils (Fig. 2), the contents of serine, asparagine, glycine, threonine, proline and valine increased considerably when the germination time increased and, with

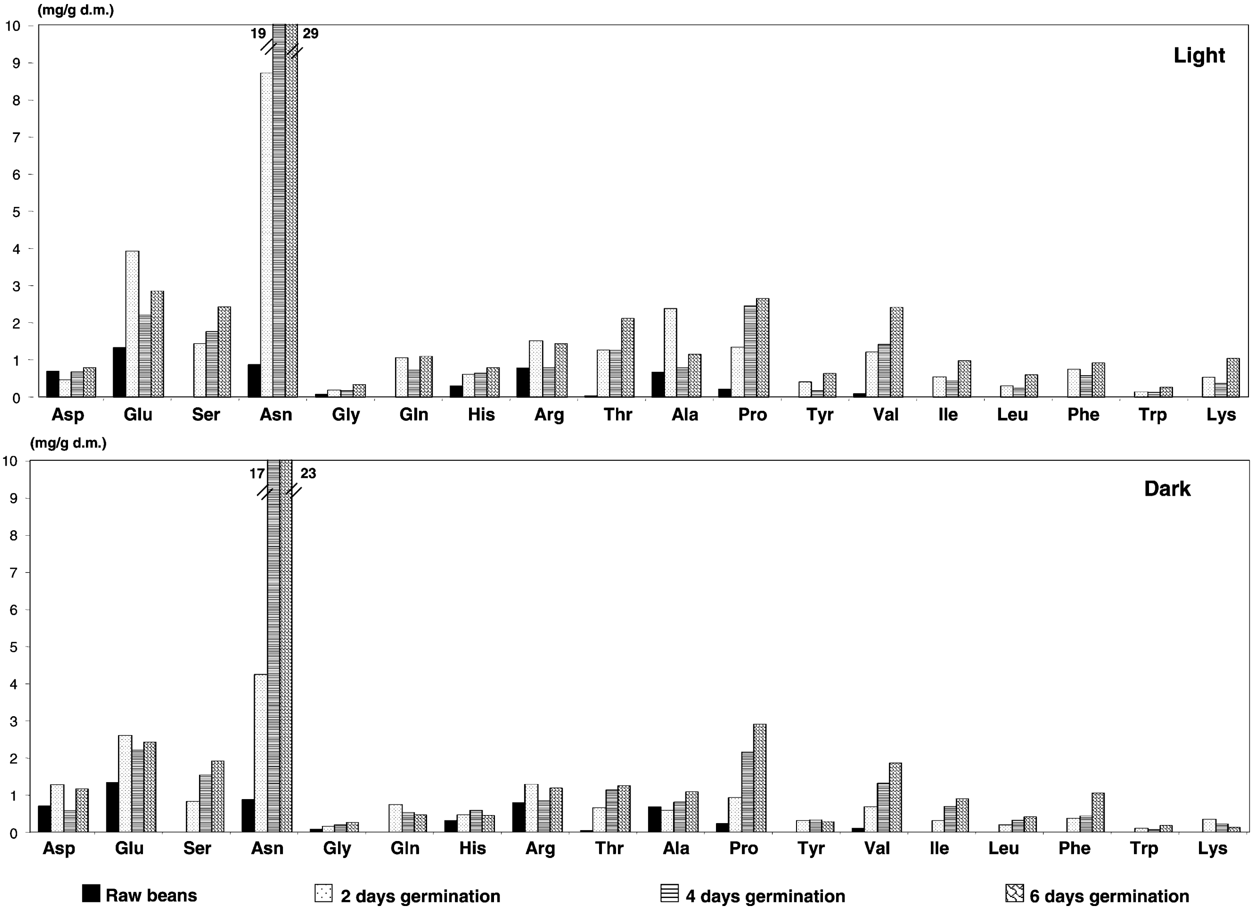

Fig. 2. Effect of germination on the free protein amino acid contents of lentils.

only few exceptions, the other FPAs showed an increasing trend. In germinating peas (Fig. 3), the proline content increased with longer germination period, while the changes of other FPAs depended on processing conditions (Figs. 1–3).

3.3. Free non-protein amino acids

The non-protein amino acids found in the seeds under study have different chemical structures and different biochemical origins: homologues or derivatives of protein amino acids (α-amino-adipic acid, homoserine, O-oxalylhomoserine, γ-aminobutyric acid (GABA), taurine, γ-hydroxy arginine), a uracil derivative (isowillardiine), isoxazolinone (β-isoxazolin-5-on-2-yl)-alanine (BIA) and a derivative of nicotinic acid (trigonelline). During the germination of leguminous seeds, some non-protein amino acids decrease while others increase or new compounds appear.

Trigonelline (*N*-methyl-nicotinic acid, a versatile plant hormone but not an amino acid) was present in all seeds studied. In beans, the trigonelline content (4.57 mg/g d.m.) decreased after germination (1.17–2.31 mg/g d.m.) and this decrease was more pronounced when the period of time increased and when the process was carried out in darkness. The content of trigonelline in lentils is quite low (0.40 mg/g d.m.) but after germination it increased up to 0.77 mg/g d.m., this increase is highest after 4 days of germination. Pea germination in the presence of light produced a slight decrease in the trigonelline content (from 0.89 mg/ g d.m. in raw peas to 0.67 mg/g d.m.) and variable changes in the dark (0.73–0.97 mg/g d.m) (Fig. 4).

α-Amino adipic acid (α-aaa) (the higher homologue of glutamate) was present in raw beans (0.92 mg/g d.m.), but it was absent in raw lentils and it was observed only in small amounts in raw peas (0.03 mg/g d.m.). Bean germination produces a considerable reduction of α-aaa after 2 days (0.06–0.14 mg/g d.m.), but the decrease was less pronounced with a longer germination (0.53–0.90 mg/g d.m.). The contents of α-aaa is lower when the process was carried out in darkness. Lentil germination causes an increase of α-aaa content, up to 0.31 mg/g d.m., and only slight influence of germination time or light was observed. Germinated peas contained higher amounts of α-aaa than the raw seeds. This increase was higher (up to 0.40 mg/g d.m.) when the process was carried out in darkness.

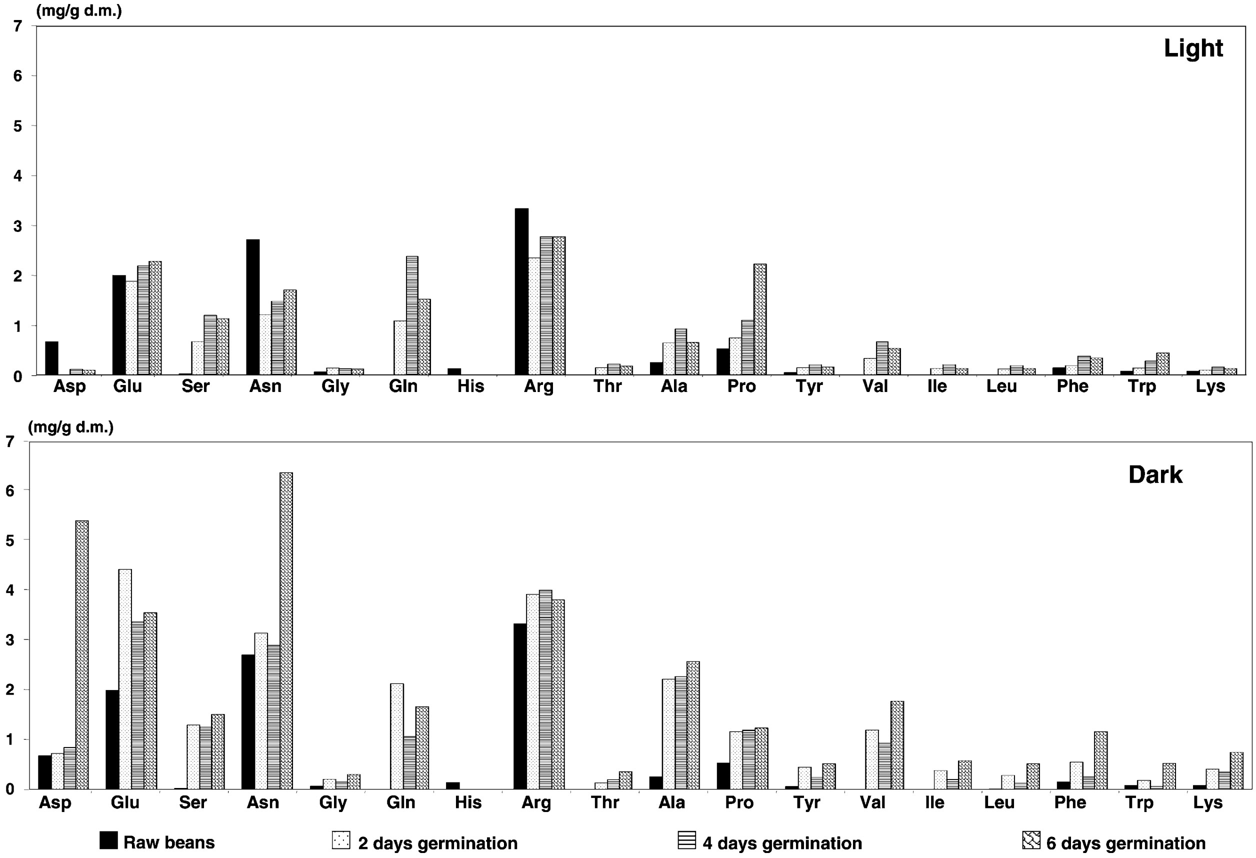

Fig. 3. Effect of germination on the free protein amino acid contents of peas.

γ-Hydroxy-arginine was detected only in lentils (1.58 mg/g d.m.) and it increased after germination (up to 2.68 mg/g d.m.). The highest content was observed when the germination was carried out in the dark and when the period of time was extended up to 6 days.

Homoserine was observed only in very low amounts in raw peas (0.01 mg/g d.m.) but its content increased up to 1500-fold after germination (15.02 mg/g d.m.). The homoserine content increased with the time of germination.

Isowillardiine was not present in any raw legumes, but appeared in germinated peas (up to 0.55 mg/g d.m.). The isowillardine content increased when the time of germination was increased and when it was conducted in darkness.

Taurine (2-amino-ethanesulphonic acid) was not found in beans but it was present in lentils and peas (0.46 and 0.34 mg/g d.m., respectively). The germination produced an increase of this amino acid, up to 0.98 mg/g d.m. for lentils and up to 0.60 mg/g d.m. for peas. The taurine content increased when the time of germination increased. Light had an increasing effect on taurine in lentils but a decreasing effect in peas.

γ-Aminobutyric acid (GABA) was not present in the raw seeds but its content increased dramatically after germination, especially after 6 days (0.44, 0.32 and 1.04 mg/g d.m. for germinated beans, lentils and peas, respectively). The presence or absence of light during germination had only slight effects.

β-(isoxazolin-5-on-2-yl)-alanine (BIA) was not present in any raw legumes studied, but appeared in germinated peas after 4 days of light germination (up to 0.95 mg/g d.m.) and increased continuously during dark germination (up to 1.49 mg/g d.m.). BIA content increased with the time of germination and when the germination process was conducted in the dark.

O-oxalylhomoserine was not present in any of the raw legumes studied, but it appeared in peas after germination (up to 6.83 mg/g d.m.). The highest amounts were observed when the germination was conducted in darkness and for longer periods, except for the 6 days of germination without light (6DD).

Table 1 summarises the contents of total FPA and total FNPA in raw and germinated legumes. In lentils and peas, total FPA content increased after germination, which was more pronounced with longer periods of germination. Total FNPA decreased after germination in beans but increased in lentils and peas. The ratio, total FNPA/total FPA, was lower in seedlings of beans and lentils (6.4–7.2% and 8.2–26.9%, respectively) than in the raw bean and lentils (11.5% and 46.6%, respectively). In the case of peas, the ratio increased even more

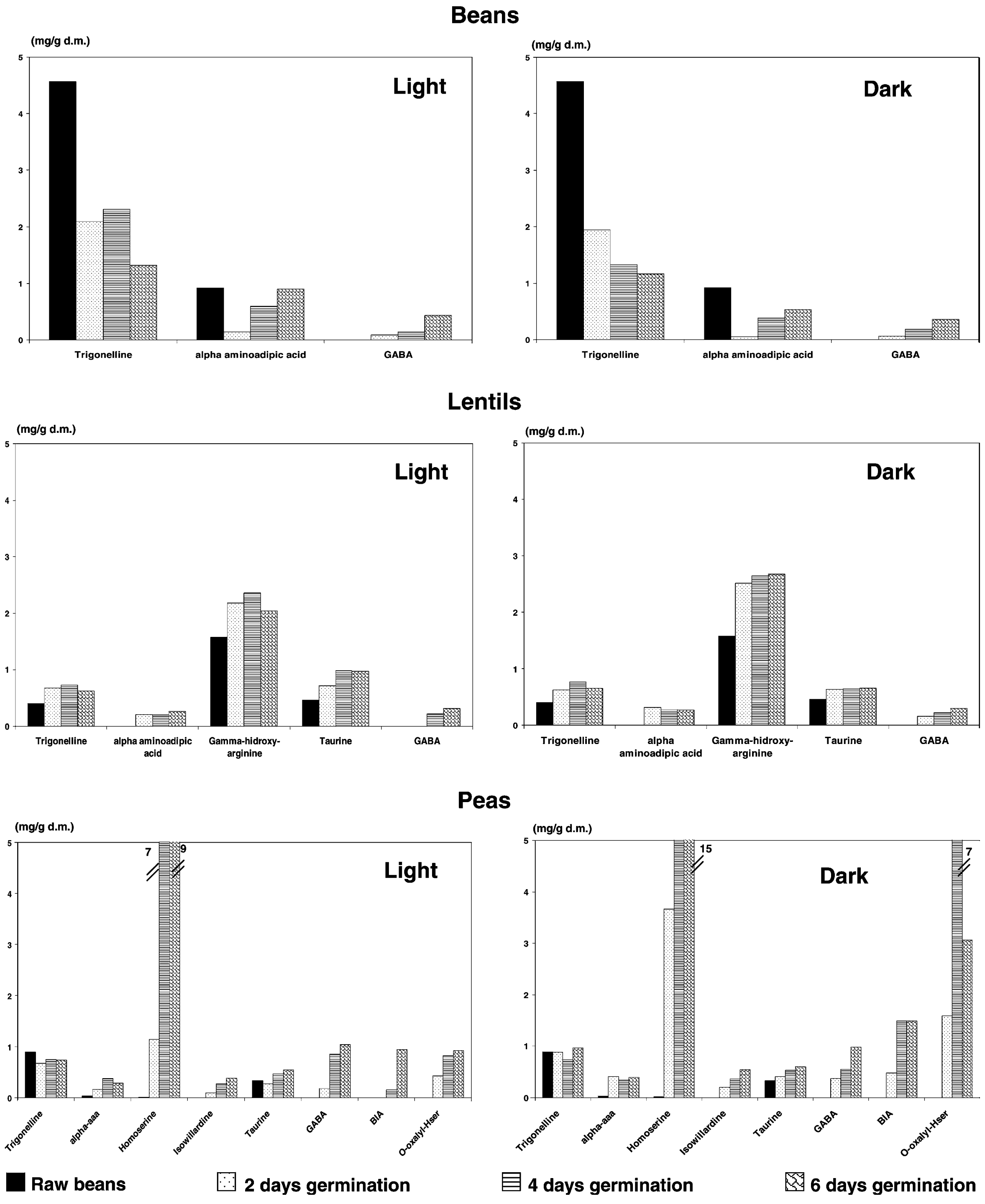

Fig. 4. Effect of germination on the free non-protein amino acid contents of legumes.

dramatically after germination of peas (28.7–96.4%) in comparison with the raw peas (12.9%).

4. Discussion

The seedlings are the site of high amino acid biosynthetic activity, resulting in high contents of free protein amino acids (Sutcliffe & Bryant, 1977), supporting the synthesis of proteins and the development of the plant. Also, storage protein can undergo proteolysis and contribute to the increase of free amino acids (Lea & Joy, 1983).

Trigonelline is present in legumes and cereals. It is not an amino acid, but its multiple functions in plant growth and development are very important (Tra-

Table 1
Effect of germination on the total free protein and non-protein amino acids, including trigonelline, in legumes[a]

	Raw legumes	2 days light	4 days light	6 days light	2 days dark	4 days dark	6 days dark
Beans							
Total FPA	48.0	35.8	47.1	39.9	29.3	26.7	31.0
Total FNPA	5.5	2.3	3.0	2.7	2.1	1.9	2.1
Lentils							
Total FPA	5.2	26.9	34.1	51.3	16.0	31.3	41.0
Total FNPA	2.4	3.8	4.5	4.2	4.3	4.6	4.6
Peas							
Total FPA	10.1	10.1	14.6	14.6	22.8	19.4	32.7
Total FNPA	1.3	2.9	11.0	14.1	8.0	16.0	23.1

[a] (mg/g d.m.); FPA = total free protein amino acid; FNPA = total free non-protein amino acid.

montano & Jouve, 1997). Under certain growth conditions, trigonelline is one of the secondary messengers in plant cells under stress, preventing oxidative stress caused by UV-B light (Kalbin, Ohlsson, Berglund, Rydstrom, & Strid, 1997) or preventing water loss (Tramontano & Jouve, 1997). Trigonelline is non-toxic and it has no any vitamin-like effect, but after a heat process it undergoes demethylation and it is converted to niacin. In this sense, foods rich in trigonelline could be a good source of niacin, to prevent or treat pellagra (Billaud & Adriand, 2001).

γ-Hydroxy-arginine has been found in different species of lentils and increases after 4 days of germination (Rozan, Kuo, & Lambein, 2001; Sulser & Sager, 1976). Our results are in agreement with those reports. γ-Hydroxy-arginine seems to be a typical lentil metabolite (Rozan et al., 2001); potentially it might interfere with the biosynthesis of nitric oxide (NO) in the human body, as an analogue of arginine, the substrate of nitric oxide synthase. The high concentration of γ-hydroxyarginine might explain the low concentration of arginine in lentil compared to bean and pea. Buga, Wei, Bauer, Fukuto, and Ignarro (1998) found that γ-hydroxy-arginine inhibits Caco-2 tumor cell proliferation.

α-Amino-adipic acid (α-aaa) was present in our samples in low amounts and it was found previously in some varieties of lentil seedlings in concentrations up to 4 mg/g d.m. (Rozan et al., 2000). Bridges, Hatalski, Shim, and Nunn (1991) found a gliotoxic activity of α-aaa in cultures of neonatal rat astrocytes.

Homoserine plays a role in the biosynthesis of threonine, isoleucine, methionine and homocysteine in plants (Rozan et al., 2000).

The isowillardiine content was 0.2% in germinated peas. Janzen, Juster, and Bell (1977) observed that isowillardiine, at 1% concentration in the normal diet of the bruchid beetle *Callosobruchus maculatus* F., inhibits the development of larvae into beetles.

The antihypertensive in vivo activities of GABA and GABA-enriched foods have been studied by other authors (Allen, 2002; Okada et al., 2000; Rhyu, Kim, Kim, Ahn, & Yang, 2000; Saikusa et al., 2001; Watanabe et al., 2002). Rozan et al. (2001) determined the GABA content in the seedlings of 5 species of lentils (up to 2.04 mg/g d.m.) and found a higher content than those obtained in this work for the germinated lentils. Germinated peas showed the highest values of GABA among the three legumes studied.

BIA is present in many *Lathyrus* species; it is the metabolic precursor of a neurotoxic amino acid β-*N*-oxalyl-L-α – β-diaminopropionic acid (β-ODAP) (Kuo, Khan, & Lambein, 1994), which is suggested to be responsible for a crippling human disease, neurolathyrism (Spencer et al., 1986). This irreversible neurodegenerative disease seems to occur only after prolonged and almost exclusive consumption of the seeds of grass pea (*Lathyrus sativus* L.) in India, Bangladesh and Ethiopia. BIA itself is slightly neurotoxic; at concentrations of 0.5–2.0 mM, it produces a concentration-dependent neurodegeneration in mouse cortical explants (Riepe, Spencer, Lambein, Ludolph, & Allen, 1995). BIA was also found in different species of germinated lentils (up to 0.43 mg/g d.m.) (Rozan et al., 2001), but was not detected in the presently studied lentils. In *L. culinaris*, BIA was metabolised to γ-glu-BIA and to four other amino acids while ODAP was not found (Kuo, Ikegami, & Lambein, 1998).

Taurine is the most abundant non-protein amino acid in the human body and is the metabolic product of cysteine after oxidation and decarboxylation (Bezkorovainy & Rafelson, 1996). It has been identified in beans, peas and lentils (Kataoka & Ohnishi, 1986; Lähdesmäki, 1986; Rozan et al., 2001). In this study, taurine was found only in lentils and peas. The metabolism and physiological role of taurine in plants is not established, but in animals taurine was reported to have multiple functions as neuromodulator, cell membrane stabilizer, antioxidant, detoxifier, bile acid conjugator and osmoregulator (Zelikovic, Chesney, Friedman, & Ahlfors, 1990). Taurine possesses in vitro antimutagenic activity comparable to major antioxidants (Sung, Jeon, & Park, 1999). Mochizuki, Oda, and Yokogoshi (1998) have shown that taurine enhanced serum HDL-cholesterol in rats while total cholesterol concentration was unaltered. Hwang, Wang, and Cheng (1998) observed that taurine might play a role in reducing the toxic effect of copper in rats.

O-oxalylhomoserine was described as an antifeedant for larvae of *Spodoptera littoralis* Boisduval (Bell, Perera, Nunn, Simmonds, & Blaney, 1996).

In a previous publication, we studied a number of nutritional and antinutritonal factors, such as soluble sugars, α-galactosides, vitamins and inositol phosphates in the same legume species during germination. That study showed mostly positive effects of germination on the nutritional quality (Vidal-Valverde et al., 2002). In the present study, analysis of lesser known secondary metabolites, that vary dramatically during germination, shows a more complicated picture of the potential benefits of germinated legume seeds for human health.

While it is general knowledge that legume seeds are rich in protein, they do not represent a well balanced food because of the low level of the sulphur amino acids, cysteine and methionine. Germination can increase the free protein amino acid content of legumes but drastic changes are observed for secondary metabolites with pharmacological or toxicological properties. Legume seeds are mostly considered as a minor part of potato- or cereal-based diets. Only grass pea (*Lathyrus sativus* L.) is consumed as a staple food by subsistence farmers in marginal lands of the Indian Sub-continent and Ethiopia, and this mostly in periods of drought and famine. Prolonged and uninterrupted consumption of this legume, as a staple food during several months, can give rise to spastic paraparesis of the legs or neurolathyrism (Getahun, Mekonnen, TekleHaimanot, & Lambein, 1999). As well as a neuro-excitatory amino acid, present in the seeds of grass pea, that is blamed for causing this paralysis, the seedlings of grass pea also contain non-protein amino acids and other metabolites. A naturally occurring cyanide (BAPN or β-aminopropionitrile), that inhibits collagen cross-linking, is blamed for the rare cases of osteo-lathyrism in Bangladesh (Haque, Hossain, Lambein, & Bell, 1977). However, most people in those countries consume grass pea seeds in their normal diet, in limited quantities, without ill effect.

The overall effect of limited consumption of legume seedlings is considered beneficial to human health because of its composition of major and minor nutrients and some beneficial non-protein amino acids. However, as is true for many of our foodstuffs, over-consumption, as a major part of the diet for long periods, might involve a health risk when toxic amino acids are present.

5. Conclusions

The effect of germination on the free protein amino acids (FPA) and non-protein amino acids (FNPA) depended on the type of legumes and the processing conditions. After bean germination, histidine, glutamate, glycine, histidine, arginine, tyrosine and tryptophan contents decreased, while asparagine, valine, isoleucine, phenylalanine and tryptophan contents varied in different ways, depending on the germination conditions. Germination of lentils produced an increase of FPAs and the appearance of new ones. The same effect was observed in peas, but histidine disappeared and the changes of aspartic acid, asparagine and arginine contents were dependant on the germination conditions. The effect of light during the germination of beans and lentils led, in general, to higher amounts of FPA but the opposite effect was observed in peas. The effect of time, during germination, on the FPA content depended, in general, on the light conditions.

The FNPA underwent marked changes during germination. Germination of beans produced a reduction of trigonelline and of α-amino adipic acid and an increase of GABA. Germination of lentils and peas produced increases of all FNPA; while the trigonelline content did increase in the germinated lentils, only slight changes were observed in germinated peas. Germination of beans under light produced the highest trigonelline and α-amino adipic acid contents, while, in lentils, it produced the highest level of taurine and the lowest level of gamma hydroxy-arginine and no effect of light was detected for the other NFPAs. Dark-germinated peas produced the highest FNPA content.

Optimising the germination conditions may further improve the nutritional quality of germinated legume seedlings. This optimisation needs, however, a separate approach for each species considered.

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