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ScienceDirect
Food Chemistry 108 (2008) 245–252
Food Chemistry
www.elsevier.com/locate/foodchem

Correlations between some nitrogen fractions, lysine, histidine, tyrosine, and ornithine contents during the germination of peas, beans, and lentils

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Received 5 July 2007; received in revised form 1 October 2007; accepted 25 October 2007

Abstract

The effect of different germination conditions, namely, germination time and total presence or absence of light, on the content of the various nitrogen fractions, three essential protein amino acids (Lys, His, and Tyr) and one non-protein amino acid (Orn), was studied in peas, beans, and lentils. The influence of light during germination on the parameters considered varied according to the legume but on the whole was less important than the influence of germination time in quantitative terms. In all three legumes, prolonging the germination time yielded flours that contained more non-protein nitrogen (NPN) and Orn and less protein nitrogen (PN) and Lys, while the changes in the His and Tyr contents varied with legume type. In addition, changes in the Lys, Tyr, and Orn contents correlated with the changes in the NPN and PN levels in the germinated legumes.

Keywords: Beans; Germination; Histidine; Legumes; Lentils; Lysine; Ornithine; Peas; Tyrosine

1. Introduction

As a food group, legumes make a major contribution to the human diet as good sources of protein, carbohydrates, several water-soluble vitamins, and minerals. Despite these nutritional benefits, legume consumption has fallen in the industrialized countries in recent years, a trend that is likely to contribute to nutritional imbalances associated with diets that are low in fibre and overly dependent on animal protein.

As the raw seeds have limited digestibility and contain certain antinutritional factors, legumes need processing before they can be eaten. A variety of methods have been put forward to that end, but there has recently been growing interest in germination, because it is a natural process with minimal energy and technical requirements and hence is not burdensome, while at the same time it helps augment the nutritive value of the seeds.

Peas, beans, and lentils are three of the main legume crops. In 1999, beans accounted for 33%, peas 20%, and lentils 5% of world legume production according to the FAO. While considerable work has been carried out to study germination in peas, beans, and lentils, the influence of the different germination conditions on protein quality in the finished products has not been studied systematically.

Most published studies have not used a germinator and have not explained in detail how temperature was regulated other than to say that germination was carried out at ambient temperature, and in most cases germination has been carried out in darkness. Very few studies have combined alternating periods of exposure to light and darkness during germination (El-Hag, Haard, & Morse, 1978; Frias, Díaz-Pollan, Hedley, & Vidal-Valverde, 1995; Frias, Díaz-Pollan, Hedley, & Vidal-Valverde, 1996; Prodanov,

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doi:10.1016/j.foodchem.2007.10.073

Sierra, & Vidal-Valverde, 1997; Pusztai & Duncan, 1971; Savelkoul, Tamminga, Leenaars, Schering, & Ter Maat, 1994; Urbano, López-Jurado, Hernández, Fernández, & Moreu, 1995). There have been very few comparative studies in which the same seeds were exposed to light germination or germinated entirely in darkness while holding other germination conditions constant (Kuo, Rozan, Lambein, Frias, & Vidal-Valverde, 2004; Vidal-Valverde, Frias, Sierra, Blázquez, & Lambein, 2002).

Consequently, this experiment was undertaken as a semi-pilot scale systematic study of the influence of the total presence or absence of light during germination and germination time on the content of the various nitrogen fractions, three essential protein amino acids (Lys, His, and Tyr) and one non-protein amino acid (Orn) in flours made from germinated peas, beans, and lentils.

2. Materials and methods

2.1. Germination of the legumes

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 Vidal-Valverde et al. (2002). For every tray, 500 g of seeds were soaked in 2.5 l of water containing 0.07% sodium hypochlorite at room temperature for 30 min. The seeds were then drained, rinsed in pH-neutral water, and soaked in distilled water for 5½ h. The imbibed seeds were then germinated at pilot scale by layering them over moist filter paper continuously watered by capillary action in a germinator (G-120 Snijders, Holland) at 20 °C and 99% relative humidity for two, four, or six days with light (batches 2DL, 4DL, and 6DL) and without light (batches 2DNL, 4DNL, and 6DNL) during the whole germination period. Thus, six trays were prepared for each legume. 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 prior to analysis.

2.2. Total nitrogen determination

The total nitrogen (TN) was analysed by the Kjeldahl method with potentiometric endpoint titration at pH 4.6. An indicator solution of 0.01 g methyl red, 0.02 g bromothymol blue, and 0.06 g bromocresol green in 100 ml 70% ethanol (v/v) was used for endpoint control. A factor of 6.25 was used to calculate the crude protein content of the samples.

2.3. Protein and non-protein nitrogen determinations

The copper sulfate method was used to determine the non-protein nitrogen (NPN). A total of 0.5 g of sample was weighed out into a precipitation tube; 50 ml of distilled water and two drops of silicone were added, and the mixture was boiled gently and shaken for 30 min. An amount of 2 ml of 10% aluminium potassium sulfate was added and the mixture heated to boiling. Next 50 ml of 3% copper sulfate was added, and the mixture was shaken until it had cooled to ambient temperature. It was then filtered, and the NPN was determined by the Kjeldahl method. The protein nitrogen (PN) was calculated as the difference between the TN and the NPN.

2.4. Lysine, histidine, tyrosine, and ornithine determination

The lysine (Lys), histidine (His), tyrosine (Tyr) and ornithine (Orn) contents were determined using the method of Sanz, Castillo, and Hernández (1996). Briefly, samples were hydrolysed with 6 M HCl in a nitrogen atmosphere at 110 °C for 24 h. The dry residue of each hydrolysate was reconstituted with Milli-Q water to a protein concentration of from 0.15 to 0.25 mg/ml. Pre-column derivatization of the amino acids was carried out using 5-dimethylaminonaphthalene-1-sulfonyl chloride (DnsCl). The dansylated derivatives were formed by combining 1 ml of protein hydrolysate, 2 ml of 40 mM Li_2CO_3 (pH 9.5), and 1 ml of DnsCl solution (4 mg/ml, 14.83 mM), in that order. The solution was mixed and heated at 60 °C for 30 min. An amount of 50 μl of methylamine solution was then added to quench the reaction.

Quantitation was performed using external standards for L-lysine (Lys), L-histidine (His), L-tyrosine (Tyr), and L-ornithine (Orn) (Sigma Chemical Company, St. Louis, MO, USA). The standards were derivatized using the same procedure as for the sample hydrolysates, except that 3 mg/ml DnsCl (11.12 mM) was used. Calibration curves were obtained by plotting the peak areas vs. concentration for each derivatized amino acid. Correlation coefficients greater than 0.998 were obtained in all cases.

Separations were carried out on a 300 × 3.9 mm i.d. column packed with Spherisorb ODS-2 (particle size 10 μm) (Sugerlabor S.A., Madrid, Spain) at a temperature of 40 °C. The mobile phase was acetonitrile: 0.01 M phosphate buffer (pH 7.0) [36:64] at a flow rate of 1.5 ml/min. Detection was carried out at 254 nm.

The HPLC apparatus consisted of two model 110B pumps, a model 210A injector, and a model 168 diode array detector (Beckman, Berkeley, CA, USA) equipped with a 20 μl loop. Peak areas were determined using a Gold System program (Beckman).

2.5. Amino acid ratios

The Lys and His ratios (g of amino acid in 16 g N of test sample/g of the same amino acid in 16 g N of reference protein × 100) for the legumes were calculated and compared with the amino acid reference standard for children (2–5 years old) proposed by FAO/WHO/UNU (1985) (5.8 g Lys and 1.9 g His in 16 g N of reference standard).

The ratio for Tyr was not calculated, because the reference standard encompasses the sum of Tyr and phenylalanine.

2.6. 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 run to establish correlations.

3. Results and discussion

3.1. Nitrogen compounds

Table 1 presents the values for total nitrogen, crude protein, and protein and non-protein nitrogen in the raw and germinated peas, beans, and lentils, expressed as percentage dry matter (d.m.). Fig. 1 shows the effect of germination on retention of the nitrogen compounds in the three legumes. In the raw legumes protein values were highest in the peas, followed by the lentils and then the beans. This finding was consistent with literature reports. The range of protein values in raw peas has been reported to be 26–32%

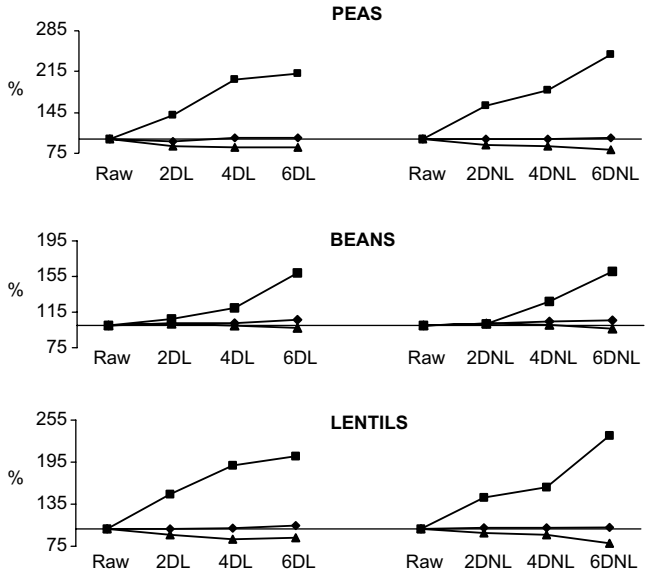

Fig. 1. Effect of germination on total (♦), non-protein (■), and protein nitrogen (▲) retention in peas, beans, and lentils.

Table 1
Total, non-protein, and protein nitrogen, and protein contents[A] (% dry matter) in raw and germinated peas, beans, and lentils

Batch	Total nitrogen	Crude protein	Non- protein nitrogen	Protein nitrogen
Peas				
Raw	4.29 ± 0.04^{bc}	26.82 ± 0.22^{bc}	0.56 ± 0.03 (13.1)^{a}	3.73
Germination with light				
2 DL	4.07 ± 0.06^{a}	25.45 ± 0.41^{a}	0.78 ± 0.00 (19.2)^{b}	3.29
4 DL	4.33 ± 0.02^{bc}	27.07 ± 0.13^{bc}	1.13 ± 0.01 (26.1)^{e}	3.20
6 DL	4.34 ± 0.07^{bc}	27.11 ± 0.42^{bc}	1.18 ± 0.04 (27.2)^{e}	3.16
Germination in darkness				
2 DNL	4.23 ± 0.11^{b}	26.42 ± 0.70^{b}	0.88 ± 0.01 (20.8)^{c}	3.35
4 DNL	4.31 ± 0.04^{bc}	26.94 ± 0.25^{bc}	1.02 ± 0.05 (23.7)^{d}	3.29
6 DNL	4.37 ± 0.02^{c}	27.30 ± 0.10^{c}	1.37 ± 0.01 (31.4)^{f}	3.00
Beans				
Raw	3.78 ± 0.10^{a}	23.60 ± 0.61^{a}	0.56 ± 0 .01 (14.8)^{a}	3.22
Germination with light				
2 DL	3.88 ± 0.01^{b}	24.23 ± 0.07^{b}	0.60 ± 0.01 (15.5)^{b}	3.28
4 DL	3.88 ± 0.04^{b}	24.24 ± 0.24^{b}	0.67 ± 0.00 (17.3)^{c}	3.21
6 DL	4.02 ± 0.06^{c}	25.11 ± 0.37^{c}	0.89 ± 0.02 (22.1)^{e}	3.13
Germination in darkness				
2 DNL	3.86 ± 0.05^{ab}	24.11 ± 0.32^{ab}	0.57 ± 0.00 (14.8)^{ab}	3.29
4 DNL	3.95 ± 0.03^{bc}	24.69 ± 0.20^{bc}	0.71 ± 0.02 (18.0)^{d}	3.24
6 DNL	4.00 ± 0.06^{c}	25.19 ± 0.05^{c}	0.90 ± 0.04 (22.5)^{e}	3.10
Lentils				
Raw	4.09 ± 0.01^{a}	25.56 ± 0.05^{a}	0.61 ± 0.02 (14.9)^{a}	3.48
Germination with light				
2 DL	4.08 ± 0.13^{a}	25.48 ± 0.82^{a}	0.91 ± 0.00 (22.3)^{bc}	3.17
4 DL	4.12 ± 0.08^{ab}	25.77 ± 0.50^{ab}	1.16 ± 0.05 (28.2)^{d}	2.96
6 DL	4.27 ± 0.07^{b}	26.70 ± 0.42^{b}	1.24 ± 0.05 (29.0)^{e}	3.03
Germination in darkness				
2 DNL	4.14 ± 0.07^{ab}	25.87 ± 0.45^{ab}	0.88 ± 0.02 (21.3)^{b}	3.26
4 DNL	4.15 ± 0.11^{ab}	25.94 ± 0.71^{ab}	0.97 ± 0.02 (23.4)^{c}	3.18
6 DNL	4.17 ± 0.13^{ab}	26.08 ± 0.80^{ab}	1.42 ± 0.05 (34.1)^{f}	2.75

[A] Values are the means of three determinations ±SD. The same superscript in the same column for each legume indicates non-significant differences ($P \leqslant 0.05$). Values in brackets are the percentage non-protein nitrogen on total nitrogen.

d.m. (Vidal-Valverde, Frias, Hernández, Martín-Alvarez, & Rodríguez, 2003). Salunkhe, Sathe, and Deshpande (1989) recorded protein values ranging between 18 and 26% d.m. in 34 varieties of bean, whereas Adsule, Kadam, and Leung (1989) estimated the percentage protein in lentils at 22–31% d.m.

NPN was 13% of the TN in the raw peas and somewhat higher, around 15% of TN, in the raw beans and lentils. Earle and Jones (1962) estimated the level of NPN in various raw legumes at 10–15%, and Mossé and Baudet (1983) recorded levels of 12–19.6%.

With respect to the effect of germination on the TN, in peas its content decreased after two days of germination with light, but levels similar to those of raw peas were reached at the end of the germination. However, germinated beans presented higher TN content than raw seeds, and only a slight increase (7%) was found after six days of germination. In lentils, TN of two and four days germinated samples was similar to the TN content in raw seeds, and only a slight increase, but not significant ($P \leqslant 0.05$), was found in six days germinated lentils in light conditions.

Germination also affected the nitrogen type and similar behaviour was observed for the three studied legumes, a gradual increase in NPN content and a slight decrease in the PN with the increase of the germination time. In peas and lentils, the germination brought about a rise on NPN close or higher than 100% in sprouts obtained after four and six days, while in beans this increment was only of 20% and 60% after four and six days of germination, respectively (Fig. 1). Therefore, in six days pea and lentil sprouts the ratio of NPN vs. TN reached values close to 30%, while in six days bean sprouts this value was of 22%.

In the case of PN, the variations found due to germination were quantitatively inferior since in peas and lentils germinated for six days only 80–85% of the PN of the raw seeds was found, while in sprouted beans processed in the same conditions 95% was observed. None of the literature studies reviewed has systematically assessed the effect of exposure to light on the nitrogen fractions in the same samples of germinated peas, beans and lentils.

The results obtained in the present experiment were in agreement with the literature findings for the effect of germination time. There was a slight increase in the TN in the germinated samples with germination time, associated with a large increase in the NPN and a smaller decrease in the PN. The increase in the TN that occurs with prolonged germination might be an effect of concentration in the germinated seeds as a result of the decrease in the dry matter that takes place concomitantly, inasmuch as there are no inputs of external nutrients. The decrease in dry weight as germination advances has been observed by a number of workers in legumes (Beevers & Guernsey, 1966) and cereals (Chavan & Kadam, 1989). The decrease appears to be attributable mainly to losses of carbon from soluble sugars and starch in the form of carbon dioxide, as a result of respiration of the seeds. Leaching of substances out of the seeds during presoaking may also be a contributing factor.

The increase in the NPN could be caused by hydrolysis of storage proteins to yield amino acids for transfer to the growing sprouts and by an increase in nucleic acids. Barceló, Rodrigo, Sabater, and Sánchez (2001) reported peak proteolytic activity in the three legumes considered here five days after the start of germination. Furthermore, the drop in the PN would seem to indicate that proteolysis outpaces protein synthesis in the growing sprouts.

3.2. Lysine, histidine, and tyrosine

Table 2 compiles the Lys, His, and Tyr values in the raw and germinated legumes. The percentages of these three amino acids in the dry matter were similar in all three raw legumes. However, when the values were expressed in terms of 16 g nitrogen, the raw beans displayed the highest proportions of these amino acids, and levels in the raw peas and lentils were similar.

Fig. 2 shows the variation of Lys, His and Tyr in germinated legumes vs. raw seeds. It was shown that Lys concentration decreased gradually with germination time in the three studied legumes, both in presence or absence of light. In contrast, the germination process caused an increase in His content of peas and beans germinated in the dark, while in those germinated in the presence of light for two and four days no significant ($P < 0.05$) differences to raw legumes were found. In lentils, the His increment was only significant ($P < 0.05$) in samples germinated for six days in light. However, Tyr content increased during germination of peas while in beans and lentils Tyr content decreased.

A literature search failed to disclose any studies dealing with variations in the total amino acids with germination time in the legumes considered. The only values reported were for samples at the end of germination, and there was substantial variability in the results. Hsu, Leung, Finney, and Morad (1980) germinated peas and lentils in a germinator in darkness for four days and found that Lys and Tyr decreased and His increased in the peas while all three amino acids increased slightly in the lentils. Urbano et al. (1995) germinated lentils of the same variety used in the present experiment under conditions of exposure to light for 12 h/d in a decantation funnel and found an increase in the Lys and His contents but no change in the Tyr levels. Mbithi-Mwikya, Ooghe, Van Camp, Ngundi, and Huyghebaert (2000) recorded a significant drop in the Lys content and no change in the His and Tyr contents in beans germinated in darkness for two days. Using five species of lentils, Rozan, Kuo, and Lambein (2001) obtained variable results for Lys, His, and Tyr after germination for four days, with Lys and His increasing in three species and decreasing in two and Tyr increasing in all five.

Changes in the amino acid content during germination could be related to protein hydrolysis, synthesis, and rearrangement. Germination involves mobilization of the protein reserves in the cotyledons coupled with the synthesis of new proteins for sprout growth. The new proteins can be

Table 2
Lysine, histidine, and tyrosine contents[A] in raw and germinated peas, beans, and lentils

Batch	Lysine (% d.m.)	Histidine (% d.m.)	Tyrosine (% d.m.)	Lysine (g/16 g N)	Histidine (g/16 g N)	Tyrosine (g/16 g N)
Peas						
Raw	1.66 ± 0.04^{c}	0.61 ± 0.00^{ab}	0.69 ± 0.05^{a}	6.20 ± 0.17^{de}	2.26 ± 0.01^{a}	2.58 ± 0.18^{a}
Germination with light						
2 DL	1.50 ± 0.06^{ab}	0.60 ± 0.03^{a}	0.73 ± 0.03^{a}	5.91 ± 0.23^{cd}	2.37 ± 0.12^{ab}	2.87 ± 0.13^{ab}
4 DL	1.51 ± 0.00^{ab}	0.65 ± 0.02^{bc}	0.92 ± 0.00^{c}	5.59 ± 0.01^{ab}	2.39 ± 0.09^{ab}	3.38 ± 0.01^{c}
6 DL	1.46 ± 0.02^{a}	0.67 ± 0.01^{cd}	0.86 ± 0.03^{bc}	5.39 ± 0.09^{a}	2.48 ± 0.04^{bc}	3.16 ± 0.10^{bc}
Germination in darkness						
2 DNL	1.67 ± 0.03^{c}	0.69 ± 0.01^{d}	0.69 ± 0.04^{a}	6.32 ± 0.10^{e}	2.60 ± 0.05^{c}	2.62 ± 0.16^{a}
4 DNL	1.56 ± 0.01^{b}	0.66 ± 0.00^{cd}	0.77 ± 0.01^{ab}	5.79 ± 0.04^{bc}	2.44 ± 0.01^{b}	2.88 ± 0.02^{ab}
6 DNL	1.49 ± 0.04^{ab}	0.68 ± 0.01^{cd}	0.82 ± 0.05^{b}	5.45 ± 0.15^{a}	2.49 ± 0.05^{bc}	3.00 ± 0.20^{b}
Beans						
Raw	1.57 ± 0.07^{b}	0.69 ± 0.03^{a}	0.70 ± 0.02^{d}	6.64 ± 0.32^{c}	2.92 ± 0.12^{ab}	2.98 ± 0.09^{d}
Germination with light						
2 DL	1.52 ± 0.09^{ab}	0.70 ± 0.02^{ab}	0.60 ± 0.03^{ab}	6.26 ± 0.36^{bc}	2.89 ± 0.09^{ab}	2.48 ± 0.14^{ab}
4 DL	1.54 ± 0.04^{ab}	0.68 ± 0.02^{a}	0.68 ± 0.00^{d}	6.36 ± 0.18^{bc}	2.82 ± 0.09^{a}	2.82 ± 0.02^{c}
6 DL	1.47 ± 0.04^{a}	0.81 ± 0.01^{d}	0.63 ± 0.01^{bc}	5.86 ± 0.14^{a}	3.22 ± 0.05^{d}	2.50 ± 0.02^{ab}
Germination in darkness						
2 DNL	1.48 ± 0.05^{ab}	0.73 ± 0.03^{bc}	0.60 ± 0.01^{ab}	6.16 ± 0.21^{ab}	3.04 ± 0.11^{bc}	2.49 ± 0.02^{ab}
4 DNL	1.52 ± 0.03^{ab}	0.76 ± 0.02^{c}	0.59 ± 0.00^{a}	6.16 ± 0.12^{ab}	3.08 ± 0.07^{cd}	2.38 ± 0.01^{a}
6 DNL	1.46 ± 0.01^{a}	0.76 ± 0.02^{c}	0.64 ± 0.04^{c}	5.86 ± 0.06^{a}	3.04 ± 0.07^{bc}	2.55 ± 0.16^{b}
Lentils						
Raw	1.59 ± 0.07^{c}	0.59 ± 0.01^{a}	0.64 ± 0.06^{c}	6.22 ± 0.26^{d}	2.31 ± 0.05^{a}	2.53 ± 0.25^{d}
Germination with light						
2 DL	1.44 ± 0.02^{b}	0.62 ± 0.01^{a}	0.54 ± 0.02^{b}	5.67 ± 0.08^{c}	2.44 ± 0.03^{ab}	2.12 ± 0.09^{c}
4 DL	1.41 ± 0.01^{b}	0.62 ± 0.00^{a}	0.49 ± 0.01^{ab}	5.48 ± 0.03^{bc}	2.41 ± 0.02^{ab}	1.91 ± 0.04^{abc}
6 DL	1.45 ± 0.05^{b}	0.66 ± 0.01^{b}	0.48 ± 0.06^{ab}	5.45 ± 0.18^{bc}	2.48 ± 0.02^{b}	1.79 ± 0.21^{ab}
Germination in darkness						
2 DNL	1.56 ± 0.03^{c}	0.62 ± 0.00^{a}	0.53 ± 0.02^{b}	6.03 ± 0.12^{d}	2.40 ± 0.02^{ab}	2.04 ± 0.06^{bc}
4 DNL	1.40 ± 0.02^{b}	0.62 ± 0.04^{a}	0.50 ± 0.03^{ab}	5.41 ± 0.07^{b}	2.38 ± 0.14^{ab}	1.93 ± 0.12^{abc}
6 DNL	1.29 ± 0.02^{a}	0.61 ± 0.03^{a}	0.44 ± 0.03^{a}	4.93 ± 0.09^{a}	2.36 ± 0.12^{ab}	1.70 ± 0.10^{a}

[A] Values are the means of three determinations ±SD. The same superscript in the same column for each legume indicates non-significant differences ($P \leqslant 0.5$).

composed of different amino acids than the stored proteins, thereby altering the pattern of amino acid content with respect to the raw legume. In keeping with this, Youssef, El-Aal, Shekib, and Ziena (1987) reported changes in the electrophoretic mobility pattern of faba bean (*Vicia faba* L.) proteins following germination.

Furthermore, the amino acids produced by hydrolysis of the protein reserves are not used solely in synthesizing new components but may also be used as an energy source, especially in the early stages of germination (Chen, Wells, & Fordham, 1975). Certain amino acids may be more readily broken down than others, and this is another potential source of alterations in the protein pattern during germination. Consistent with this, an increase in biogenic amines with germination time, brought about by enzymatic decarboxylation of amino acids, has been observed in various legumes (Shalaby, 2000). Gamarnik and Frydman (1991) for germinated soybeans and Torrigiani and Scoccianti (1995) for germinated chickpeas both reported considerably higher levels of the diamine cadaverine with germination time. Cadaverine, produced by enzymatic decarboxylation of Lys, is concentrated mainly along the embryonic axis, and these authors suggested that it could play a role in sprouting and cell division.

A statistical study was carried out to determine whether the variations in the levels of these three amino acids in the germinated seeds were related to the variations in the type of nitrogen, and the linear regression results are summarized in Table 3. There was an inverse linear correlation between the Lys content and NPN levels in all three legumes ($P \leqslant 0.05$) and a direct correlation between the Lys content and PN levels that was statistically significant in the peas and the lentils but not in the beans. Accordingly, the NPN content increased and the Lys content decreased with germination time in the peas and lentils, possibly attributable to use of Lys in synthesizing other amino acids or as an energy source. No linear correlations between the His content and NPN or PN levels were found.

The variations in the Tyr content were correlated significantly ($P \leqslant 0.05$) with the NPN, directly in the peas and inversely in the lentils. The opposite was true for Tyr and the PN, being inversely correlated in the peas and directly correlated in the lentils. There were no significant correlations in the beans. The increase in the NPN and the decrease in the PN with germination time along with higher Tyr concentrations in the peas may mean that the Tyr produced by hydrolysis during germination has not been modified. In the lentils in contrast, the increase in the NPN and

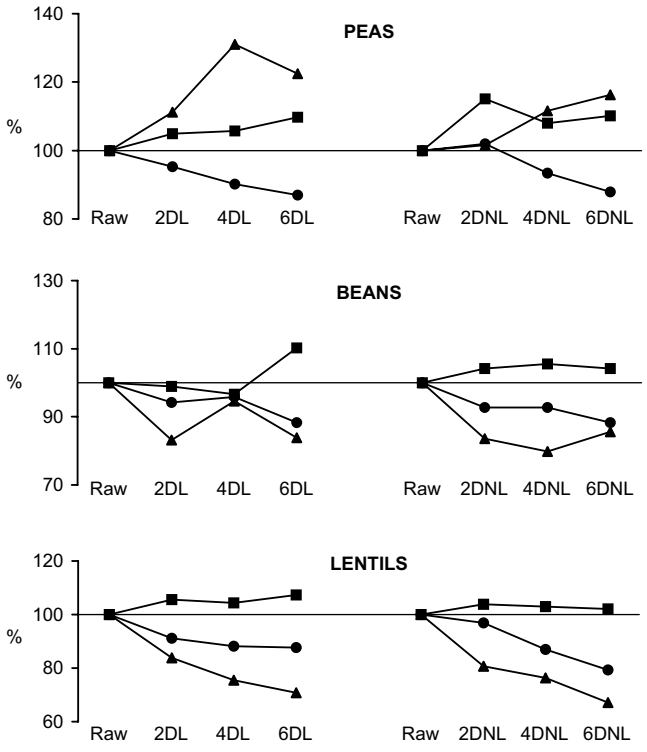

Fig. 2. Effect of germination on lysine (♦), histidine (■), and tyrosine (▲) retention in peas, beans, and lentils.

Table 3
Linear regression analysis for lysine, histidine, and tyrosine (g per 16 g of nitrogen) and non-protein and protein nitrogen (g per 100 g dry matter) in raw and germinated peas, beans, and lentils

Amino acid	Non-protein nitrogen		Protein nitrogen	
	r	*p*-Value	*r*	*p*-Value
Lysine				
Peas	−0.8372	0.0188	0.7735	0.0413
Beans	−0.8342	0.0196	0.5552	0.1958
Lentils	−0.9194	0.0034	0.9321	0.0022
Histidine				
Peas	0.5328	0.2183	−0.5877	0.1652
Beans	0.6139	0.1425	−0.4027	0.3704
Lentils	0.3870	0.3910	−0.3083	0.5012
Tyrosine				
Peas	0.7063	0.0761	−0.6724	0.0979
Beans	−0.3200	0.4841	−0.1154	0.8053
Lentils	−0.9435	0.0014	0.9020	0.0055

the decrease in the PN, coupled with the drop in the Tyr levels in the samples, may signal that this amino acid was used in synthesizing other amino acids or as an energy source.

To assess the effect of germination on the Lys and His contents, the proportion of these amino acids in the samples was calculated and compared to the FAO/WHO/UNU amino acid standard (1985). Table 4 shows that the proportion of Lys exceeded 100 in all three raw legumes, indicating that this amino acid is non-limiting in the seeds. However, in all three legumes the Lys index values in the germinated samples were generally lower than those for the raw legumes, the proportion of this amino acid decreasing with germination time. After germination for six days, the index value was higher than 100 only in the beans. The proportion of His in the three raw legumes was likewise higher than 100 and on the whole tended to increase with germination time, particularly in the beans.

3.3. Ornithine

This non-protein amino acid was present in all the samples of lentils but in none of the samples of peas and beans. Table 5 presents the Orn values expressed as mg/100 g of the dry matter and mg/g NPN in the raw lentils and in the seeds germinated under the different experimental conditions. All the germinated samples had higher Orn levels than the raw legume, and levels in the samples germinated in the presence of light differed from those in the samples germinated in darkness.

In the lentils germinated under conditions that included exposure to light, Orn levels increased in the batches germinated for two and four days and then declined in the batch germinated for six days. In the batches germinated in darkness, Orn levels increased gradually over the germination period when expressed as percentage of the dry matter and were higher than in the batches exposed to light except in the samples germinated for two days. On the other hand, when the results were expressed as a proportion of the NPN, the values in the batches germinated for four and six days were similar.

Regression analysis was carried out to reveal any relationships between the variations in Orn levels and the NPN, PN, and the three protein amino acids considered. The results have been compiled in Table 6. There was a direct linear correlation between the Orn and the NPN in the samples and an inverse linear correlation between the Orn and the PN. There was also a highly significant inverse linear correlation between the Orn and the Lys and Tyr levels, but there was no correlation with the His content. Accordingly, the NPN and Orn increased and the Lys and Tyr decreased with germination time.

The presence of such other non-protein amino acids as γ-hydroxyarginine, γ-hydroxyornithine, taurine, and α-aminobutyric acid in the raw seeds has been described (Kuo et al., 2004; Rozan et al., 2001; Sulser & Sager, 1976), but there have been no reports of Orn. Rozan et al. (2001) and Kuo et al. (2004) studied the effect of germination on the levels of various non-protein amino acids in lentils. Sprouting distinctly increased the content of certain non-protein amino acids, while others that were undetectable in the raw seeds became detectable. Rozan et al. (2001) also reported important qualitative and quantitative differences in the non-protein amino acids present in different species of lentils.

According to Dennis, Turpin, Lefebvre, and Layzell (1998), most non-protein amino acids in plants are intermediate metabolites used in the biosynthesis of protein amino

Table 4
Percentage ratio of lysine and histidine in raw and germinated peas, beans, and lentils to standard FAO/WHO/UNU protein

Batch	Peas		Beans		Lentils	
	Lysine	Histidine	Lysine	Histidine	Lysine	Histidine
Raw	106.9	119.0	114.5	153.7	107.2	121.6
Germination with light						
2 DL	101.9	124.7	107.9	152.1	97.8	128.4
4 DL	96.4	125.8	109.7	148.4	94.5	126.8
6 DL	92.9	130.5	101.0	169.5	94.0	130.5
Germination in darkness						
2 DNL	109.0	136.8	106.2	160.0	104.0	126.3
4 DNL	99.8	128.4	106.2	162.1	93.3	125.3
6 DNL	94.0	131.0	101.0	160.0	85.0	124.2

Table 5
Ornithine[A] in raw and germinated lentils

Lentils	Ornithine	
	mg/100 g dry matter	mg/g of non-protein nitrogen
Raw	9.76 ± 0.61^{a}	16.0 ± 0.7^{a}
Germination with light		
2 DL	73.2 ± 2.9^{c}	80.0 ± 2.8^{c}
4 DL	174 ± 1.1^{e}	150.1 ± 7.4^{e}
6 DL	164.8 ± 6.2^{d}	132.8 ± 3.6^{d}
Germination in darkness		
2 DNL	57.2 ± 2.1^{b}	65.0 ± 3.9^{b}
4 DNL	178.5 ± 3.8^{e}	183.4 ± 3.7^{f}
6 DNL	260.3 ± 3.4^{f}	183.9 ± 6.2^{f}

[A] Values are the means of three determinations ±SD. The same superscript in the same column indicates non-significant differences ($P \leqslant 0.05$).

Table 6
Linear regression analysis for ornithine and protein and non-protein nitrogen, lysine, histidine, and tyrosine in lentils

	r	*p*-Value
Nitrogen (*% dry matter*)		
Non-protein	0.8102	0.0000
Protein	−0.7527	0.0001
Amino acid (*g/16 g N*)		
Lysine	−0.8872	0.0000
Histidine	0.1632	0.4797
Tyrosine	−0.7870	0.0000

acids and are concentrated in the seeds, particularly in legumes.

Orn is an intermediate metabolite used to synthesize the amino acid proline from arginine, alkaloids, and prolamines, and it is also involved in the urea cycle. Like all non-protein amino acids, it may be present only in a single family, genus, or species (Bell, 1971).

Ugalde, Maher, Nardella, and Wallsgrove (1995) observed that the Orn metabolism in plants appeared to employ the same reactions as the urea cycle in animal tissues and also appeared to have the same function, the detoxifying of the ammonium ions in the form of urea. Furthermore, Lea and Joy (1983) pointed out that Orn may replace amides as a means of nitrogen transport during germination, which might explain the rise in levels during germination. Nevertheless, no study on legumes suggesting the possible existence of a common metabolic pathway for the three amino acids Lys, Tyr, and Orn that might account for the correlations between them that have been found.

Acknowledgment

This work was supported by the Spanish Commissión Interministerial de Ciencia y Tecnología (ALI 96-0408).

References

Adsule, R. N., Kadam, S. S., & Leung, H. K. (1989). Lentil. In D. K. Salunkhe & S. S. Kadam (Eds.), *CRC Handbook of world food legumes. Nutritional chemistry, processing, technology and utilization* (Vol. II, pp. 131–152). Boca Raton, FL: CRC.

Barceló, J., Rodrigo, G. N., Sabater, B., & Sánchez, R. (2001). *Fisiología vegetal*. Madrid: Pirámide.

Beevers, L., & Guernsey, F. S. (1966). Changes in some nitrogenous components during the germination of pea seeds. *Plant Physiology, 41*, 1455–1458.

Bell, E. A. (1971). Comparative biochemistry of non-protein amino acids. In J. B. Harbone, D. Boulter, & B. L. Turner (Eds.), *Chemotaxonomy of the leguminosae*. London: Academic Press.

Chavan, J. K., & Kadam, S. S. (1989). Nutritional improvement of cereals by sprouting. *Critical Reviews in Food Science and Nutrition, 28*, 401–437.

Chen, L. H., Wells, C. E., & Fordham, J. R. (1975). Germinated seeds for human consumption. *Journal of Food Science, 40*, 1290–1294.

Dennis, D. T., Turpin, D. H., Lefebvre, D. D., & Layzell, D. B. (1998). *Plant metabolism*. Edinburgh: Addison Wesley Longman.

Earle, F. R., & Jones, O. (1962). Analysis of seed samples from 113 plant families. *Economic Botany, 16*, 221–225.

El-Hag, N., Haard, N. F., & Morse, R. E. (1978). Influence of sprouting on the digestibility coefficient, trypsin inhibitor and globulin proteins of red kidney beans. *Journal of Food Science, 43*, 1874–1875.

FAO/WHO/UNU, (1985). Energy and protein requirements. Report of the Joint FAO/WHO/UNU Expert Consultation. Technical Report Series 724. Geneva, Switzerland: FAO, WHO and UNU.

Frias, J., Díaz-Pollan, C., Hedley, C. L., & Vidal-Valverde, C. (1995). Evolution of trypsin inhibitor activity during germination of lentils. *Journal of Agricultural and Food Chemistry, 43*, 2231–2234.

Frias, J., Díaz-Pollan, C., Hedley, C. L., & Vidal-Valverde, C. (1996). Evolution and kinetics of monosaccharides, disaccharides and α-

galactosides during germination of lentils. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 202*, 35–39.

Gamarnik, A., & Frydman, R. B. (1991). Cadaverine, an essential diamine for the normal root development of germinating soybean (Glycine max) seeds. *Plant Physiology, 97*, 778–785.

Hsu, D., Leung, H. K., Finney, P. L., & Morad, M. M. (1980). Effect of germination on nutritive value and baking properties of dry peas, lentils, and faba beans. *Journal of Food Science, 45*, 87–92.

Kuo, Y.-H., Rozan, P., Lambein, F., Frias, J., & Vidal-Valverde, C. (2004). Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes. *Food Chemistry, 86*, 537–545.

Lea, P. J., & Joy, K. W. (1983). Amino acid interconversion in germinating seeds. In C. Nozzolillo, P. J. Lea, & F. A. Loewus (Eds.), *Mobilization of reserves in germination. Recent Advances in Phytochemistry* (Vol. 17, pp. 77–109). New York: Plenum Press.

Mbithi-Mwikya, S., Ooghe, W., Van Camp, J., Ngundi, D., & Huyghebaert, A. (2000). Amino acid profiles after sprouting, autoclaving, and lactic acid fermentation of finger millet (*Eleusine coracan*) and kidney beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry, 48*, 3081–3085.

Mossé, J., & Baudet, J. (1983). Crude protein content and amino acid composition of seeds: Variability and correlations. *Plant Foods for Human Nutrition, 32*, 225–245.

Prodanov, M., Sierra, I., & Vidal-Valverde, C. (1997). Effect of germination on the thiamine, riboflavin and niacin contents in legumes. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 205*, 48–52.

Pusztai, A., & Duncan, I. (1971). Changes in proteolytic enzyme activities and transformation of nitrogenous compounds in the germinating seeds of kidney bean (*Phaseolus vulgaris*). *Planta (Berl.), 96*, 317–325.

Rozan, P., Kuo, Y.-H., & Lambein, F. (2001). Amino acids in seeds and seedlings of the genus Lens. *Phytochemistry, 58*, 281–289.

Salunkhe, S. K., Sathe, S. K., & Deshpande, S. S. (1989). French bean. In D. K. Salunkhe & S. S. Kadam (Eds.), *CRC Handbook of world food legumes. Nutritional chemistry, processing, technology and utilization* (Vol. II, pp. 23–63). Boca Raton, FL: CRC.

Sanz, M. A., Castillo, G., & Hernández, A. (1996). Isocratic high-performance liquid chromatographic method for quantitative determination of lysine, histidine and tyrosine in foods. *Journal of Chromatography A, 719*, 195–201.

Savelkoul, F. H. M. G., Tamminga, S., Leenaars, P. P. A. M., Schering, J., & Ter Maat, D. W. (1994). The degradation of lectins, phaseolin and trypsin inhibitors during germination of white kidney beans, *Phaseolus vulgaris* L. *Plant Foods for Human Nutrition, 45*, 213–222.

Shalaby, A. R. (2000). Changes in biogenic amines in raw and germinating legume seeds and their behavior during cooking. *Nahrung, 44*, 23–27.

Sulser, H., & Sager, F. (1976). Identification of uncommon amino acids in the lentil seed (*Lens culinaris* Med.). *Experientia, 32*, 422–423.

Torrigiani, P., & Scoccianti, V. (1995). Regulation of cadaverine and putrescine levels in different organs of chick-pea and seedlings during germination. *Physiologia Plantarum, 93*, 512–518.

Ugalde, T. D., Maher, S. E., Nardella, N. E., & Wallsgrove, R. M. (1995). Amino acid metabolism and protein deposition in the endosperm of wheat: Synthesis of proline via ornithine. In R. M. Wallsgrove (Ed.), *Amino acids and their derivatives in higher plants* (pp. 77–86). Cambridge: Cambridge University Press.

Urbano, G., López-Jurado, M., Hernández, J., Fernández, M., Moreu, M. C., & Frias, J. (1995). Nutritional assessment of raw, heated, and germinated lentils. *Journal of Agricultural and Food Chemistry, 43*, 1871–1877.

Vidal-Valverde, C., Frias, J., Hernández, A., Martín-Alvarez, P. J., Sierra, I., & Rodríguez, C. (2003). Assessment of nutritional compounds and antinutritional factors in pea (*Pisum sativum*) seeds. *Journal of the Science of Food and Agriculture, 83*, 298–306.

Vidal-Valverde, C., Frias, J., Sierra, I., Blázquez, I., Lambein, F., & Kuo, Y.-H. (2002). New functional legume foods by germination: Effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology, 215*, 472–477.

Youssef, M. M., El-Aal, M. H. A., Shekib, L. A. E., & Ziena, H. M. (1987). Effects of dehulling, soaking and germination on chemical composition, mineral elements and protein patterns of faba beans (*Vicia faba* L.). *Food Chemistry, 23*, 129–138.