JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 7391−7398 **7391**

Influence of Lupin (*Lupinus luteus* L. cv. 4492 and *Lupinus angustifolius* L. var. *zapaton*) and Fenugreek (*Trigonella foenum-graecum* L.) Germination on Microbial Population and Biogenic Amines

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Microbial population and bioactive amine profile and levels of two lupin species (*Lupinus luteus* L. cv. 4492 and *Lupinus angustifolius* L. var. *zapaton*) and fenugreek (*Trigonella foenum-graecum* L.) seeds as affected by germination were investigated. Microbial population increased considerably mainly in the first stage of germination (2 days), then small changes in bacterial numbers were observed up to 5 days to levels between 7.8 and 8.9 log colony-forming units/g. Microorganisms belonging to the Enterobacteriaceae family were dominant for the legumes tested. Ungerminated legume seeds contained putrescine, cadaverine, histamine, tyramine, spermidine, and spermine. Bioactive amine levels found in fenugreek seeds were between 3- and 4-fold higher than those found in lupin seeds. The highest total amine levels were found in fenugreek seeds [162 mg/kg of dry weight (dw)], followed by *L. angustifolius* var. *zapaton* seeds (84 mg/kg of dw) and, finally, *L. luteus* cv. 4492 (46 mg/kg of dw) seeds. The concentration of individual amines showed a gradual rising trend during the germination period in all tested sprouts, reaching levels >3 times higher than those found in ungerminated seeds. After 5 days of germination, the fenugreek sprouts contained the highest amount of total bioactive amines. Tyramine was the predominant amine in both lupin varieties, whereas cadaverine was the main bioactive amine detected in fenugreek. The results of this work thus indicated that microbial population and biogenic amine levels in the studied lupin and fenugreek sprouts are not a risk for healthy consumers or for individuals with restricted activity of detoxification enzymes.

KEYWORDS: Bioactive amines; fenugreek; lupin; microbial population

INTRODUCTION

The use of seed sprouts as food originating in Far Eastern countries has spread over the past few decades to parts of the Western world (*1*). In general, sprouts have a fresh and healthy image because sprouting increases the content and availability of essential nutrients, whereas levels of antinutrients decrease, resulting in a higher nutritional value (*2*−*5*). However, they are also a suitable medium for bacterial growth, which has led to numerous outbreaks of foodborne diseases (*4*, *6*, *7*).

Seeds routinely contain high numbers of microbial flora [10^3−10^6 colony-forming units (cfu)/g], including coliforms and fecal coliforms, which appear to be part of the normal seed flora (*8*). Sprouting conditions of high moisture and warm temperatures as well as an increase in nutrient content and availability seem to provide ideal conditions for bacterial growth (*9*−*11*).

In the Western world, most consumers and retailers do not cook sprouts and, because bacteria on the seed surface can become internalized during sprouting, washing sprouts is probably an ineffective way to eliminate spoilage and pathogenic bacteria (*12*). The severity of this problem is shown by the elaboration of a specific recommendation developed in 1997 by the National Advisory Committee of Microbiological Criteria for Foods (*13*). The U.S. Food and Drug Administration (FDA) also issued guidelines to enhance sprouts' safety (*14*). It is generally accepted that microbial populations exceeding 5×10^6 cfu/g or 5×10^6 cfu/mL may display measurable metabolites not only in terms of spoilage parameters but also in relation to toxic metabolites such as some biogenic amines.

It has long been known that certain amines fulfill a number of important metabolic and physiological functions in living organisms. They are formed during normal metabolic processes and are, therefore, present in foods. They can be classified on the basis of the number of amine groups, chemical structure, and biosynthesis or physiological functions. They are usually generated by decarboxylation of amino acids (*15*). Amines such as putrescine, spermidine, spermine, and also cadaverine are essential components of living cells and are important in the regulation of nucleic acid function and protein synthesis.

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10.1021/jf061238v CCC: $33.50
Published on Web 08/29/2006

Furthermore, the biogenic amines, histamine, tyramine, serotonine, putrescine, cadaverine, and phenylethylamine, are neuroactive and vasoactive (*16*−*18*).

Most fruits and vegetables contain small amounts of polyamines with spermidine as a predominant one (*19*). Some biogenic amines occur naturally in the plant kingdom, but others can be introduced during production and processing because of decarboxylase activity from microorganisms (*20*, *21*).

The presence of bioactive amines in foods and beverages is important because of their influence on physiological activities. Some of them are required in cellular metabolism and growth and, particularly, in rapidly growing tissues. Similarly, during periods of wound healing, regeneration, and compensatory growth, amines are also required (*16*, *18*, *22*, *23*). However, high levels of biogenic amines can cause distinct pharmacological and toxic effects, with symptoms such as skin irritations, headache, dizziness, vomiting, and diarrhea (*24*). In the intestinal tract of mammals, a fairly efficient detoxification system exists that is capable of metabolizing normal dietary biogenic amine intakes. The enzymes monoamine oxidase and diamine oxidase play important roles in this detoxification process, although the detoxification system fails to eliminate the high amounts of biogenic amines ingested with certain spoiled or fermented foods (*25*). However, information is still lacking on the levels of amines in foodstuffs and on the influence of many factors including plant species and variety, type of tissue, germination, conditions of growth, stage of development, degree of ripening, and processing and storage conditions. To assess the possible risk of orally ingested biogenic amines, their occurrence and content in foods must be known.

It is, therefore, of special importance to obtain information about levels of biogenic amines and microorganisms in food and their evolution during food processing because practically no information is available about the presence and evolution of either of these in fresh prepacked vegetable-type products, including legume sprouts. The aim of the present study is to investigate the influence of germination on microbial population and bioactive amine profiles and levels present in two lupin species and fenugreek seeds.

MATERIALS AND METHODS

Samples. Lupin seeds (*Lupinus luteus* L. cv. 4492 and *Lupinus angustifolius* L. var. *zapaton*) were supplied by Agrarian Research and Technology Development Service from the Agriculture and Commerce Council of the Junta de Extremadura (Spain). Fenugreek seeds (*Trigonella foenum-graecum* L.) were purchased from a herbalist shop. Seeds were cleaned and stored in polyethylene containers at 4 °C until used for germination experiments.

Reagents. Media for bacterial counts such as peptone water Tryptic Soy Agar (TSA, Scharlau Chemie), Violet-Red Bile Agar (VRBA, pH 7.4), and Slanetz Bartley Agar were supplied by Scharlau Chemie. Bioactive amine standards putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, tyramine, spermidine trihydrochloride, and spermine tetrahydrochloride were purchased from Fluka. For amine derivatization, dansyl chloride (DCl) was acquired through the Sigma Co.

Seed Germination. Ten grams of seeds was soaked with 50 mL of 0.07% sodium hypochlorite for 30 min. These seeds were drained and washed with distilled water until neutral pH. Afterward, seeds were soaked in distilled water (50 mL) for 5.5 h, shaking every 30 min. The imbibed seeds were germinated in a pilot scale germinator by layering them over a moist filter paper in a germination tray. The tray was placed in a seed germinator G-120 model (ASL Snijders International S.L.), and seeds were continuously watered by capillary. Germination was carried out at 25 °C in darkness for 5 days. Sprouts were harvested at 2, 3, 4, and 5 days. Germination rate was >90% of seeds.

Microbiological Determinations. Five grams of fresh sprouts and seeds were aseptically placed in a flask with 45 mL of peptone water to achieve a 10^{-1} dilution and stirred with vortex for 1 min. Serial dilutions were made up in 0.1% buffered peptone water in tubes.

Determination of Total Mesophilic and Psychrotrophic Aerobic Bacteria. Determination of total mesophilic and psychrotrophic aerobic bacteria counts was carried out in fresh sprouts and raw seeds. Appropriate serial dilutions with buffered peptone water were surface-plated on TSA. Plates were incubated at 32 °C for mesophilic bacteria counts at 48 h and at 8 °C for psychrotrophic bacteria counts at 10 days. After incubation, plates with 25−250 cfu were enumerated.

Determination of Total and Fecal Coliforms. Total and fecal coliforms were determined at 2, 3, 4, and 5 days in fresh sprouts and in raw seeds. Appropriate serial dilutions with buffered peptone water were plated on VRBA (pH 7.4) and incubated at 37 °C (for total coliforms) or 44 °C (fecal coliforms) for 24 h under anaerobic conditions.

Determination of Fecal Streptococci. Fecal Streptococci were determined at 2, 3, 4, and 5 days in fresh sprouts and in raw seeds. Appropriate serial dilutions with buffered peptone water were surface-plated on Slanetz Bartley agar and incubated at 37 °C for 24 h.

Bioactive Amine Determination. Determination of bioactive amines was carried out by acid extraction, derivatization, and HPLC quantification using the method of Moret et al. (*26*). For this purpose, 5 g of fresh sprout or seed flour was directly weighed into a centrifuge tube and homogenized with 20 mL of 0.1 M HCl in an Ultra-Turrax T25 homogenizer for 2 min at 30000 rpm. The resulting homogenate was centrifuged at 12000 rpm for 20 min at 4 °C. Supernatant was collected and the residue re-extracted in the same conditions. The two combined extracts were filtered through Whatman no. 1 paper and diluted to 100 mL in a volumetric flask.

For amine derivatization, 1 mL aliquot of the diluted extract was mixed with 0.5 mL of saturated $NaHCO_3$, and 1 mL of dansyl chloride reagent (20 mg/mL in acetone) was added to the seed extract. The mixture was then transferred to an incubator and kept at 40 °C in darkness under agitation for 60 min. The residual dansyl chloride was removed by adding 200 μL of a proline solution (100 mg/mL), vortexed for 1 min, and left to react at room temperature in darkness for 15 min. Finally, the sample was extracted twice with a 1 mL aliquot of diethyl ether. The combined extracts were dried under nitrogen flow, and the residue was redissolved in 1 mL of acetonitrile and filtered through a 0.45 μm PVDF Millipore filter for injection.

A stock standard aqueous solution of amines was prepared by adding an accurately weighed amount of each standard (ca. 50 mg) to a 25 mL volumetric flask. Standards were derivatized as described for the samples and were used as external standards. Calibration curves were obtained for standard amines and r values were always >0.990.

The chromatographic system consisted of an Alliance Separation Module 2695 (Waters, Milford, MA), a photodiode array detector 996 settled at 254 nm (Waters, Milford, MA), and a personal computer running Empower 2 for Windows chromatographic software (Waters). The sample (20 μL) was injected onto a C_{18} Kromasil 250 × 4.6 mm i.d., 5 μm size (Waters) column equipped with an ODS2 guard column (Waters), both thermostated at 30 °C.

The mobile phase for DCl derivatives consisted of bidistilled water (solvent A) and acetonitrile (solvent B). The gradient elution program was maintained at 65% of B for 1 min, ramped at 80% (10 min), 90% (12 min), 100% of B (16 min), and held until the end of the run (23 min) with a flow rate of 0.8 mL/min.

Statistical Analyses. All analyses were performed in duplicate using three test samples, and data were expressed as mean ± standard deviation (SD). Data were subjected to multifactor analysis of variance with the use of the least significance difference test with the Statgraphic 5.0 program (Statistical Graphics Corp., Rockville, MD).

RESULTS AND DISCUSSION

Influence of Lupin and Fenugreek Germination on Microbial Population. Table 1 shows the influence of germination time on the microbial population of *L. luteus* cv. 4492 and *L. angustifolius* var. *zapaton* seeds, respectively. The

Table 1. Influence of Germination Time on Microbial Population of Lupin (*L. luteus* cv. 4492 and *L. angustifolius* var. *zapaton*) Seeds[a]

	counts (log cfu/g of fresh sprout)				
	0	2	3	4	5
L. luteus cv. 4492					
mesophilic bacteria	6.57 ± 0.04a	7.12 ± 0.12b	7.22 ± 0.20b	8.43 ± 0.16c	8.93 ± 0.03d
psychrotrophic	4.99 ± 0.07a	5.79 ± 0.17b	6.20 ± 0.08c	6.85± 0.14d	7.25 ± 0.10e
total coliforms	3.62 ± 0.04a	5.10 ± 0.22b	5.07 ± 0.10b	5.45 ± 0.54b	6.50 ± 0.28c
fecal coliforms	3.08 ± 0.13a	3.81 ± 0.05b	4.89 ± 0.16c	4.32 ± 0.03cd	4.60± 0.23d
fecal Streptococci	<2	<2	<2	<2	<2
L. angustifolius var. zapaton					
mesophilic bacteria	6.50 ± 0.28a	7.25 ± 0.38b	7.09 ± 0.13b	7.41 ± 0.11bc	7.81 ± 0.06c
psychrotrophic	4.81 ± 0.06a	6.59 ± 0.17b	6.77 ± 0.204b	6.81± 0.09b	6.57 ± 0.10b
total coliforms	4.22 ± 0.31a	5.63 ± 0.21b	5.60 ± 0.00b	5.93 ± 0.04bc	6.22 ± 0.15c
fecal coliforms	2.62 ± 0.22a	3.49 ± 0.21b	4.46 ± 0.22c	5.28 ± 0.47cd	5.34± 0.50d
fecal Streptococci	<2	<2	<2	<2	<2

[a] Results are expressed as mean value ± SD of three repetitions. Rows with different letters are significantly different ($P \leq 0.05$).

Table 2. Influence of Germination Time on Microbial Population in *T. foenum-graecum* Seeds[a]

	counts (log cfu/g of fresh sprout)				
	0	2	3	4	5
mesophilic bacteria	6.34 ± 0.32a	7.36 ± 0.36b	8.33 ± 0.49c	8.72 ± 0.05cd	9.13 ± 0.21d
psychrotrophic	4.12 ± 0.73a	6.39 ± 0.20b	6.63 ± 0.07bc	7.47 ± 0.30cd	8.22± 0.32d
total coliforms	4.52 ± 0.34a	5.73 ± 0.08b	5.79 ± 0.03b	6.22 ± 0.46b	7.87 ± 0.15c
fecal coliforms	3.62 ± 0.03a	4.57 ± 0.10b	5.50 ± 0.29c	5.58 ± 0.39c	6.13 ± 0.24c
fecal Streptococci	<2	<2	<2	<2	<2

[a] Results are expressed as mean value ± SD of three repetitions. Rows with different letters are significantly different ($P \leq 0.05$).

numbers of total aerobic mesophilic bacteria in raw seeds were 6.57 and 6.50 log cfu/g for *L. luteus* cv. 4492 and *L. angustifolius* var. *zapaton*, respectively. Total aerobic plate counts increased considerably from the second day of germination, and then no significant ($P \leq 0.05$) changes in bacterial number were observed up to 3 or 4 days of germination in *L. luteus* cv. 4492 and *L. angustifolius*, respectively. Finally, the largest count was recorded at the fifth day of germination, at which levels of 8.9 and 7.8 log cfu/g for *L. luteus* cv. 4492 and *L. angustifolius* var. *zapaton*, respectively, were found. The number of psychrotrophic bacteria in seeds was ∼5 log cfu/g for raw lupin species. This microbial population showed a rise of ∼1 log cycle in 2-day sprouts that continued growing until the end of germination in *L. luteus* cv. 4492, whereas in *L. angustifolius* var. *zapaton*, the psychrotrophic bacterial population was maintained in 5-day sprouts. Maximum levels recorded were 7.3 and 6.6 log cfu/g for *L. luteus* cv. 4492 and *L. angustifolius* var. *zapaton*, respectively. Total coliforms counted in both raw lupin seeds was ∼4 log cfu/g. Seed germination led to gradual increases of about 3 and 2 log cycles after 5 days for *L. luteus* cv. 4492 and *L. angustifolius* var. *zapaton*, respectively, to a maximum level of ∼6 log cfu/g in both lupin species. However, total coliform numbers were maintained after 3 and 4 days of germination. Fecal coliform contamination of raw seeds was 3 log cfu/g for *L. luteus* and *L. angustifolius* var. *zapaton*. In lupin sprouts, fecal coliform numbers showed an upward trend to >1 log cycle during germination to levels of ∼5 log cfu/g. The amount of fecal Streptococci in raw lupin seeds showed levels of <2 log cfu/g, which were maintained unchanged during the whole germination process.

Table 2 shows the influence of germination time on the microbial population of *T. foenum-graecum* seeds. The number of total aerobic mesophilic bacteria in raw seeds was 6.34 log cfu/g, similar to those found in lupin seeds. Total aerobic plate counts increased continuously until the end of germination, and maximum numbers of total mesophilic bacteria were recorded in 5-day fenugreek sprouts (9.1 log cfu/g, which comprised up to almost 3 log cycles). Psychrotrophic bacteria number in raw fenugreek seeds was ∼4 log cfu/g. This microbial population suffered a sharp increase of ∼4 log cycles after 5 days of germination, and maximum levels recorded were 8.22 log cfu/g. Total coliform counts present in fenugreek seeds corresponded to ∼4 log cfu/g, and germination led to a gradual increase of ∼3 log cycles after 5 days to a maximum level of ∼7.9 log cfu/g. However, coliform numbers were maintained between the third and fourth days of germination. Fecal coliform contamination of raw seeds corresponded to 3.6 log cfu/g of seeds. Fecal coliforms presented an upward trend to 2 log cycles after 3 days of germination to maximum counts of ∼6 log cfu/g. The amount of fecal Streptococci present in raw seeds was below 2 log cfu/g, and no changes were observed during germination.

Results obtained for raw seeds before sprouting correspond to those recorded by other authors for different kinds of seeds (*27*−*30*). Microbiological analyses have shown that alfalfa and mungbean seeds routinely contained high numbers of microbial flora (10^2-10^6 cfu/g), including coliforms (10^4 cfu/g) and fecal coliforms (10^2-10^3 cfu/g), and that these organisms appeared to be part of the normal seed flora (*4*). Seeds of different plants differ in the microbial population, and these differences can be caused by the different compositions of the seed coat and different cultivation and storage conditions (*28*), although no microbiological data have been published in lupin and fenugreek seeds.

The small amount of bacteria present in raw seeds can immediately grow during sprouting, because germination seems to provide ideal conditions for bacterial growth (*28*). Within the first two sprouting days microbial populations increased approximately 2 log cycles in rice seeds (*28*), 3 log cycles on alfalfa seeds and mungbeans (*10, 31*), and 4 log cycles in kidney beans (*30*). Several factors have been identified that contribute to the rapid proliferation of bacteria on sprouts; these include

Table 3. Influence of Germination Time on Bioactive Amine Levels in Lupin and Fenugreek Sprouts[a]

	days	PUT	CAD	HIS	TYR	SPD	SPM	water (%)
L. luteus var. 4492	0	3.36 ± 0.03a	5.78 ± 0.04a	2.68 ± 0.04a	6.92 ± 0.17a	6.37 ± 0.20a	3.60 ± 0.07a	4.52 ± 0.01
	2	4.41 ± 0.23b	7.72 ± 0.18b	5.30 ± 0.35b	12.06 ± 0.14b	7.46 ± 0.12b	6.67 ± 0.11b	66.39 ± 0.69
	3	6.77 ± 0.23c	9.85 ± 0.28c	7.58 ± 0.15c	13.88 ± 0.29c	9.36 ± 0.14c	8.81 ± 0.10c	72.81 ± 0.01
	4	7.98 ± 0.24d	14.51 ± 0.30d	11.03 ± 0.16d	19.44 ± 0.25d	11.78 ± 0.22d	12.66 ± 0.20d	78.69 ± 0.89
	5	10.80 ± 0.20e	18.91 ± 0.32e	12.58 ± 0.19e	21.88 ± 0.14e	15.27 ± 0.30e	13.02 ± 0.10e	80.42 ± 0.04
L. angustifolius var. *zapaton*	0	3.49 ± 0.03a	6.01 ± 0.03a	3.44 ± 0.11a	5.70 ± 0.21a	4.93 ± 0.12a	4.69 ± 0.14a	6.27 ± 0.03
	2	4.90 ± 0.06b	8.47 ± 0.17b	3.99 ± 0.03b	10.21 ± 0.37b	6.66 ± 0.21b	7.02 ± 0.26b	67.24 ± 0.11
	3	6.35 ± 0.15c	10.80 ± 0.07c	6.90 ± 0.14c	14.10 ± 0.24[c]	10.09 ± 0.34c	8.98 ± 0.34c	74.09 ± 0.60
	4	7.36 ± 0.10d	12.28 ± 0.19d	8.70 ± 0.25d	20.60 ± 0.23d	11.18 ± 0.32d	12.20 ± 0.18d	76.90 ± 0.37
	5	11.07 ± 0.21e	17.30 ± 0.23e	13.02 ± 0.22e	23.98 ± 0.45e	15.06 ± 0.24e	17.52 ± 0.44e	85.06 ± 0.85
T. foenum-graecum	0	13.74 ± 0.07a	22.29 ± 0.19a	17.02 ± 0.49a	16.94 ± 0.36a	20.07 ± 0.16a	18.23 ± 0.05a	5.37 ± 0.04
	2	16.56 ± 0.44b	32.95 ± 0.54b	17.01 ± 0.45a	17.75 ± 0.38b	20.21 ± 0.36a	18.45 ± 0.49a	79.49 ± 0.10
	3	19.25 ± 0.62c	36.72 ± 1.21c	17.44 ± 0.42ab	18.19 ± 0.63b	20.21 ± 0.52a	18.72 ± 0.40ab	81.13 ± 0.39
	4	13.74 ± 0.07d	42.89 ± 0.95d	17.45 ± 0.53ab	20.57 ± 0.30c	20.10 ± 0.51a	18.61 ± 0.58ab	84.51 ± 0.56
	5	24.63 ± 0.61e	66.43 ± 0.98e	18.19 ± 0.63b	21.26 ± 0.49d	20.11 ± 0.50a	19.12 ± 0.38b	88.80 ± 0.73

[a] Results are expressed as mean value ± SD of three repetitions. Letters a-d following entries indicate statistical significance at $P \leq 0.05$. PUT, putrescine; CAD, cadaverine; HIS, histamine; TYR, tyramine; SPD, spermidine; SPM, spermine.

enzymatic, nutritional, and environmental factors (*4*). Mungbean, alfalfa seeds, and soybeans contain trypsin inhibitors, which may provide a defense mechanism by which seeds inhibit the trypsin-like enzymes in bacteria. Levels of trypsin inhibitors in seeds decrease during germination, thereby enabling the microbial flora to proliferate (*10*, *32*). Similarly, the content of some nutrients in seeds (mono- and disaccharides and amino acids) increase 10-fold during germination, providing available substrates for microbial growth. Finally, the high levels of moisture required and the warm conditions resulting from heat generated during the sprouting process create a favorable environment for bacterial growth (*4*). There are numerous papers showing that the highest growth of bacteria takes place at the beginning of sprouting (*1*, *33*−*36*). Kimanya et al. (*30*) reported that the absence or low levels of pathogens in sprouts are not sufficiently competitive with the background flora during germination to increase to levels which lead to toxin production.

Skowronek et al. (*37*) reported that Enterobacteriaceae and *Pseudomonas* spp. represented the dominant groups during sprouting and comprised up to 95% of the total microbial population of mungbean and lentil sprouts and >99% for radish sprouts after 2 days. Earlier studies have also described Enterobacteriaceae as the dominant microorganism (*38*). Splittstoesser et al. (*31*) found that the microbial population of commercial sprouts was very similar to that germinated in the laboratory under aseptic conditions. These authors concluded that the bacterial species found on sprouts probably originated from the seeds rather than being due to the sanitary conditions of commercial sprout production.

Lupin and fenugreek sprouts contained microbial counts between 10^8 and 10^9 log cfu/g for total mesophilic bacteria, which are the usual counts for minimally processed germinated seeds and do not necessarily indicate a public health concern or a lack of quality. The prevailing species are commonly found in plants in the field. The number of psychrotrophic microorganisms was very similar to those of commercial alfalfa and bean sprouts (*8*), suggesting that lupin and fenugreek sprouts could have the same shelf life when stored at refrigerated temperatures. Coliforms were also part of the epiphytic microflora of the seeds, and a large majority of thermotolerant strains cannot be considered as fecal indicators, even indole producers (*28*). Finally, fecal Streptococci numbers were too low to affect human health. Nevertheless, because microorganisms are related to biogenic amine production, these toxic compounds were also evaluated.

Influence of Lupin and Fenugreek Germination on Profile and Levels of Biogenic Amines. Table 3 shows the individual bioactive amine levels of two lupin species (*L. luteus* L. cv. 4492 and *L. angustifolius* L. var. *zapaton*) and fenugreek (*T. foenum-graecum* L.) seeds. Because the moisture content of the different samples varies, biogenic amine levels were expressed on a dry weight basis. Of the bioactive amines investigated putrescine, cadaverine, histamine, tyramine, spermidine, and spermine were detected in raw seeds of lupin and fenugreek. The presence of these bioactive amines in legumes such as lupin, lentil, mungbean, soybean, broad bean, and chickpea seeds was described in the literature (*37*, *39*−*41*). However, agmatine was not detected in lupin and fenugreek seeds, whereas this amine has been found in soybean and lentil seeds (*39*, *41*). There were significant differences in the levels of individual amines between lupin species. Significantly lower putrescine, cadaverine, histamine, and spermine levels were observed in *L. angustifolius* var. *zapaton* seeds compared with *L. luteus* cv. 4492 seeds. Moreover, bioactive amine levels found in fenugreek seeds were between 3- and 4-fold higher than those found in lupin seeds. It has been reported that levels of amines, which seem to be dependent on the seed type and amine levels found in the tested legume seeds, are in the concentration range given by Shalaby (*40*) for broad bean, chickpea, and lupin seeds. However, Glória et al. (*39*) reported lower amine levels for different soybean cultivars, whereas Simon-Sarkadi and Holzapfel (*41*) described higher ones for mungbean, lentil, and radish. No data have been found for fenugreek seeds.

The presence of spermidine and spermine in lupin and fenugreek seeds was expected, as these polyamines are widely distributed in the plant kingdom, together with their diamine precursor, putrescine. According to the literature (*21*), polyamines play a critical role in several processes, including root growth, somatic embryogenesis, control of intracellular pH, flower and fruit development, and response to abiotic stress, such as potassium deficiency, osmotic shock, drought, and pathogen infection. Furthermore, these amines are associated with cell wall and membrane, preventing deterioration because of radical scavenging properties.

Cadaverine is a diamine that can be formed by the decarboxylation of lysine. It has restricted occurrence, being found mainly in species of the Leguminosae family (*19*, *21*, *22*). Its presence in lupin and fenugreek, which belong to this botanic family, is in accordance with the literature.

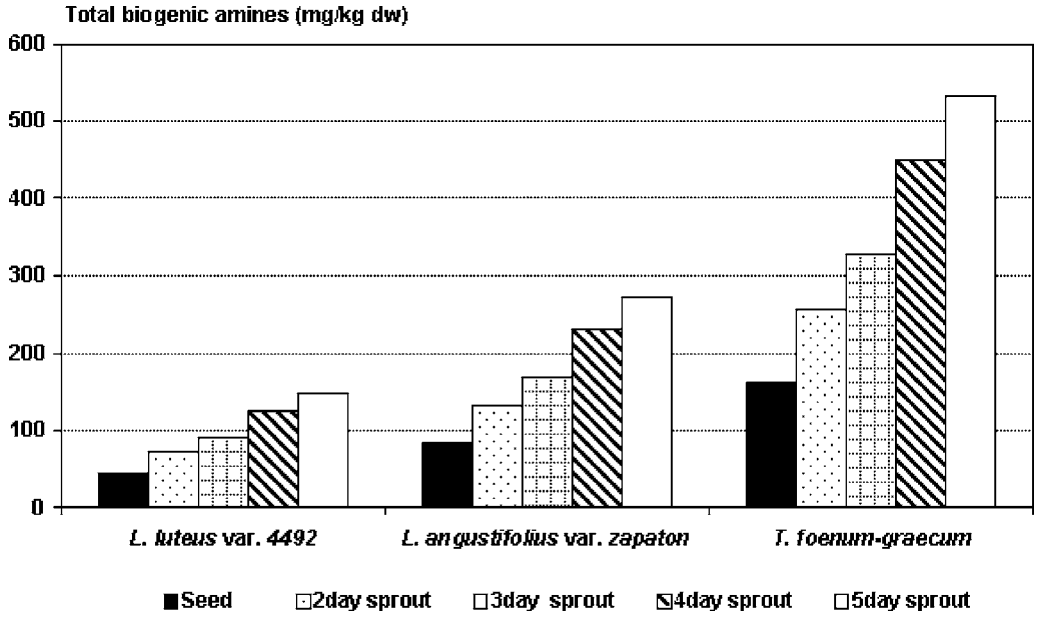

Figure 1. Total bioactive amine levels in lupin (*L. luteus* L. cv. 4492 and *L. angustifolius* L. var. *zapaton*) and fenugreek (*T. foenum-graecum* L.) sprouts.

The influence of germination time on individual bioactive amine levels of two lupin species (*L. luteus* L. cv. 4492 and *L. angustifolius* L. var. *zapaton*) and fenugreek (*T. foenum-graecum* L.) seeds is shown in **Table 3**. The concentration of putrescine showed a rising trend during the germination period in all tested grains. The amounts detected at the end of the germination period were 11 and 25 mg/kg of dry weight (dw) for lupin and fenugreek, respectively. Cadaverine content in legume seeds during the germination process followed a similar pattern as that found for putrescine. The amounts detected at the end of germination time (5 days) were 19, 17, and 66 mg/kg of dw for *L. luteus* cv. 4492, *L. angustifolius* var. *zapaton*, and fenugreek, respectively. The concentration of histamine rose gradually (up to ~13 mg/kg of dw) in lupin species and increased significantly (up to 18 mg/kg of dw) at the end of the germination period in fenugreek. Tyramine increased gradually until the end of the experiment, reaching 22, 24, and 21 mg/kg of dw in *L. luteus* cv. 4492, *L. angustifolius* var. *zapaton*, and fenugreek, respectively. Spermidine content showed a continuous increase (up to ~15 mg/kg of dw) in the two lupin species, but the germination process had no significant effect ($P \leq 0.05$) on changes in spermidine levels in fenugreek. Concerning the spermine content of the germinated legumes studied, its concentration showed a pattern of increasing changes from early stages of germination to the end of germination for both lupin and fenugreek. Spermine concentration achieved 13, 18, and 19 mg/kg of dw in 5-day sprouts of *L. luteus* cv. 4492, *L. angustifolius* var. *zapaton*, and fenugreek, respectively.

The influence of germination on total levels of amines in lupin and fenugreek seeds is shown in **Figure 1**. The highest total amine levels were recorded in fenugreek seeds (162 mg/kg of dw), followed by *L. angustifolius* var. *zapaton* (84 mg/kg of dw) and, finally, *L. luteus* cv. 4492 (46 mg/kg of dw) seeds. These levels are lower than those found by Simon-Sarkadi and Holzapfel (*41*) for mungbean, lentil, and radish. The total bioactive amines showed an increasing trend throughout the germination period in all tested legume seeds, reaching levels >3 times higher than the original ones. After 5 days of germination, the fenugreek sprouts contained the highest amount of total bioactive amines. The pattern observed for total bioactive amines was similar to those found in the literature for lupin, chickpea, and broad bean (*40*).

Contributions of each amine to total levels in lupin (*L. luteus* cv. 4492 and *L. angustifolius* var. *zapaton*) and fenugreek (*T. foenum-graecum*) seeds at different stages of germination are indicated in **Figure 2**. Tyramine was the predominant amine in both lupin varieties, followed by cadaverine. The mean contribution of the other amines to total levels indicated the predominance of spermidine and spermine, followed by histamine and putrescine with the smallest contribution. However, the contribution pattern of each amine to total levels differed when fenugreek was considered. In the latter case, cadaverine was the predominant amine, followed by spermidine and spermine, respectively. The mean contribution of putrescine, cadaverine, and histamine to total levels was affected by the day of germination. The higher contribution of spermidine than spermine to total levels in lupin and fenugreek seeds was also observed by other authors (*17*, *19*, *20*, *39*) for soybean, coffee, and some vegetables.

The increment in total bioactive amines observed during the early stage of germination was previously reported in different seeds by several authors (*42*, *43*). According to Simon-Sarkadi and Holzapfel (*41*), Shalaby (*40*), and Glória et al. (*39*) amines are endogenously produced during germination process. Polyamines have been reported to be related to protein and nucleic acid synthesis (*44*). During the germination period, protein is synthesized rapidly and increasing levels are expected. Glória et al. (*40*) attributed the significantly higher levels of the polyamines spermidine, spermine, and putrescine during germination to the greater cellular multiplication period and growth. The concentration of these amines is expected to increase in tissues with a high rate of development. However, small changes in the spermidine and spermine concentration were observed during the germination period of radish as found here for fenugreek. The presence of cadaverine in lentil, mungbean, and radish sprouts was reported by Simon-Sarkadi and Holzapfel (*41*). Shalaby (*40*) observed an increase in cadaverine levels during the germination of some legume seeds (bean, chickpea, and lupin), reaching a maximum value on the fifth day. The presence of cadaverine in sprouts could be associated with its role in elongation of the root and the increase in cell size (*21*). However, Simon-Sarkadi and Holzapfel (*41*) also observed a significant increase in Enterobacteriaceae and *Pseudomonas* spp., which represent the dominant groups and

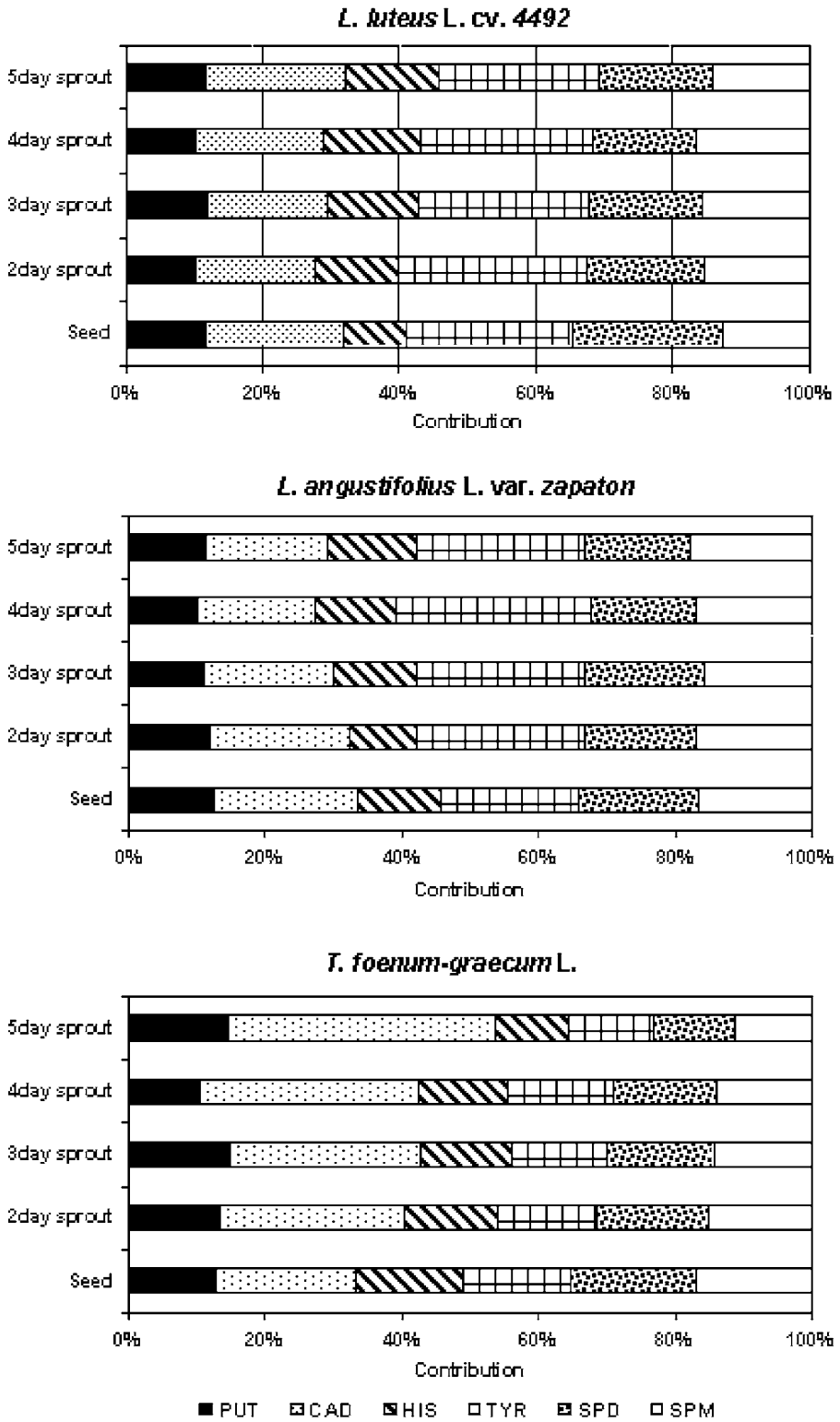

Figure 2. Contribution of individual amine to total levels in lupin (*L. luteus* cv. 4492 and *L. angustifolius* var. *zapaton*) and fenugreek (*T. foenum-graecum*) seeds at different stages of germination.

comprise up to 95% of total microbial population of mungbean and lentil sprouts and >99% for radish sprouts. These microorganisms are known to have a high decarboxylase activity, which plays a vital part in the metabolism of biogenic amines, especially putrescine and cadaverine. Germination occurs in a warm and moist environment, conducive to the rapid proliferation of microorganisms (*40*). Therefore, the increase in cadaverine levels could be explained by these two factors. On the other hand, some strains of lactic acid bacteria are tyramine and histamine producers (*45*).

Nout (*46*) proposed, as acceptable levels for fermented foods, 50−100 and 100−800 mg/kg of dw for histamine and tyramine, respectively. Similar levels exist in the legislation of different countries, also for nonfermented foods. Putrescine is regarded as an indicator of microbial spoilage in fish and meat products, with a limit of acceptability in the range of 11.3−20 mg/kg of

dw (*41*). The results of the present work indicate that the lupin and fenugreek sprouts studied here did not present levels of biogenic amines that would constitute a risk for healthy consumers. However, for individuals with restricted activity of detoxification enzyme monoamine oxidase (MAO; EC 1.4.3.4.), such as patients treated with some psychoactive drugs, a 6 mg intake of tyramine within a 4 h period can be deleterious (*47*). Using the water content of sprouts, shown in **Table 3**, amine levels during human intake of fresh sprouts can be calculated. An intake of 100 g of fresh sprouts of lupin and fenugreek would not be a risk for either healthy individuals or patients treated with psychoactive drugs.

ACKNOWLEDGMENT

We are indebted to Dr. M. C. Polo of Instituto de Fermentaciones Industriales for advice on determination of biogenic amines.

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Received for review May 3, 2006. Revised manuscript received July 27, 2006. Accepted July 28, 2006. We acknowledge AGL-2004-00886/ ALI project for financial support.

JF061238V