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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).