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Changes in flax (*Linum usitatissmum*) seed nitrogenous compounds during germination

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Abstract

The changes in the nitrogenous compounds of flaxseed during an 8-day germination period were studied. The dry matter content of the seeds was reduced by 35% at the end of the germination. During the germination period, a relatively small decrease was observed in total nitrogen content, but there was an increase in the content of non-protein nitrogen from 9 to 33.5% of the total amount. An increase in the total content of free amino acids was also observed. Among individual amino acids, glutamine showed a marked change during the germination period indicating that it is the favoured amide donor in the developing flax seedlings. An increase in the water-soluble protein and a decrease in the salt-soluble protein fractions was also observed. The content of polyamines, namely agmatine, spermidine and putrescine, which are important in controlling cellular metabolism and growth, was also increased during the germination period. After day 8 of germination, the contents of cyanogenic glycosides, linustatin and neolinustatin in the seeds were reduced by 40 and 70%, respectively. Trypsin inhibitor content was very low in flaxseed and only trace amounts were present after 8 days of germination.

Keywords: Flaxseed; Nitrogenous compounds; Germination; Amino acids; Cyanogenic glycosides; Polyamines; Trypsin inhibitors

1. Introduction

Flax (*Linum usitatissimum* L.) is one of the world's oldest arable crops which has regained popularity as a potential food ingredient because of its nutritionally valuable components. In recent years, potential use of flaxseed for production of value-added products has been investigated. Presence of biologically-active phytochemicals such as α-linolenic acid, lignans and soluble fibre has generated new and increased interest about the nutritional and pharmaceutical value of flaxseed. In particular, use of whole flaxseed in bakery products and breakfast cereals has increased in recent years (Caragay, 1992; Carter, 1993; Cunnane & Thompson, 1995).

Germination is a widely used processing method for grain legumes and cereals, especially in the Orient and Far East Asia. It has been reported, for most of the legumes and cereals, that soaking and germination may reduce their content of antinutritional components (Chavan & Kadam, 1989; Ghorpade & Kadam, 1989; Savelkoul, Van der Poel, & Tamminga, 1992; Bau, Villaume, Nicolas, & Méjean, 1997) and, at the same time, the availability and digestibility of their proteins may increase (Chavan & Kadam, 1989; Savelkoul et al., 1992). Germination of seeds mobilizes reserves from the seed to the growing seedling; increased metabolic activities in turn result in chemical changes in the macromolecules. Increased enzymic activities in the germinating seeds are usually accompanied by interconversion and production of new compounds. However, no information is available, in the literature, on the effect of germination on the nitrogenous constituents of flaxseed.

The nitrogenous compounds of flaxseed include proteins, non-protein nitrogen compounds, such as amino acids, enzymes, polyamines and cyanogenic glycosides, among others. As a major fraction of seed constituents, proteins of flaxseed may undergo considerable change during germination as a result of increased metabolic

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activities. Changes of nitrogenous compounds that occur during germination of flaxseed are presented in this paper.

2. Materials and methods

2.1. Germination of flaxseed

Whole flaxseeds (variety, Somme) were surface-sterilized by soaking in 0.5% (w/v) Javex (NaClO content of 4%, v/v) solution for 15 min, then washed well with demineralized water and spread on enamel-coated plates (30×18×5 cm) with paper towels saturated with demineralized water underneath. The trays were labelled with sampling dates and arranged as for a complete randomized design (CRD; Montgomery, 1984) on a table kept in the laboratory with an even light supply (375 lux). Seeds in trays were covered with paper towels and allowed to germinate. The average temperature of the room was 22 ± 1°C and the relative humidity was 78 ± 2%. The trays containing seeds were supplied with ample water to maintain approximately 100% relative humidity within each tray throughout the germination period. Demineralized water was used to maintain the moisture content during the germination period. Samples of seedlings were withdrawn on days 0, 2, 4, 6 and 8 for further analyses; day 0 was designated for seeds after pretreatments. About 25 seedlings were randomly selected from each tray for each sampling day and the length of the seedlings was measured. The rest of the seedlings were lyophilized and dried samples were stored at 4 ± 1°C. Dried seedlings were ground using a coffee grinder to pass through a 2-mm mesh screen and used for chemical analyses.

Moisture content of the seedlings was determined by drying in a forced-air oven at 104 ± 1°C for 18 h until a constant weight was reached. Moisture content was calculated as percentage weight loss (moisture) of the samples during drying (AOAC, 1990). Defatting of germinated flaxseed samples was carried out by extracting the samples with hexane (1:2, w/v, six times).

2.2. Total and non-protein nitrogen content

Total nitrogen content of the samples was determined by Kjeldahl analysis (AOAC, 1990). For non-protein nitrogen (NPN) content, approximately 1 g of defatted flaxseed sample was extracted with a 12% (w/v) solution of trichloroacetic acid (TCA, 20 ml) using a wrist-action shaker for 30 min at room temperature. The extracted nitrogenous compounds were quantitated by Kjeldahl analysis. The content of NPN of each sample was then calculated as the percentage ratio of the dissolved nitrogen in TCA to the total nitrogen content of the sample (Naczk, Diosady, & Rubin, 1985).

2.3. Separation of protein classes based on their solubility characteristics

Protein classes of germinated and ungerminated flaxseeds were determined according to their solubility (successively in distilled water, 5% (w/v) NaCl, 70% (v/v) ethanol at 65°C and 0.2% (w/v) NaOH) using a modified Osborne classification procedure as described by Lund and Sandstrom (1943).

2.4. Amino acid composition

Samples (5–10 mg) of defatted germinated seedlings were hydrolysed in 1 ml of 6 M HCl containing 0.5% (v/v) phenol at 110°C for 24 h under a nitrogen atmosphere (Blackburn, 1978). Hydrochloric acid in the hydrolysate was removed under vacuum and the dried sample was reconstituted with a sodium citrate buffer (0.2 M, pH 2.2) for analysis. Amino acids in the hydrolysate were separated, identified and quantitated using a Beckman 121 MB amino acid analyzer (Beckman Instruments, Palo Alto, CA) equipped with a cation-exchange resin column (Benson D-X 8.25, bed size 200×2.8 mm) at the Amino Acid Facility, Department of Biochemistry, Memorial University of Newfoundland.

To determine the content of sulphur-containing amino acids, samples were oxidized by performic acid (Blackburn, 1978) prior to their hydrolysis in a 6 M HCl solution. Cysteine and methionine were measured as cysteic acid and methionine sulphone, respectively. For tryptophan analysis, samples were hydrolysed in 1 ml of 3 M mercaptoethanesulphonic acid (Penke, Ferenczi, & Kovacs, 1974) for 22 h at 110°C under nitrogen and then neutralized with LiOH and adjusted to pH 2.2.

2.5. Individual free amino acids and their contents

Germinated flaxseed samples (1–2 g) were extracted with 20 ml of 6% (v/v) perchloric acid (PCA) solution by homogenization using a Polytron homogenizer (10,000 rpm, 2 min) at 5°C. Extracts were centrifuged at 4100×$\boldsymbol{g}$ for 20 min and solids were re-extracted with another 10 ml of PCA. The supernatants were combined and their pH was adjusted to 10 using a 33% (w/v) KOH solution. The resultant solution was centrifuged to remove any precipitate and the supernatant was decanted and its pH adjusted to 2 with 3 M HCl. The total volume of the extract was brought to 25 ml with distilled water. The extract was filtered through a 0.45-μm nylon filter to eliminate any turbidity. One millilitre of lithium–citrate buffer (pH 2.2, 0.3 M) was added to 2 ml of the filtered extract and the resultant solution was analyzed for individual amino acids using a Beckman 121 MB amino acid analyzer.

2.6. Identification and quantification of polyamines

Defatted samples (1–2 g) were extracted with 10 ml of a 6% (v/v) perchloric acid by homogenization at 5°C (10,000 rpm, 1 min), using a Polytron homogenizer. Insoluble meal particles and precipitated proteins were removed by centrifugation at 2000×***g*** for 15 min. The supernatant was first filtered through a layer of glass wool and then a 0.45-μm nylon filter and diluted to 25 ml with HPLC-grade water. The polyamines in each solution were separated on a 5-μm particle size Beckman Ultrasphere C_{18}-IP analytical column (4.6×250 mm) coupled with a guard column (ODS-C_{18}, 5 μm, 2.1×70 mm). A gradient solvent system consisting of a 0.1 M sodium acetate (pH 4.5) containing 10 mM octanesulphonic acid (OSA) and 10% (v/v) methanol and 0.2 M sodium acetate (pH 4.5) containing 10 mM OSA and acetonitrile (10:3, v/v) and 10% (v/v) methanol was used (Seiler & Knodgen, 1985). Post-column derivatisation of separated polyamines was carried out with *o*-phthalaldehyde/2-mercaptoethanol reagents. Polyamine derivatives were detected using a fluorescence detector (Model 420, Waters, Milford, MA); excitation at 345 nm and emission at 455 nm were monitored. A 10-μl flow cell maintained at 1 ml/min flow rate was used. Peaks were identified and recoveries calculated by spiking the sample with known amounts of polyamines agmatine, cadaverine, histamine, putrescine, spermidine and spermine (Sigma Chem. Co., St. Louis, MO) which were dissolved in 0.2 M PCA. A Beckman (Beckman Scientific, Irvine, CA) HPLC system, consisting of a pump (Beckman) for post-column derivatisation and two other pumps (Model 510, Waters, Milford, MA) with a mixing chamber for solvent gradient and an autosampler (Model 231-401, Mandel Scientific, Toronto, ON), were used.

2.7. Cyanogenic glycoside content

The content of cyanogenic glycoside of the seedling samples was determined as described by Amarowicz, Wanasundara, and Shahidi (1993) and Wanasundara, Amarowicz, Kara, and Shahidi (1993) for flaxseed samples.

2.8. Trypsin inhibitors (TI)

The germinated, dried and ground flaxseed samples were extracted with a 0.05 M potassium phosphate buffer containing 0.5 M NaCl (pH 8.0, 1:50, w/v) for 2 h using a water bath shaker at 22 ± 1°C. Affinity chromatography with trypsin–sepharose 4B (cyanogen bromide activated) was used to determine trypsin inhibitors in the extracts. The content of TI in samples was calculated as mg TI per gram of crude protein (Roozen & de Groot, 1991).

2.9. Statistical analysis

All experiments carried out in this study were replicated three times. Mean values with standard deviations (SD) were reported when and where necessary. Analysis of variance (ANOVA) was performed and differences in mean values were determined using Tukey's studentized test at $p < 0.05$ and employing ANOVA and Tukey procedures of statistical analytical system (SAS, 1990), respectively.

3. Results and discussion

Table 1 presents the content of dry matter and length of seedlings in flaxseeds over an 8-day germination period. The content of dry matter decreased drastically as the seedlings grew to about 6 cm in height. A 35% loss of dry matter was observed on day 8 of the germination. At the termination stage of the germination experiment two cotyledons opened up and most of them were green. The decrease in the percentage of dry matter is a combination of increased water content of the germinated seeds as well as the actual loss of dry matter. It is well known that, during the initial stages of germination, seed reserves are utilized to supply energy and other requirements for the growth of new cells. Since no external nutrients were added, only water and oxygen were consumed by the sprouting seeds. However, a net loss of dry matter occurred as a result of oxidation and breakdown of the stored macromolecules (Chavan, Kadam, & Salunkhe, 1981), as there was a decrease in the amount of major seed components such as lipids and proteins.

3.1. Changes in nitrogenous components

3.1.1. Total and non-protein nitrogen and free amino acids

Changes in the contents of total nitrogen and non-protein nitrogen (NPN) in flaxseed during 8 days of germination are summarised in Fig. 1. Although a

Table 1
Changes in the contents of dry matter and length of seedlings during germination of flaxseed[a]

Germination period (days)	Dry matter content (%)	Length of seedling (mm)
0 (ungerminated)	73.5 ± 3.5[c]	0.0
2	67.6 ± 2.7[c]	3.5 ± 1.1
4	55.4 ± 1.2[b]	20.0 ± 4.3
6	53.8 ± 2.4[b]	45.5 ± 3.9
8	47.5 ± 2.0[a]	59.2 ± 6.1

Values in the same column with different superscripts are significantly ($p < 0.05$) different from one another.

[a] Mean ± SD (three replicates).

relatively small decrease was observed in the total nitrogen content, the amount of NPN was increased from 9 to 33.5% of the total nitrogen content of the seeds. Thus, the true protein content of seedlings was actually decreased during the germination. The relative increase in the total nitrogen content observed in the early stages of germination may possibly be due to the initial loss of lipids and other major reserves of the seed. A parallel increase in the content of total free amino acids (Table 2) was also observed as the NPN content of seedlings increased during the germination period, thus indicating increased proteolytic activity. The proteins of the cotyledons are hydrolysed to amino acids which are used for the synthesis of new proteins for the newly developing parts. However, utilization of liberated free amino acids after germination does not occur quantitatively, since there is an increase in the free amino acid content (El-Mahdy & El-Sebay, 1985; Finney, 1983). The process of amino acid catabolism in germinating seeds involves removal of nitrogen from the carbon skeleton, which then undergoes breakdown or interconversion. The carbon skeleton may provide the basis for an alternate amino acid, a respiratory substrate or other non-nitrogenous metabolites such as keto acids or sugars. Reactions involved in removal of nitrogen from amino acids are transamination and deamination. Transamination results in transfer of an amino group to an alternate keto acid, whereas deamination removes nitrogen from the compound to produce ammonia, which is then reassimilated in amide synthesis (Lea & Joy, 1983). Examination of the free amino acid profile of the developing flax seedlings showed that the content of almost all the identified free amino acids was increased during germination (Table 2). The content of

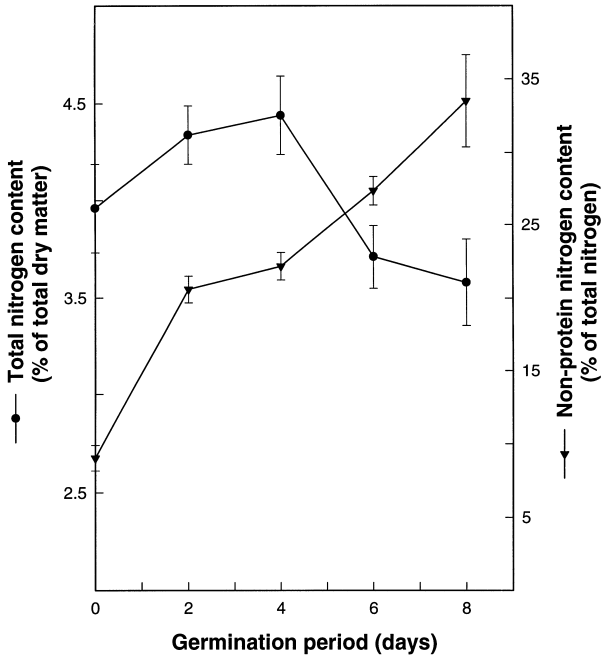

Fig. 1. Changes of the contents of total nitrogen and non-protein nitrogen during germination of flaxseed.

Table 2
Content of free amino acids (mg/g protein) of germinating flaxseed

Amino acid	Duration of germination (days)				
	0	2	4	6	8
Alanine	0.24 ± 0.01	2.46 ± 0.11	2.43 ± 0.10	3.97 ± 0.20	4.89 ± 0.01
Arginine	1.35 ± 0.09	3.74 ± 0.07	3.16 ± 0.10	4.36 ± 0.13	4.65 ± 0.09
Asparagine	0.31 ± 0.01	0.88 ± 0.01	1.10 ± 0.11	2.00 ± 0.21	3.48 ± 0.05
Aspartic acid	0.43 ± 0.01	0.80 ± 0.03	0.67 ± 0.05	0.92 ± 0.01	1.06 ± 0.04
Cystine	0.06 ± 0.00	0.14 ± 0.10	0.25 ± 0.01	0.33 ± 0.00	0.42 ± 0.03
Glycine	0.19 ± 0.01	0.96 ± 0.08	1.51 ± 0.02	2.58 ± 0.01	6.61 ± 0.10
Glutamine	0.06 ± 0.01	5.11 ± 0.04	8.83 ± 0.12	10.36 ± 0.11	12.58 ± 0.11
Glutamic acid	0.44 ± 0.10	3.17 ± 0.09	3.52 ± 0.11	4.50 ± 0.10	5.83 ± 0.10
Histidine	0.15 ± 0.08	1.24 ± 0.03	1.38 ± 0.08	2.00 ± 0.08	2.47 ± 0.10
Lysine	0.20 ± 0.01	1.14 ± 0.06	1.19 ± 0.02	1.69 ± 0.01	1.95 ± 0.01
Leucine	0.01 ± 0.00	1.84 ± 0.01	1.69 ± 0.10	2.57 ± 0.02	2.63 ± 0.12
Isoleucine	0.04 ± 0.00	1.16 ± 0.10	1.06 ± 0.04	1.32 ± 0.00	1.48 ± 0.00
Methionine	0.01 ± 0.00	0.57 ± 0.02	0.48 ± 0.05	0.55 ± 0.01	0.52 ± 0.00
Phenylalanine	0.13 ± 0.01	1.38 ± 0.06	1.03 ± 0.01	1.57 ± 0.01	1.58 ± 0.01
Proline	0.27 ± 0.02	1.04 ± 0.04	2.35 ± 0.30	4.38 ± 0.10	4.60 ± 0.07
Serine	0.08 ± 0.01	1.92 ± 0.01	1.92 ± 0.01	3.42 ± 0.11	5.08 ± 0.10
Threonine	0.11 ± 0.01	0.99 ± 0.03	1.01 ± 0.00	1.30 ± 0.01	1.62 ± 0.01
Tryptophan	0.37 ± 0.08	0.93 ± 0.05	1.21 ± 0.06	1.79 ± 0.06	1.95 ± 0.08
Tyrosine	0.10 ± 0.00	1.11 ± 0.00	1.14 ± 0.10	1.27 ± 0.00	1.36 ± 0.09
Valine	0.06 ± 0.01	1.43 ± 0.00	1.44 ± 0.01	1.83 ± 0.01	2.32 ± 0.10
Total content	4.61 ± 0.47	31.08 ± 0.94	37.37 ± 1.40	52.71 ± 1.19	72.38 ± 1.22
Ammonia	2.27 ± 0.20	5.77 ± 0.50	11.62 ± 0.22	20.11 ± 0.61	32.10 ± 0.35

glutamine on days 2, 4, 6 and 8 of germination was 16, 23, 19 and 17% of total free amino acids, respectively. At the same time, increased ammonia content in the samples suggests deamination and synthesis of glutamine in the germinating seeds. Glutamine may be the favoured form of amide donor in developing flax seedlings. Small amounts of amides are directly incorporated into new proteins and most are used in nucleic acid or amino acid synthesis (El-Mahdy & El-Sebay, 1985; Lea & Joy, 1983).

3.1.2. Protein fractions

Studies on the solubility of flax proteins indicated that water-soluble (albumins) and salt-soluble (globulins) proteins were predominant and present in nearly equal amounts in the ungerminated seeds (Table 3). However, during the germination, an increase in the content of the water-soluble fraction and a decrease in the salt-soluble fraction was observed. It may be assumed that globulin breakdown products became part of the nitrogen determined as albumins (Balasubramaniam & Sadasivam, 1989). The alcohol- and alkali-soluble proteins comprised 7–8% of the total proteins of flaxseed. It was also noticeable that alkali-soluble proteins (glutelins) increased as germination proceeded while the content of alcohol-soluble proteins did not change. Since albumins are mostly enzymic proteins (Bewley & Black, 1978), synthesis of enzymes during germination might be responsible for the apparent increase in the content of the water-soluble fraction. An increase in the content of free amino acids is another indication of the increased protease activity and proteolytic degradation during the germination process.

3.1.3. Amino acid composition

Amino acid composition of germinated and ungerminated flax proteins is presented in Table 4. The contents of individual amino acids in ungerminated flaxseed were within the range reported in previous studies (Sosulski & Sarva, 1973; Bhatty & Cherdkiatgumchai, 1990; Wanasundara & Shahidi, 1994). The high content of non-essential amino acids, especially aspartic acid, glutamic acid and arginine may not be nutritionally important, but may be useful in seed metabolism/enzyme synthesis or function (Finney, 1983).

3.1.4. Polyamines

The content of polyamines in flax seedlings during germination is shown in Table 5. The only polyamines present were agmatine and spermidine; putrescine, a diamine, was also present. The content of putrescine was higher than that of both polyamines present before the commencement of germination. As the germination proceeded, the contents of agmatine and spermidine were increased by approximately 50 and 200 folds, respectively. Meanwhile, the content of putrescine decreased by a factor of three.

Polyamines of plants are fundamentally important in the control of cellular metabolism and growth, and their content may reach high levels in rapidly growing tissues (Janne, Poso, & Raina, 1978; Smith, 1985). Decarboxylation of arginine serves as the basis for biosynthesis of agmatine, putrescine and spermidine in plants. Initially, arginine is decarboxylated to agmatine by arginine decarboxylase and then hydrolysed to putrescine by agmatinase (Bardocz, 1995). Spermidine is formed sequentially from putrescine by spermidine synthase (Smith, 1985). Polyamines are reported to enhance protease activity and to increase the mobility of reserve

Table 4
Amino acid composition (g/16 g N) of germinating flaxseed

Amino acid	Duration of germination (days)		
	0	4	8
Essential amino acids			
Histidine	2.50 ± 0.11	2.67 ± 0.08	2.53 ± 0.10
Isoleucine	4.54 ± 0.30	4.92 ± 0.09	3.74 ± 0.04
Leucine	6.54 ± 0.26	7.06 ± 0.06	6.62 ± 0.20
Lysine	4.55 ± 0.18	5.49 ± 0.10	5.20 ± 0.10
Methionine + cysteine	6.09 ± 0.02	5.02 ± 0.10	3.84 ± 0.08
Phenylalanine + tyrosine	7.50 ± 0.08	7.50 ± 0.13	6.01 ± 0.08
Threonine	4.37 ± 0.20	4.61 ± 0.20	4.03 ± 0.09
Tryptophan	0.70 ± 0.02	1.59 ± 0.01	1.28 ± 0.02
Valine	5.46 ± 0.06	5.85 ± 0.20	4.87 ± 0.06
Non-essential amino acids			
Alanine	5.02 ± 0.10	5.99 ± 0.09	5.88 ± 0.11
Arginine	10.03 ± 0.43	9.77 ± 0.15	6.48 ± 0.40
Aspartic acid + asparagine	10.65 ± 0.50	10.72 ± 0.58	8.87 ± 0.50
Glycine	7.44 ± 0.82	6.03 ± 0.08	7.19 ± 0.41
Glutamic acid + glutamine	20.76 ± 1.09	17.02 ± 0.90	15.04 ± 1.00
Proline	3.98 ± 0.23	4.96 ± 0.09	4.85 ± 0.03
Serine	5.02 ± 0.09	5.45 ± 0.11	5.31 ± 0.09

Table 3
Protein fractions of germinating flaxseed

Duration of germination (days)	Fraction of total nitrogen (%)				
	Water-soluble	Salt-soluble	Alcohol-soluble	Alkali-soluble	Residue
0 (ungerminated)	36.9 ± 1.0	30.2 ± 0.9	3.5 ± 0.8	3.9 ± 0.9	27.5 ± 2.7
4	61.0 ± 2.0	12.9 ± 1.1	3.5 ± 0.6	5.3 ± 1.2	17.3 ± 1.5
2	61.2 ± 2.1	17.3 ± 1.1	3.5 ± 1.0	3.9 ± 1.1	14.2 ± 2.3
6	58.6 ± 1.5	8.7 ± 0.8	4.6 ± 1.2	9.8 ± 1.5	18.3 ± 2.4
8	55.9 ± 1.4	8.1 ± 1.0	4.1 ± 1.0	12.5 ± 2.6	19.4 ± 3.2

Table 5
Polyamine content of germinating flaxseed [a]

Polyamine	Duration of germination (days)				
	0	2	4	6	8
Agmatine $NH_2(CH_2)_4NHCH(NH)NH_2$	0.08 ± 0.01	1.18 ± 0.10	2.44 ± 0.12	3.32 ± 0.11	4.21 ± 0.44
Putrescine $NH_2(CH_2)_4NH_2$	0.21 ± 0.02	0.09 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
Spermidine $NH_2(CH_2)_3NH(CH_2)_4NH_2$	0.03 ± 0.01	0.29 ± 0.05	2.54 ± 0.09	4.71 ± 0.23	5.86 ± 0.15

Mean ± SD of three replicates.
[a] μmol/g of dry matter.

proteins and the growth of radish seedlings (Srivastava, Kansara, & Mungre, 1985). Foods such as beer, cheese, chocolate, fish, sauerkraut and wine provide large quantities of polyamines (220–450 μmol putrescine, 100–180 μmol spermidine and 70 μmol spermine), in addition to their biosynthesis in situ (1–2 nmol putrescine/h/g of wet tissue in active organs). Polyamines are essential for the maintenance of the high metabolic activity of the normally functioning healthy gut and also play a role in repairing damage to gut tissues caused by deleterious components of food and/or by bacteria. However, concerns have been expressed about needs to minimize polyamine intake in order to slow down the growth of tumours (Bardocz, 1995). Therefore, it would be important to assess the polyamine content of rapidly growing tissues, such as sprouting seeds, especially those intended for human consumption. No previous studies have been carried out on identification and quantification of polyamines of germinated seeds intended for consumption.

3.1.5. Cyanogenic glycosides and trypsin inhibitors

The cyanogenic glycosides of the flaxseed variety used in this study were linustatin and neolinustatin (Table 6). Their contents in the seeds decreased by 40 and 70% on day 8 of germination, respectively. There is little information available on the fate of cyanogenic glycosides during germination of seeds. However, it has been demonstrated that linamarin in seeds of *Hevea brasiliensis* is metabolized into non-cyanogenic compounds without any liberation of HCN (Selmar, Lieberei, & Bichl, 1988). Linustatin is the other cyanogenic glycoside present in this seed. According to Selmar et al. (1988) linamarin is first converted to the diglycoside linustatin which is the translocation form of cyanogenic glycosides that may not be broken down by β-glycosidase (linamarase) during transportation. During the germination phase of leaf expansion, linustatin is transported out to the growing parts of seedlings, where the enzyme diglucosidase splits off gentiobiose. The HCN produced by dissociation of the resulting acetone cyanohydrin is immediately fixed by β-cyanoalanine synthase to β-cyanoalanine. The β-cyanoalanine, so produced, may be hydrolysed to afford asparagine. This pathway of degradation of linustatin suggests that cyanogenic glycosides are not solely stored secondary metabolites and may function as a source of nitrogen when required, such as in certain developmental stages. From a nutritional point of view the disappearance of cyanogenic glycosides during sprouting reduces the risk of HCN production, thus improving the nutritional quality of sprouts.

The content of trypsin inhibitors in flaxseed was very low compared to those of legumes and only trace amounts were present in the germinated samples (Table 6). This is a very good indication that there are no commonly found protease (trypsin) inhibitors in germinated flax.

Food utilization of flaxseed has recently been encouraged as it contains clinically valuable phytochemicals such as α-linolenic acid, seicoisolariciresinol diglucoside (SDG) and soluble fibre (Caragay, 1992; Cunnane & Thompson, 1995). Besides consuming whole seeds as such or incorporating them into existing food products, germination may provide an alternate means for utilization of flaxseed. This study shows reduction in the contents of cyanogenic glycosides and trypsin inhibitors which may improve the nutritional value of

Table 6
Changes in the contents of cyanogenic glycosides, phytic acid and trypsin inhibitors during germination of flaxseed [a]

Component	Duration of germination (days)				
	0	2	4	6	8
Cyanogenic glycosides (mg/g dry matter)					
Linustatin	2.70 ± 0.50	3.50 ± 0.67	3.00 ± 0.55	2.79 ± 0.20	1.60 ± 0.10
Neolinustatin	3.09 ± 0.90	3.60 ± 0.80	2.75 ± 0.18	0.65 ± 0.10	0.28 ± 0.07
Trypsin inhibitors (mg TI/g crude protein)	13.3 ± 1.40	ND	ND	ND	ND

ND: Not detected.
[a] Mean ± SD of three replicates.

flaxseed. The fate of other macromolecules of flaxseed during germination has been reported elsewhere (Wanasundara, Wanasundara, & Shahidi, 1998).

4. Conclusions

The total content of nitrogen of flaxseed did not show a significant ($p < 0.05$) change due to germination; however, the content of non-protein nitrogen in the samples was increased. A decrease in the amount of salt-soluble nitrogen fraction showed a reduction in the content of storage proteins of flaxseed and an increase in their water-soluble fraction, which supports the conversion of large protein molecules to small ones. A reduction in the content of linustatin and neolinustatin was also observed. A large increase in the amines, namely agmatine, putrescine and spermidine, occurred during the germination. Presence of trace amounts of trypsin inhibitor in germinated flaxseed is a further important nutritional characteristic.

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