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Total Chemically Available (Free and Intrachain) Lysine and Furosine in Pea, Bean, and Lentil Sprouts

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The effect of the germination of peas, beans, and lentils under differing conditions of illumination for different times on parameters linked to the Maillard reaction (chemically available free and intrachain lysine, lysine availability, and furosine) was evaluated. The chemically available free lysine content in the raw seeds of the three legumes was quite small compared to the chemically available intrachain lysine content, and furosine was detectable only in the beans and the lentils. The effect of germination was to increase lysine availability compared with levels in the raw seeds in all of the germinated samples, the smallest increase taking place in the lentils. In addition, furosine became detectable in all of the germinated samples. Quantities varied depending on the germination conditions but in all cases were higher than the quantities observed in the raw seeds. Linear correlations were observed to exist between some of the parameters considered in the three legumes tested.

KEYWORDS: Available lysine; beans; furosine; germination; lentils; peas

INTRODUCTION

The low digestibility of legumes, combined with the presence of antinutritional factors, means that legumes require processing before they can be eaten. Germination of the seeds is one of the oldest processing methods and one in which there has been renewed interest in recent years.

During germination reserves are mobilized, and as a result polymeric molecules are converted into simpler molecules that can be transported to the embryo and used as energy sources and as substrates for synthesis in the new sprout. Accordingly, proteins and starches undergo enzymatic hydrolysis, raising the concentrations of free amino acids and soluble carbohydrates, many of the latter being reducing sugars. These substances can interact with each other in the germinating seeds, with adverse effects on the nutritional value of the resulting flours.

The high protein content of legumes is one reason why they are so nutritional, and their proteins are rich in lysine. During processing some of the lysine residues may become nutritionally unavailable, because its highly reactive ϵ-amino group reacts with other components in the food, mainly with reducing sugars through the Maillard reaction, also known as nonenzymatic glycosylation or nonenzymatic browning. If there is free and/or N-terminal lysine in the food, the α-amino group may also take part in the reaction.

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For all these reasons, information on the proportion of lysine available in legumes is indispensable. Little work on this aspect has been published in the literature, and even less attention has focused on the effects of germination on the proportion of available lysine. This circumstance led Tharanathan and Mahadevamma (*1*) to observe that this question required systematic investigation.

The basic chemical method for determining available lysine was developed by Carpenter (*2*). The method derivatizes the available lysine in a foodstuff with fluorodinitrobenzene (FDNB) to form *N*-ϵ-(2,4-dinitrophenyl)-L-lysine (ϵ-DNP-Lys), which can then be measured spectrophotometrically following hydrolysis of the sample and extraction with an organic solvent. A series of chromatographic methods for separating and quantifying ϵ-DNP-Lys were developed on the basis of this reaction. All of these methods have one feature in common, namely, that they determine only the available intrachain lysine, not the free and/or N-terminal lysine, which may or may not be part of the available lysine. To overcome this limitation, Hernández et al. (*3*) developed a method that was also able to quantify *N*,*N*′-di(2,4-dinitrophenyl)-L-Lys (α,ϵ-diDNP-Lys). The sum of the two values for the available intrachain and available free lysine yielded the total chemically available lysine content.

In addition, the early stages of the Maillard reaction between lysine and glucose or carbohydrates that contain glucose with a free reducing group yield the Amadori product fructosyllysine. Acid hydrolysis of this compound produces the nonprotein

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amino acid furosine [ϵ-*N*-(2-furoylmethyl)-L-lysine]. Determining the furosine is helpful in evaluating the unavailable lysine in foods.

Accordingly, the object of the present experiment was to assess the degree of interaction between lysine and soluble reducing sugars during germination, under varying conditions, of three commonly consumed legumes, i.e., peas, beans, and lentils, by analyzing the total chemically available lysine and furosine.

MATERIALS AND METHODS

Legume Germination. Peas (*Pisum sativum* L. var. *esla*), beans (*Phaseolus vulgaris* L. var. *la granja*), and lentils (*Lens culinaris* L. var. *castellana*) were used in the germination experiments.

Germination of the legumes was carried out according to Vidal-Valverde et al. (*4*). For every tray, 500 g of seeds was soaked in 2.5 L of water containing 0.07% sodium hypochlorite at room temperature for 30 min. The seeds were then drained, rinsed to a neutral pH, and soaked in distilled water for 5.5 h. The imbibed seeds were germinated at pilot scale by layering them over moist filter paper continuously watered by capillary action in a model G-120 germinator (Snijders, Holland) at 20 °C and 99% relative humidity for 2, 4, or 6 days. Thus, six trays were prepared for each legume, half germinated in total darkness and half germinated entirely with light. The sprouted seeds were freeze-dried and ground to a size small enough to pass through a 0.60 mm sieve for analysis.

The germinated legume flours were packaged in heat-sealed vacuum bags and refrigerated at 4 °C in plastic jars containing silica gel.

Three replications of all sample processing and all determinations were carried out.

Total Nitrogen Determination. The total nitrogen was analyzed by the Kjeldahl method with potentiometric end point titration at pH 4.6. An indicator solution of 0.01 g of methyl red, 0.02 g of bromothymol blue, and 0.06 g of bromocresol green in 100 mL of 70% ethanol (v/v) was used for end point control.

Total Chemically Available Lysine Determination. The total chemically available lysine determination was carried out using the method of Hernández et al. (*3*).

Derivatization was performed by pouring a quantity of sample accurately weighed out, such that it contained about 2.5 mg of total lysine, into a 250 mL Pyrex screw-cap flask. Next, 10 mL of 8% $NaHCO_3$ was added, and the resulting suspension was stirred for 10 min. Then 15 mL of ethanol and 0.1 mL of 2,4-dinitrofluorobenzene (FDNB) (Sigma Chemical Co., St. Louis, MO) were added to the reaction mixture, in that order. The resulting solution was covered by aluminium foil to protect it from light and stirred at room temperature for 2 h. Finally, the ethanol was evaporated away completely in a hot water bath heated to 90 °C.

After derivatization, the protein was hydrolyzed using 6 M HCl. The volume of acid was adjusted to yield a ratio of 1 mL of HCl/mg of protein in the sample and to neutralize the added $NaHCO_3$. The mixture was sonicated for approximately 20 min to remove the CO_2 generated by the neutralization reaction, an essential step to prevent excess pressure from building up during hydrolysis, which could otherwise cause the flask to break. Additionally, sufficient empty head space was left in the flask as a further safety precaution. Nitrogen was bubbled in the screw-cap flask and then closed to carry out hydrolysis at 110 °C for 24 h.

After the hydrolysate had cooled to room temperature, 50 mL of acetonitrile was added to the flask, and the mixture was sonicated for 5 min. It was then filtered through no. 52 Whatman paper, and the flask was washed with 50 mL of acetonitrile and 50 mL of Milli-Q water, in that order. The volume was made up to 250 mL with Milli-Q water. All sample hydrolysates were stored chilled at 4 °C in hermetically sealed brown tinted bottles.

A 5 mL aliquot of the resulting solution was evaporated to dryness at 40 °C in a rotary evaporator. The dry residue was reconstituted with Milli-Q water–acetonitrile (1:4), taking care to add the acetonitrile first and to dissolve the residue by sonication before the water was added. The mixture was injected onto the chromatograph without any further filtration.

Quantitation was carried out using two external standards, one being ϵ-DNP-lysine and the other being α,ϵ-diDNP-lysine (Sigma Chemical Co., St. Louis, MO). The standard solution was prepared by dissolving an appropriate quantity of α,ϵ-diDNP-lysine in acetonitrile. When dissolved, an appropriate quantity of ϵ-DNP-lysine was added to 10 mL of Milli-Q water for proper solubilization and added to the standard solution, and the mixture was made up to 200 mL with acetonitrile. The standard solution was diluted with Milli-Q water to concentrations ranging from 5 to 30 μg/mL for ϵ-DNP-lysine and from 0.5 to 2.0 μg/mL for α,ϵ-diDNP-lysine. The calibration curve was obtained by plotting the peak areas against concentration. All of the dilutions of the standard solutions and samples were injected twice.

Separations were carried out on μ-Bondapak C_{18} (300 × 3.9 mm i.d.) columns (particle size: 10 μm) (Waters, Milford, CT) using a guard column (20 × 3.9 mm i.d.) having identical features. The columns were heated to 50 °C. The mobile phase for the gradient was solvent A [0.01 M acetate buffer (pH 5)] and solvent B (acetonitrile). Separation was performed under a gradient of 20% B for 8 min, 20–35% B in 1.5 min, 35% B for 14.5 min, 35–20% B in 1.5 min, and a step at 20% B for 11 min to reequilibrate the column to the initial conditions between runs. The flow rate was 2 mL/min. Detection was carried out at 360 nm.

The HPLC apparatus consisted of a model 110B pump and a model 210A injector from Beckman (Berkeley, CA) equipped with a 20 μL loop and a 168 diode array detector (Beckman). Peak areas were determined using a GOLD System (Beckman).

Furosine Determination. The furosine determination was carried out using the method of Serrano et al. (*5*).

About 0.1 g of sample, accurately weighed out, was poured into a 250 mL Pyrex screw-cap flask, and an appropriate quantity of 10 M HCl acid was added (1 mL of acid/mg of protein). Hydrolysis was carried out in a nitrogen atmosphere at 110 °C for 24 h. After the hydrolysate had cooled to room temperature, it was filtered through no. 52 Whatman paper, and the flask was washed with Milli-Q water. All of the liquids were collected in a 100 mL volumetric flask that was then topped with Milli-Q water.

The hydrolysate was purified using Sep-Pak C_{18} cartridges (WAT020515; Waters, Milford, MA). The cartridges were prewetted with 5 mL of methanol and 10 mL of Milli-Q water before use. Aliquots of 5 mL of filtered hydrolysate were gradually loaded onto the cartridge, and the displaced liquid was collected in an evaporation flask, care being taken to keep air from entering the cartridge. Next, the furosine retained on the cartridge was eluted using 5 mL of 3 M HCl, the eluate being collected in the same flask. The solution thus obtained was evaporated to dryness in a rotary evaporator at 30 °C. The dry residue was reconstituted with 0.4 mL of a solvent comprising acetonitrile–Milli-Q water–formic acid (20:79.8:0.2).

Quantitation was performed using an external standard. A stock solution with about 140 $\mu g \cdot mL^{-1}$ pure furosine standard (Neosystem, Strasbourg, France) was prepared by dissolving the total amount of a commercial vial in 0.1 M HCl. This stock solution was refrigerated at 4 °C until use.

An appropriate aliquot of the stock solution was evaporated to dryness in a rotary evaporator, and the dry residue was reconstituted with an appropriate volume of acetonitrile–Milli-Q water–formic acid (20:79.8:0.2). Eight standard dilutions ranging in concentration from 0.5 to 5 $\mu g \cdot mL^{-1}$ were prepared from the reconstituted solution using that same solvent. A calibration curve was obtained by plotting the peak areas on the $\mu g \cdot mL^{-1}$ furosine injected. All standard solutions and samples were injected twice.

Separations were carried out on a Spherisorb ODS2 5 μm column (250 × 4.6 mm i.d.) (Phenomenex, Torrance, CA) warmed to 30 °C. The mobile phase was 5 mM sodium heptanesulfonate with 20% acetonitrile and 0.2% formic acid at a flow rate of 1.2 mL/min. Detection was carried out at 280 nm. The HPLC apparatus was the same as the one used to perform the chemically available lysine determination.

Table 1. Chemically Available Lysine Content[a] in Raw and Germinated Peas, Beans, and Lentils

legume	chemically available lysine		
	intrachain (g/16 g of N)	free and/or N-terminal lysine (mg/16 g of N)	total (g/16 g of N)
peas			
raw	5.44 ± 0.05[b]	41.00 ± 1.27[a]	5.48 ± 0.05[b]
germinated with exposure to light			
2 days	5.54 ± 0.08[b]	105.85 ± 4.31[b]	5.65 ± 0.07[b]
4 days	4.98 ± 0.06[a]	194.00 ± 7.35[e]	5.17 ± 0.05[a]
6 days	5.01 ± 0.00[a]	163.70 ± 5.94[d]	5.18 ± 0.01[a]
germinated in darkness			
2 days	5.38 ± 0.28[b]	149.10 ± 4.10[c]	5.52 ± 0.29[b]
4 days	4.98 ± 0.05[a]	148.45 ± 4.88[c]	5.12 ± 0.05[a]
6 days	4.77 ± 0.05[a]	258.25 ± 5.44[f]	5.03 ± 0.06[a]
beans			
raw	5.16 ± 0.27[ab]	76.40 ± 2.69[a]	5.23 ± 0.27[ab]
germinated with exposure to light			
2 days	5.23 ± 0.25[ab]	103.04 ± 3.62[b]	5.33 ± 0.25[ab]
4 days	5.06 ± 0.25[ab]	178.70 ± 4.95[d]	5.24 ± 0.24[ab]
6 days	4.84 ± 0.21[a]	184.62 ± 6.76[d]	5.03 ± 0.21[a]
germinated in darkness			
2 days	5.65 ± 0.24[c]	118.50 ± 4.52[c]	5.77 ± 0.24[c]
4 days	5.42 ± 0.23[bc]	196.94 ± 1.44[e]	5.61 ± 0.23[bc]
6 days	4.96 ± 0.06[a]	235.47 ± 3.60[f]	5.20 ± 0.05[a]
lentils			
raw	4.89 ± 0.10[e]	1.78 ± 0.12[a]	4.90 ± 0.10[d]
germinated with exposure to light			
2 days	4.48 ± 0.12[cd]	138.45 ± 5.24[c]	4.62 ± 0.13[bc]
4 days	4.36 ± 0.17[bc]	149.86 ± 7.18[c]	4.51 ± 0.18[ab]
6 days	4.34 ± 0.16[bc]	272.65 ± 7.06[e]	4.62 ± 0.16[bc]
germinated in darkness			
2 days	4.23 ± 0.17[ab]	100.22 ± 4.55[b]	4.33 ± 0.17[a]
4 days	4.62 ± 0.08[d]	183.87 ± 5.82[d]	4.80 ± 0.08[cd]
6 days	4.04 ± 0.07[a]	268.06 ± 14.41[e]	4.31 ± 0.07[a]

[a] Values are the means of three determinations ± SD. Different superscripts in the same column for each legume indicate significantly different values ($P < 0.05$).

Statistical Analysis. All statistical analyses were performed using the Statgraphics Statistical Graphics System, version 5.0. Analysis of variance (ANOVA) was used to evaluate significant differences. The least significance difference (LSD) test was used to compare differences among the means at the 0.05 level. Regression analysis was carried out to establish correlations.

RESULTS AND DISCUSSION

Chemically Available Lysine. Table 1 presents the chemically available free and intrachain lysine and the total chemically available lysine values in the raw and germinated peas, beans, and lentils, while **Table 2** sets out the percentage lysine availability in the three legumes calculated using the total chemically available lysine and total lysine contents, the latter as determined in an earlier study carried out using the same samples used here (*6*).

Chemically available free and intrachain lysine was present in the raw seeds of all three legumes, though the proportion of chemically available free lysine was extremely low. The peas had the highest chemically available intrachain lysine content, and the beans had the highest chemically available free lysine content.

Table 2. Percentage Lysine Availability in the Raw Legumes and Sprouts

legume	lysine availability (%)		
	peas	beans	lentils
raw	88.4	78.8	78.8
germinated with exposure to light			
2 days	95.6	85.1	81.5
4 days	92.5	82.4	82.3
6 days	96.1	85.8	84.8
germinated in darkness			
2 days	87.3	93.7	71.8
4 days	88.4	91.1	88.7
6 days	92.3	88.7	87.4

Lysine availability was higher in the raw peas than the beans and lentils. Only around 12% of the lysine in the peas was unavailable, compared with about 21% in the beans and the lentils.

A literature search disclosed few studies dealing with lysine availability in the raw seeds of legumes, and those that were found assessed only the available intrachain lysine using spectrophotometry, much less accurate than chromatography.

Hendriks et al. (*7*) recorded an available lysine value of 1.69% of dry matter (DM) and a total lysine value of 1.74% of dry matter in peas (var. *finale*), that is, 97.1% availability. Wu et al. (*8*) found an available lysine content of 6.21 g/16 g of N and a total lysine content of 6.77 g/16 g of N in raw beans, i.e., 91.7% lysine availability. Working with that same legume, El-Adawy et al. (*9*) reported an unusually low available lysine value of 2.9 g/16 g of N and similarly reported uncharacteristically low available lysine values of 1.3 g/16 g of N in soybeans (*Glycine max*) and 3.1 g/16 g of N in sweet lupin (*Lupinus albus*).

Working with blackgram (*Phaseolus mungo*), Venugopal and Rama-Rao (*10*) obtained an available lysine value of 4.10 g/16 g of N but did not report a total lysine value. The same is true of Reyes-Moreno et al. (*11*), who recorded an available lysine value of 3.04 g/16 g of N in raw chickpea (*Cicer arietinum*) seeds, without stating the total lysine value. Petzke et al. (*12*) found percentage availability levels from 65% to 96% in various tropical seeds.

No possible reasons for the presence of unavailable lysine in raw legume seeds have been found in the literature.

The only reports on this subject dealt with ageing of cereal grain and legume seeds during extended storage, but none of those reports published any available lysine determinations (*13–19*). Amadori and advanced Maillard products are the most often studied in relation to the Maillard reaction and in all cases used nonspecific methods for its analysis.

The findings indicated an increase in Maillard products and a decrease in certain oligosaccharides in the seeds with storage time, without any concomitant increase in the corresponding monosaccharides. As a consequence, the authors of these reports concluded that the monosaccharides produced by hydrolysis took part in the Maillard reaction, which was deemed to be a cause of reduced seed viability. Accordingly, Bernal-Lugo and Leopold (*14*) found lower sucrose and raffinose levels in maize (*Zea mays* L.), and Sun and Leopold (*17*) found a decrease of stachyose in soybean (*G. max*). Begnami and Cortelazzo (*18*) observed that with storage the starch grains in bean seeds were more numerous but smaller in size. On the other hand, Murthy and Sun (*19*) reported an increase in glucose in mungbean (*Vigna radiata* L.) axes.

Additionally, Taylor et al. (*20*) reported that the Maillard reaction could cause browning in lima bean coats, which contained both proteins and reducing sugars.

Thus, the unavailable lysine in raw legumes may be natural in origin, or it may have formed during storage prior to acquisition and processing. In any case, another possibility that requires study is that the chemical methods for analyzing the available lysine may underestimate levels in the raw legume seeds taking into account the complex structure of the storage proteins.

Bewley and Black (*21*), Kigel and Galili (*22*), and various review papers (*23, 24*) have all pointed out that many legume globulins and lectins are glycoproteins and that enzymatic N-glycosylation occurs at the asparagine residues present in the Asp-X-Ser/Thr sequence, X being any amino acid except proline. Those same authors have likewise pointed out that glycosylation does not take place at all potential glycosylation sites because of the steric inaccessibility of enzymes. As a result, the glycosylation rate varies from species to species. In addition, legumins and some lectins contain inter- and intrachain disulfide bonds, and insoluble proteinaceous inclusions can be found embedded in the soluble protein matrix of the protein bodies.

The result of all this is that lysine residues having a free ϵ-amino group could be inaccessible to chemical reagents, which might prevent this lysine from being quantified as part of the available lysine, thereby resulting in underestimation by the analytical method employed.

From **Table 1** the effect of germination can be seen to lower the chemically available intrachain lysine content in the peas and lentils in comparison with the raw seeds, except in the case of the 2-day-old pea sprouts. Generally speaking, the longer the germination time, the greater the decrease. Conversely, there was a sharp increase in the chemically available free and/or N-terminal lysine in both of these legumes with germination time. The value of the chemically available free lysine was four to six times greater in the 6-day-old peas than in the raw seeds, and in the lentils the increase was much greater, around 150 times the value in the raw seeds. The overall effect was a decrease in the total chemically available lysine in the 4- and 6-day-old pea sprouts and similar levels in the 2-day-old sprouts compared with the levels in the raw seeds. In the lentils, all of the samples had lower levels of total chemically available lysine, though the difference was small, between 2% and 12%.

There were no significant differences in the chemically available intrachain lysine content between the germinated samples and the raw seeds in the beans (**Table 1**), except in the case of the 2-day-old bean sprouts germinated in darkness, in which the level increased. On the other hand, the chemically available free lysine levels rose with germination time as in the peas and the lentils, though quantitatively the increase was smaller. The result was that there were no differences in the total chemically available lysine content between the bean sprouts and the raw seeds except for the sample germinated in darkness for 2 days, in which the content was higher.

Linear regression analysis was performed to establish the relationships between the changes of chemically available lysine and the changes of nitrogen (protein and nonprotein) (*6*) during germination, and the results are presented in **Table 3**.

A direct and highly significant linear correlation between the nonprotein nitrogen content and the available free lysine content was observed in all three legumes considered here. The correlation was inverse in the case of the protein nitrogen. A direct and highly significant linear correlation with the protein nitrogen also existed for the available intrachain lysine in the three legumes, while the available intrachain lysine had an inverse correlation with the nonprotein nitrogen, though the correlation was not significant in the beans.

Table 3. Linear Regression between the Chemically Available Free and/or N-Terminal (mg/16 g of N) and Intrachain (g/16 g of N) Lysine and Nonprotein and Protein Nitrogen (% DM) in Peas, Beans, and Lentils

	nonprotein nitrogen		protein nitrogen	
chemically available lysine (%)	*r*	*p*-value	*r*	*p*-value
free and/or N-terminal lysine				
peas	0.9605	0.0000	−0.8902	0.0000
beans	0.8584	0.0000	−0.5733	0.0066
lentils	0.9188	0.0000	−0.7837	0.0000
intrachain lysine				
peas	−0.8761	0.0000	0.8649	0.0001
beans	−0.3019	0.1835	0.4779	0.0284
lentils	−0.6487	0.0015	0.6965	0.0005

The resulting conclusion is that nonprotein nitrogen and chemically available free lysine levels increased while protein nitrogen and chemically available intrachain lysine levels decreased in all three legumes with germination time due to hydrolysis of the storage proteins.

Table 2 summarizes the effects of sprouting on lysine availability. In the peas the lysine availability values were between 4% and 8% higher in the seeds exposed to light during sprouting than in the seeds germinated in darkness. Values were higher in all of the bean sprouts than in the raw seeds, the highest values being attained in the samples germinated in darkness, 3–8% higher than in the germinated with light, depending on germination time. Finally, values were also higher in the lentil sprouts than in the raw seeds, except in the sample germinated in darkness for 2 days. Thus, of the three legumes considered, the lentils underwent the smallest increase of the lysine availability during germination, since its value did not exceed 90% in any of the samples.

There have been very few studies on the influence of sprouting on the available lysine content or on lysine availability in legumes. Venugopal and Rama-Rao (*10*) established the available lysine content in blackgram (*P. mungo*) seeds germinated for 18–66 h and observed an increase in the available lysine from the start of germination to a maximum at 42 h, after which the available lysine value gradually fell with additional germination time. Fernández and Berry (*25*) used an in vivo method to assess lysine availability in chickpeas germinated for 1 and 2 days. They found a value of 87.8% in the raw seeds and reported that the value then decreased by 2% and 10%, respectively, in the seeds germinated for 1 and 2 days. However, they indicated that the decrease could be due to the heat treatment (autoclaving for 30 min) undergone by the samples before analysis.

The enhanced lysine availability in the germinated legumes studied here as compared to the raw legumes suggests that the Maillard reaction does not take place during sprouting to any appreciable extent. The increase in availability can thus be attributed either to an increase in the total chemically available lysine or to a decrease in the total lysine, or to both. The total lysine decreased in these three legumes with germination time (*6*), while the total chemically available lysine decreased in the peas and lentils and held steady in the beans.

Furosine. Table 4 presents the values for furosine produced by acid hydrolysis in the raw and germinated peas, beans, and lentils.

No furosine was detectable in the raw pea seeds, which had the highest percentage lysine availability of the three legumes considered. The absence of furosine could be attributable to the formation of trace amounts of the corresponding Amadori product only and/or to the reaction of the lysine with sugars

Table 4. Furosine[a] in Raw and Germinated Peas, Beans, and Lentils

legume	furosine (mg/16 g of N)		
	peas	beans	lentils
raw	ND[b]	21.47 ± 0.39^{c}	6.33 ± 0.11^{a}
germinated with exposure to light			
2 days	9.03 ± 0.09^{b}	15.73 ± 0.02^{a}	40.34 ± 1.57^{b}
4 days	11.23 ± 0.64^{c}	19.86 ± 0.38^{b}	83.59 ± 3.12^{e}
6 days	16.53 ± 0.46^{d}	27.56 ± 1.30^{d}	40.62 ± 0.39^{b}
germinated in darkness			
2 days	6.68 ± 0.07^{a}	14.56 ± 0.14^{a}	46.01 ± 0.93^{c}
4 days	31.97 ± 0.24^{f}	19.08 ± 0.91^{b}	58.51 ± 2.12^{d}
6 days	19.44 ± 0.74^{e}	68.28 ± 1.55^{e}	116.74 ± 4.50^{f}

[a] Values are the means of three determinations ± SD. Different superscripts in the same column for each legume indicate significantly different values ($P < 0.05$). [b] ND = not detected.

other than glucose to form other Amadori products that do not generate furosine from the hydrolysis.

Different quantities of furosine were found in the raw seeds of the beans and the lentils, even though these two legumes had similar lysine availability levels. This result suggests that a higher proportion of lysine reacted with glucose in the beans.

Therefore, it can be suggested that qualitative differences in the reducing sugars present in legumes during ripening and/or storage are responsible for the formation of different Amadori compounds in each species.

Literature studies dealing with the identification and quantification of reducing sugars in raw legumes have reported no glucose to be present in peas (*26*) or lentils (*27–29*) and present only in low concentrations in beans (*30, 31*). Vidal-Valverde et al. (*4*) were unable to detect glucose in any of the three legumes used in the present experiment.

Lysine could react with glucose during seed formation and/or maturation. Sugar transport from the mother plant to the cotyledons takes place in the form of sucrose, that is the substrate for starch synthesis, the principal form of energy storage in the raw seeds (*21, 22*). In the course of these complex stages, lysine could react with glucose as well as with glucose-containing oligosaccharides to form the Amadori compounds that subsequently give rise to furosine.

In contrast with the raw seeds, furosine was recorded in all of the legume sprouts. In the peas germinated while being exposed to light, the amount of furosine increased with germination time, whereas in the peas sprouted in darkness, the maximum furosine value was observed after germination for 4 days. The amount of furosine that formed in the 2- and 4-day-old bean sprouts was less than in the raw seeds. This was followed by an increase in the 6-day-old sprouts, the increase being greater in the sample germinated in darkness. In all of the lentil sprouts the quantities of furosine produced were higher than in the raw seeds. When the seeds were exposed to light during germination, the quantity produced was similar in the 2- and 6-day-old sprouts and in both cases was lower than the amount formed in the 4-day-old sprouts. However, when germination took place in darkness, furosine formation increased with germination time.

Regression analysis was performed to establish the possible correlations between furosine formation and the chemically available free and intrachain lysine in the three legumes (**Table 5**). There was a direct linear correlation between the amount of furosine and the chemically available free lysine and an inverse correlation with the chemically available intrachain lysine.

Table 5. Linear Regression between Furosine (mg/16 g of N) and the Chemically Available Free and/or N-Terminal (mg/16 g of N) and Intrachain (g/16 g of N) Lysine in Pea, Bean, and Lentil Sprouts

chemically available lysine	furosine	
	r	*p*-value
free and/or N-terminal lysine		
peas	0.5289	0.0518
beans	0.6611	0.0011
lentils	0.6635	0.0010
intrachain lysine		
peas	–0.6694	0.0088
beans	–0.4289	0.0523
lentils	–0.7118	0.0003

Accordingly, the increase in the amount of chemically available free lysine as germination progressed, which was associated with an increase in the amount of furosine, suggests that the lysine released by hydrolysis during mobilization of the storage proteins is nutritionally available.

At the same time, the decrease in the chemically available intrachain lysine and the increase in furosine formation with germination time might mean that there was a rise in the percentage of unavailable lysine. However, it should be noted that the decrease in the chemically available intrachain lysine is also caused by hydrolysis of the storage proteins that takes place during sprouting.

Different hypotheses could be postulated with respect to the increase in furosine and thus the higher levels of Maillard products which give rise to furosine in the germinated seeds. One hypothesis would be that the intrachain lysine actually reacts with glucose on mobilization of the seed's reserves. For the three legumes considered here, various workers have observed glucose formation or increases in glucose levels during sprouting (*4, 27, 28, 30–32*). Moreover, it could be that the Amadori products that contain glucose present in the raw seeds were not metabolized during sprouting and were concentrated in the germinated seeds as a consequence of the dry weight lost during germination (*33–35*).

SAFETY

FDNB is a potent irritant with toxic effects that may cause skin sensitization. Use of nitrile gloves offers the best protection for the skin during laboratory work (*36*).

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