Nutritional Evaluation of Ethanol-Extracted Lentil Flours

M. Ángeles Sanz,[†] Inmaculada Blázquez,[‡] Isabel Sierra,[‡] M. Ángeles Medrano,[†] Juana Frias,[‡] Concepción Vidal-Valverde,[‡] and Amelia Hernández*,[†]

Departamento de Nutrición y Bromatología, Universidad de Alcalá, Campus Universitario, 28871 Alcalá de Henares (Madrid), Spain, and Instituto de Fermentaciones Industriales (CSIC), C/Juan de la Cierva 3, 28006 Madrid, Spain

Lentil flours were extracted with 80% ethanol at 25 and 50 °C for 1, 2, or 3 h. The various nitrogen fractions, soluble carbohydrates, three amino acids (Lys, His, and Tyr), available lysine, protein digestibility, and vitamins B_1 and B_2 were analyzed to evaluate the effect of extraction. Extraction resulted in an increase in the total nitrogen content of the extracted flours, with extraction temperature affecting the nature of the nitrogen (protein or nonprotein) content. There was also a large reduction in the oligosaccharides of the raffinose family, although the effect of temperature was appreciable only in the case of stachyose. There was hardly any effect on the concentrations of the amino acids analyzed or on protein digestibility; however, a positive correlation between protein digestibility and the available lysine was recorded in the samples. The vitamin B_1 and B_2 contents underwent variable decreases depending on extraction temperature.

Keywords: *Lentils; ethanol extraction; protein quality; soluble carbohydrates; vitamins B_1 and B_2*

INTRODUCTION

As good sources of protein, carbohydrates, several water-soluble vitamins, and minerals, legumes in general make a major contribution to human nutrition. However, the nutritive value of legumes is limited by certain antinutritional factors that can affect acceptability and nutrient availability. Removal of undesirable components is essential for optimal utilization of legumes as human food and enhanced acceptability and nutritional quality. A number of different processing methods have been put forward to that end, the most conventional being soaking, boiling, germination, and fermentation. Other methods less widespread, including extraction, have also been developed.

Extraction may involve removal of one or more unwanted components in either a single step or multiple steps and may or may not be intentional, depending on the process involved (*1*). Extraction is not intentional when it comprises one stage of a processing method. For instance, extraction using 60−80% aqueous alcohol to immobilize the protein component is a step in the preparation of protein concentrates (*2*).

Alternatively, extraction may be intentional, the primary purpose being to remove some of the unwanted components from the legumes. Very little work has been conducted in this respect. Extraction using different alkaline solutions to reduce tannins, trypsin inhibitor activity, and hemagglutinating activity has been tested (*3*−*5*). Solvent extraction and a combination of solvent extraction and heat treatment have been described to remove lipid-derived off-flavors (*6*). Alcohol extraction has been proposed as a possible alternative processing method to remove growth-depressing factors in soybean meal (*7*−*9*). Tolman (*10*) studied the effect of extraction performed at different temperatures using different concentrations of ethanol on certain antinutritional factors, protein denaturation, and functional properties in raw peas and pea protein isolate.

Oligosaccharides of the raffinose family are one of the primary antinutritional factors in lentils and are known to be extractable in 80% aqueous ethanol, so it was decided to study the effect of extraction on such sugars. An additional advantage of this method of solvent extraction is the possibility of removing color and flavor components and residual lipids, thereby improving the aroma and flavor profiles of the resulting product and extending shelf life. However, in addition to measuring the effect of this extraction method on the antinutritional factors of the final product, it is also necessary to evaluate the other essential nutrients, namely, proteins and vitamins, to know the effect on its nutritive value.

Accordingly, the objective of the study was to obtain lentil flours prepared by means of extraction using aqueous ethanol (80% v/v) at two different working temperatures, namely, ambient temperature (25 °C) and 50 °C, for three different working times, 1, 2, and 3 h, and then to evaluate the effect of extraction on the various nitrogen fractions (total, protein, and nonprotein nitrogen), monosaccharides, disaccharides, and α-galactosides, three amino acids (lysine, histidine, and tyrosine), available lysine, protein digestibility, and two vitamins (B_1 and B_2).

MATERIALS AND METHODS

Ethanol Extraction of the Lentils. *Lens culinaris* var. *vulgaris* cv. Magda-20 was used in the extraction experiments. The seeds were comminuted in a mill under refrigeration to prevent heating, and the ground lentils were then sieved through a screen with a pore size of 0.6 mm.

For the extraction performed at ambient temperature, 100 g of ground, sieved lentil flour was extracted in 300 mL of 80%

* Author to whom correspondence should be addressed (fax 34 91 8854660; e-mail amelia.hernandez@uah.es).

[†] Universidad de Alcalá.

[‡] Instituto de Fermentaciones Industriales (CSIC).

10.1021/jf001293i CCC: $20.00
Published on Web 03/20/2001

ethanol. After initial stirring by hand with a rod, stirring was continued automatically using a magnetic stirrer at 1000 rpm. The container was protected from light during the extraction procedure. The hot extraction was performed in the same conditions just described above, except that the ethanol was heated to 50 °C before being added to the sample, heating at 50 °C was carried out during the procedure, and the container was fitted with a cooling coil to prevent evaporation losses during extraction. Three extraction times (1, 2, and 3 h) were tested at each extraction temperature. Extractions were performed in duplicate.

At the end of the extraction time, extracted flour samples were separated from the extraction liquids by vacuum filtration. The extracted samples were freeze-dried, and the extraction liquids were discarded. Residual moisture in the freeze-dried samples was determined by drying in an oven at 100 °C.

Total Nitrogen Determination. The total nitrogen was analyzed by using the Kjeldahl method with endpoint potentiometric 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 endpoint control. The color of this solution changes at pH 4.6. A factor of 6.25 was used to calculate the crude protein content of the samples.

Protein and Nonprotein Nitrogen Determination. The copper sulfate method was used to determine the nonprotein nitrogen. An amount 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% aluminum potassium sulfate was added, and the mixture was 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 nonprotein nitrogen was determined by using the Kjeldahl method. The protein nitrogen was calculated as the difference between the total and nonprotein nitrogen.

Soluble Nitrogen Determination. The soluble nitrogen was determined at three pH values, 3, 5, and 7. An amount of 0.4 g of sample was weighed out in a centrifugation tube, and 30 mL of distilled water was added. The pH was adjusted using 0.1 M NaOH or 0.1 M HCl, depending on the assayed pH. The mixture was shaken continuously for 1 h at ambient temperature. The pH was monitored continuously throughout the procedure and readjusted as necessary. The mixture was then centrifuged at 20000*g* for 15 min and filtered through Whatman No. 1 paper. The filtrate and the liquids from washing the residue were collected in a Kjeldahl flask, and soluble nitrogen was determined using the method already described above for total nitrogen.

Monosaccharide, Disaccharide, and α-Galactoside Determinations. Analysis of monosaccharides (glucose and fructose), disaccharides (sucrose), and α-galactosides (raffinose, ciceritol, and stachyose) was carried out using the method described by Frias et al. (*11*). The extraction of the soluble sugars was performed using 80% ethanol and boiled under reflux for 15 min. Quantitation was carried out by HPLC with refraction index detection (HPLC-RI).

Lysine, Histidine, and Tyrosine Determinations. The total lysine, histidine, and tyrosine contents of the lentils were determined after hydrolysis with 6 M HCl at 110 °C for 24 h. Amino acid were analyzed by HPLC as dansylated derivatives using the method of Sanz et al. (*12*).

Available lysine determination. The available lysine content of the lentils was determined using 1-fluoro-2,4-dinitrobenzene (FDNB) according to the method of Castillo et al. (*13*). After reaction with FDNB, the proteins were hydrolyzed using 6 M HCl at 110 °C for 24 h. The available lysine was determined by HPLC.

In Vitro Digestibility. In vitro digestibility of the samples was determined according to the pH-stat method developed by Pedersen and Eggum (*14*). A three-enzyme solution was made up to contain 23100 units of trypsin (EC 3.4.21.4) (porcine pancreatic trypsin, type IX, Sigma T-0134), 186 units of chymotrypsin (EC 3.4.21.1) (bovine pancreatic chymotrypsin, type II, Sigma C-4129), and 0.052 unit of peptidase (porcine intestinal peptidase, grade K, Sigma P-7500) per milliliter. The pH was adjusted to 8.00 at 37 °C, and the enzyme solution was frozen at −30 °C until use. The activity of this solution was determined daily using an aqueous suspension of sodium caseinate (Sigma C-8654).

An amount of 10 mL of sodium caseinate or sample suspension (1 mg of N/mL of water) was placed in a titration cell at 37 °C, and the pH was adjusted to 8.0 and held at that level for 10 min. An automatic titration was then run using the pH-stat procedure, with the addition of 1 mL of the enzyme solution. The amount of 0.100 M NaOH required to keep the pH at 7.98 for exactly 10 min was used to determine uncorrected true protein digestibility (%UTD) employing the following equation:

$$\%\mathrm{UTD} = 76.14 + 47.77B$$

where B = mL of added 0.100 M NaOH. The %UTD values were corrected using the value obtained for the sodium caseinate solution (*15*).

Vitamin B_1 and B_2 Determinations. A single extraction procedure for vitamins B_1 and B_2 was carried out according to the method of Vidal-Valverde et al. (*16*). Vitamin B_1 was quantified by HPLC with postcolumn derivatization and fluorescence detection according to the method of Sierra and Vidal-Valverde (*17*). Vitamin B_2 was determined by HPLC with fluorescence detection as described by Sierra and Vidal-Valverde (*17*).

Statistical Analysis. All statistical analyses were performed using the Statgraphics Statistical Graphics System, version 5.0 (Statistical Graphics Corp., Rockville, MD). Multifactor analysis of variance was used to determine significant differences and regression analysis to determine the correlations.

Table 1. Total, Protein, and Nonprotein Nitrogen and Crude Protein Content[a] (Grams per 100 g of DM) in Lentils Extracted Using Ethanol

lentils	total nitrogen	crude protein	nonprotein nitrogen	protein nitrogen
control	4.42 ± 0.03[a]	27.62 ± 0.17[a]	0.57 ± 0.05[a]	3.85[a]
extracted at ambient temperature				
1 h	4.56 ± 0.02[b]	28.50 ± 0.17[b]	0.81 ± 0.05[b]	3.75[b]
2 h	4.56 ± 0.03[b]	28.53 ± 0.22[b]	0.84 ± 0.03[b]	3.72[b]
3 h	4.56 ± 0.04[b]	28.50 ± 0.26[b]	0.84 ± 0.02[b]	3.72[b]
extracted at 50 °C				
1 h	4.54 ± 0.02[b]	28.40 ± 0.13[b]	0.45 ± 0.01[c]	4.09[c]
2 h	4.51 ± 0.01[b]	28.21 ± 0.04[b]	0.38 ± 0.01[c]	4.13[c]
3 h	4.51 ± 0.02[b]	28.18 ± 0.17[b]	0.47 ± 0.06[c]	4.04[c]

[a] Values are the means of four determinations ± SD. Means with different superscripts in the same column are significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Total, Protein, and Nonprotein Nitrogen. Table 1 presents the total nitrogen, crude protein, protein nitrogen, and nonprotein nitrogen values for the ethanol-extracted lentil flours and the control lentil flour.

Total nitrogen content in the control flour was 4.42% and that of crude protein, 27.62%. This latter value was within the range of 19.5−31% reported by Bhatty (*18*) for lentils grown in Canada. Nonprotein nitrogen in the control flour made up 12.9% of the total nitrogen.

With regard to the effect of extraction on the total nitrogen content and the crude protein content, Table 1 shows that there was a significant increase (2−3%) in the values at both extraction temperatures but that the values were unaffected by the extraction time employed. Grant et al. (*8*) also recorded an increase in protein concentration of ∼1% after ethanol extraction

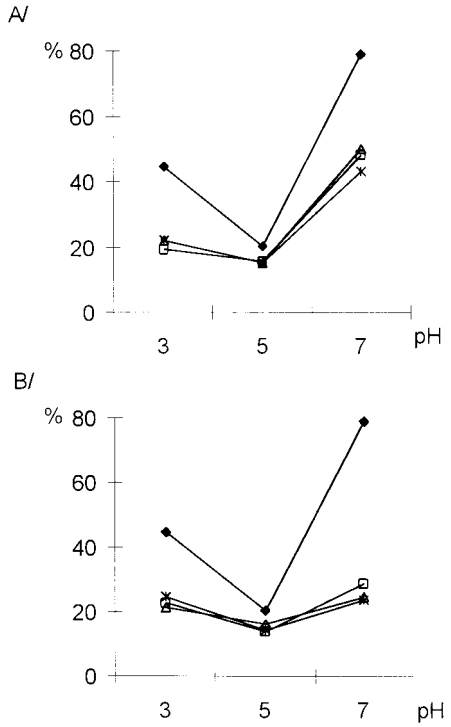

Figure 1. Nitrogen solubility (percent) in lentils extracted at (A) ambient temperature and (B) 50 °C: (◆) control; (△) 1 h; (∗) 2 h; (□) 3 h.

of defatted soybean meal at 60 °C. Tolman (*10*) found an increase of 2.5% in the crude protein content of peas following ethanol extraction at 50 °C.

Extraction also exerted a significant effect ($P < 0.05$) on both the protein nitrogen and the nonprotein nitrogen contents. Ambient temperature extraction resulted in a substantial increase in the nonprotein nitrogen content compared with the control flour, the increase ranging from 42 to 47% depending on the extraction time, indicating that ∼18% of the total nitrogen in the samples was nonprotein nitrogen. Conversely, hot extraction at 50 °C brought about a decrease in the nonprotein nitrogen content of the extracted flours to below the value for the control flour, with losses of 18−33%. In the case of hot extraction, the proportion of nonprotein nitrogen in the total nitrogen ranged between 8 and 10%, depending on the extraction time.

Tolman (*10*) reported, without furnishing data, that the nonprotein nitrogen fractions were more readily separated from denatured proteins. If this is the case, it may mean that the nonprotein nitrogen is less soluble in the ethanol on extraction of the lentil flours at ambient temperature, because of the lower level of protein denaturation. In contrast, during hot extraction, the higher temperature and higher level of denaturation may be conducive to extraction, thereby bringing about a decrease in the nonprotein nitrogen content of the samples.

Soluble Nitrogen. Figure 1 shows the percentage soluble nitrogen values obtained at the three pH values considered (3, 5, and 7) in the control flour and in the lentil flours extracted using ethanol. The solubility profile for the control flour was in agreement with the findings reported by other workers for various raw legumes (*19*−*25*).

The percentage soluble nitrogen values were lower in all of the extracted flours than in the control flour at all the pH values tested. Although soluble nitrogen values in the extracted flours were lowest at pH 5, the values at that pH were nonetheless proportionally the highest with respect to the value for the control flour, nearly 75% of the control value for the ambient temperature extractions and 67−80% for the hot extractions. In comparison, the soluble nitrogen values for all of the extractions performed at pH 3 were only ∼50% of the initial level. The effect of extraction temperature was only significant at pH 7, at which the soluble nitrogen was 55−63% of the value for the control flour in the samples extracted at ambient temperature and only 30−35% of the value for the control flour in the samples extracted at 50 °C.

Organic solvents, which have a dielectric constant lower than that of water, alter the tertiary structure of the proteins, which are denatured as a result of a competitive reaction for the intermolecular hydrophobic interactions, interfering with the hydrogen bridges and thereby altering hydration (*26*). Mattil (*27*) regarded solubility as a measure of protein denaturation.

The fact that alcohols tend to denature proteins and lower protein solubility (*9*) and the fact that denatured proteins are ordinarily less soluble than native proteins around their isoelectric point may account for the lower level of soluble nitrogen at pH 3 and 5 as well as the fact that time and temperature were not determining factors at those pH values. Tolman (*10*) likewise reported denaturation of pea proteins by ethanol to be more marked than that brought about by temperature. Nevertheless, in that same study a larger decrease in the protein dispersibility index was also recorded when the extraction temperature was increased from 50 to 80 °C. That index obtains at each foodstuff's natural pH, which in the case of peas is ∼7. This is consistent with the higher level of protein denaturation in the lentils caused by ethanol at pH 7.0 recorded in this experiment at the higher extraction temperature.

Monosaccharides, Disaccharides, and α-Galactosides. Table 2 summarizes the effects of ethanol extraction on the soluble carbohydrate content. The values for these sugars in the unextracted lentils, that is, the control flour, were in the range of values previously published in the literature (*28*−*32*). In the control flour stachyose was the sugar present in the highest amounts, followed by sucrose, ciceritol, and raffinose, whereas only small amounts of fructose were present. Glucose was not detected in the control flour.

After 80% ethanol extraction, the total soluble sugar content decreased sharply after large decreases in the contents of the individual sugars. The fructose content decreased after ethanol extraction, with no significant differences between extraction at ambient temperature and at 50 °C, although larger decreases (75%) were recorded after 3 h at ambient temperature. The decrease in sucrose (77%) after ethanol extraction for 1 h at 50 °C was larger than the decrease (73%) recorded after 1 h at ambient temperature. After 2 h, the observed decreases were similar (79%) at both temperatures tested, and the maximum decrease (82%) in the sucrose content was attained only after extraction at ambient temperature for 3 h.

In the case of the α-galactosides, raffinose and ciceritol behaved similarly, with the contents of those sugars decreasing after ethanol extraction, although no significant differences were found between the ambient temperature and 50 °C extraction temperature after 3

Table 2. Changes in Soluble Carbohydrates[a] (Grams per 100 g of DM) in Lentils Extracted Using Ethanol

lentils	fructose	sucrose	raffinose	ciceritol	stachyose	α-galactosides	soluble carbohydrates
control	0.08 ± 0.00[a]	2.94 ± 0.03[a]	0.52 ± 0.01[a]	2.20 ± 0.04[a]	3.74 ± 0.04[a]	6.46 ± 0.05[a]	9.48 ± 0.05[a]
extracted at ambient temperature							
1 h	0.04 ± 0.01[b]	0.80 ± 0.02[b]	0.22 ± 0.03[b]	0.17 ± 0.01[b]	0.35 ± 0.04[b]	0.74 ± 0.03[b]	1.58 ± 0.04[b]
2 h	0.06 ± 0.02[b]	0.62 ± 0.02[c]	0.18 ± 0.01[c]	0.12 ± 0.01[cd]	0.37 ± 0.02[b]	0.67 ± 0.03[c]	1.36 ± 0.05[c]
3 h	0.02 ± 0.01[c]	0.58 ± 0.01[d]	0.11 ± 0.01[d]	0.11 ± 0.01[c]	0.31 ± 0.01[c]	0.53 ± 0.01[d]	1.13 ± 0.02[de]
extracted at 50 °C							
1 h	0.05 ± 0.02[b]	0.68 ± 0.09[e]	0.16 ± 0.02[e]	0.13 ± 0.02[d]	0.24 ± 0.01[d]	0.53 ± 0.04[d]	1.26 ± 0.15[f]
2 h	0.04 ± 0.01[b]	0.62 ± 0.07[c]	0.13 ± 0.05[d]	0.13 ± 0.03[d]	0.24 ± 0.06[d]	0.54 ± 0.01[d]	1.16 ± 0.21[df]
3 h	0.04 ± 0.00[b]	0.64 ± 0.03[c]	0.13 ± 0.01[d]	0.11 ± 0.02[c]	0.16 ± 0.03[e]	0.40 ± 0.07[e]	1.08 ± 0.10[e]

[a] Values are the means of four determinations ± SD. Means with different superscripts in the same column are significantly different ($P < 0.05$).

Table 3. Lysine, Histidine, and Tyrosine Contents[a] in Lentils Extracted Using Ethanol

	% DM			g/16 g of nitrogen		
lentils	lysine	histidine	tyrosine	lysine	histidine	tyrosine
control	1.80 ± 0.00[a]	0.76 ± 0.01[a]	0.80 ± 0.01[ab]	6.54 ± 0.02[a]	2.75 ± 0.03[a]	2.92 ± 0.03[ab]
extracted at ambient temperature						
1 h	1.93 ± 0.06[b]	0.74 ± 0.01[a]	0.84 ± 0.01[c]	6.79 ± 0.26[a]	2.59 ± 0.07[b]	2.95 ± 0.01[a]
2 h	1.87 ± 0.03[b]	0.77 ± 0.01[a]	0.84 ± 0.00[c]	6.55 ± 0.16[a]	2.70 ± 0.02[ab]	2.94 ± 0.03[a]
3 h	1.85 ± 0.00[b]	0.81 ± 0.07[a]	0.83 ± 0.01[bc]	6.48 ± 0.05[a]	2.68 ± 0.00[ab]	2.92 ± 0.02[ab]
extracted at 50 °C						
1 h	1.87 ± 0.00[b]	0.77 ± 0.00[a]	0.82 ± 0.00[abc]	6.60 ± 0.01[a]	2.71 ± 0.00[ab]	2.89 ± 0.04[ab]
2 h	1.84 ± 0.03[b]	0.77 ± 0.01[a]	0.81 ± 0.01[abc]	6.52 ± 012[a]	2.73 ± 0.04[a]	2.87 ± 0.04[ab]
3 h	1.91 ± 0.09[b]	0.79 ± 0.02[a]	0.80 ± 0.02[a]	6.77 ± 0.37[a]	2.80 ± 0.09[a]	2.83 ± 0.09[b]

[a] Values are the means of four determinations ± SD. Means with different superscripts in the same column are significantly different ($P < 0.05$).

h (a decrease of 79% for raffinose and a decrease of 95% for ciceritol). Stachyose also decreased after ethanol extraction, with larger reductions at 50 °C. A decrease of 96% was obtained after 3 h.

Oligosaccharides of the raffinose family are known to be extractable in 80% aqueous ethanol, and indeed the procedure employed for oligosaccharide determination involves this extraction during 15 min of boiling. However, data on extraction at different temperatures would appear to be lacking. The results presented in Table 2 indicate that ethanol extraction brings about a substantial reduction in the oligosaccharides of the raffinose family (raffinose, ciceritol, and stachyose) and that temperature did not make any appreciable difference except in the case of stachyose.

Total and Available Lysine, Histidine, and Tyrosine. Table 3 presents the values for the amino acids lysine, histidine, and tyrosine in the control flour and in the extracted flours. The values have been expressed as percentage dry matter (DM) and as grams of amino acid per 16 g of total nitrogen.

The values of the three amino acids in the control flour were within the ranges published in the literature, namely, 6.3−7.0 for lysine, 2.1−2.6 for histidine, and 2.2−3.1 for tyrosine (*33*−*37*).

According to the data shown in the Table 3, extraction caused hardly any alteration in the values for the amino acids considered and likewise did not affect the proportions of those amino acids in the protein, regardless of extraction temperature and duration.

Various workers have reported that the values for essential amino acids remain similar to those in the original seeds during the production of concentrates and isolates from legumes, a procedure that includes a preliminary extraction step performed using alcohol (*9*, *38*, *39*).

Table 4. Available Lysine Content[a] (Grams per 16 g of Nitrogen) and Lysine Availability (Percent) in Lentils Extracted Using Ethanol

lentils	available lysine	availability
control	5.02 ± 0.07[ab]	76.8[ab]
extracted at ambient temperature		
1 h	4.70 ± 0.19[ac]	69.2[c]
2 h	4.85 ± 0.15[abc]	74.0[abc]
3 h	4.57 ± 0.00[c]	70.5[ac]
extracted at 50 °C		
1 h	4.83 ± 0.27[abc]	73.2[abc]
2 h	5.12 ± 0.06[b]	78.5[b]
3 h	4.79 ± 0.04[abc]	70.8[ac]

[a] Values are the means of four determinations ± SD. Means with different superscripts in the same column are significantly different ($P < 0.05$).

Table 4 sets out the values for available lysine, expressed as grams per 16 g of nitrogen, and percentage lysine availability calculated as a percentage of the total lysine.

Lysine availability in the control flour was ∼77%, which means that ∼23% of the lysine in the seeds is present in a form in which it is not available for use, either for natural reasons or as a result of the processing undergone by the seeds prior to marketing. Petzke et al. (*40*) determined the available lysine content in selected tropical crop seeds using the method of Booth (*41*). The lysine availability values ranged from 65 to 96% in the different species considered.

Table 4 shows that extraction at ambient temperature brought about a decrease in the available lysine content after 3 h ($P < 0.05$). Differences in availability were not significant except after 1 h, when a slight decrease was observed. The hot ethanol-extracted flours showed no significant differences either in available lysine content or in lysine availability. Only a significant decrease in lysine availability between extraction for 2 and 3 h was found.

Table 5. In Vitro Protein Digestibility[a] (Percent) and pH Values for Lentils Extracted Using Ethanol

lentils	pH	digestibility
control	6.38	92.39 ± 0.17[ab]
extracted at ambient temperature		
1 h	6.61	91.43 ± 0.53[c]
2 h	6.59	92.03 ± 0.00[a]
3 h	6.63	91.42 ± 0.26[c]
extracted at 50 °C		
1 h	6.79	92.83 ± 0.05[b]
2 h	6.60	92.39 ± 0.08[ab]
3 h	6.67	91.91 ± 0.12[ac]

[a] Values are the means of four determinations ± SD. Means with different superscripts in the same column are significantly different ($P < 0.05$).

The lysine and histidine scores (grams of amino acid in 16 g N of test sample/grams of the same amino acid in 16 g of N of reference protein × 100) for the lentil flours can be calculated against the pattern of amino acid requirements for preschool children (2−5 years) proposed by FAO/WHO/UNU (*42*) (5.8 g of lysine and 1.9 g of histidine in 16 g N of pattern). The tyrosine score was not calculated, because the reference standard encompasses the sum of tyrosine and phenylalanine. Two lysine scores were calculated, one based on the total lysine and the other based on the available lysine.

The histidine score and the score based on the total lysine were >100 for all of the flours, and the values for the extracted flours were similar to the value for the control flour (144.7 and 112.8 for histidine and lysine scores, respectively).

When calculated on the basis of the available lysine content, the scores for all of the flours were <100. The scores for the flours extracted at ambient temperature (76.0, 83.6, and 78.8 for extraction for 1, 2, and 3 h, respectively) were lower than the values for the control flour (86.6). The scores for the hot-extracted flours (83.3, 88.3, and 82.6 for extraction for 1, 2, and 3 h, respectively) were higher.

In Vitro Digestibility. Table 5 gives the in vitro digestibility values for the control flour and for the flours extracted under the different conditions tested.

Digestibility values varied hardly at all in the flours extracted at ambient temperature, with the highest value for the sample extracted for 2 h. Hot extraction yielded also values similar to the value for the control flour in the flours extracted for 1 h and for 2 h and a slightly lower value in the flour extracted for 3 h.

The results of the statistical analysis indicated a highly significant correlation between protein digestibility and the available lysine in the samples ($r = 0.7698$, $n = 7$, $P = 0.0429$) and a significant correlation between digestibility and lysine availability ($r = 0.6737$, $n = 7$, $P = 0.0971$). Protein digestibility increased as lysine availability increased.

The literature review did not turn up any information on the effect of alcohol extraction on protein digestibility in lentils.

On treating soybean with ethanol, Hancock et al. (*9*) recorded an increase in in vivo digestibility. Those workers tentatively attributed that increase to the fact that processing brought about a 45.7% reduction in trypsin inhibitor. Grant et al. (*8*) failed to find any correlation between the trypsin inhibitor content and the apparent protein digestibility in raw soybeans extracted using ethanol.

Vitamins B_1 and B_2. The vitamin B_1 and B_2 contents in the control flour (Table 6) were 0.208 and 0.156 mg/100 g of DM, respectively. Comparing those results with the literature values showed the vitamin B_1 content to be in consonance with the literature reports (0.1−0.32 mg/100 g of DM) but the vitamin B_2 content to be lower (*43*). This may have been due to the fact that most literature values have been obtained using the method of the AOAC, a fluorometric method that may overestimate the value of vitamin B_2 as a result of interference by other fluorescent components in the food. There is no overestimation when the HPLC method is used to make the determinations.

Table 6. Vitamin B_1 and Vitamin B_2 Contents[a] (Milligrams per 100 g of DM) in Lentils Extracted Using Ethanol

lentils	vitamin B_1	vitamin B_2
control	0.208 ± 0.02[a]	0.156 ± 0.01[a]
extracted at ambient temperature		
1 h	0.175 ± 0.01[b]	0.144 ± 0.01[ab]
2 h	0.171 ± 0.01[b]	0.140 ± 0.01[b]
3 h	0.174 ± 0.01[b]	0.134 ± 0.02[b]
extracted at 50 °C		
1 h	0.192 ± 0.01[c]	0.127 ± 0.01[c]
2 h	0.189 ± 0.02[c]	0.117 ± 0.02[c]
3 h	0.191 ± 0.02[c]	0.125 ± 0.01[c]

[a] Values are the means of four determinations ± SD. Means with different superscripts in the same column are significantly different ($P < 0.05$).

Table 6 also presents the values for both vitamins in the lentil flours prepared by ethanol extraction under the different conditions tested. Extraction brought about a significant reduction ($P < 0.05$) in the vitamin B_1 content in the lentil flours; the decrease was higher at ambient temperature (16%) than at 50 °C (8%). The reason may be that hot extraction removed more of the components soluble in the ethanol (sugars, antinutritional factors, etc.), leading to a percentage increase in the vitamin B_1 content as compared to the flours that underwent extraction at ambient temperature. Extraction time was observed not to exert any significant influence on the vitamin B_1 content ($P > 0.05$).

The vitamin B_2 content varied according to the extraction conditions. For hot extraction at 50 °C, the content fell by 21% after 3 h, compared with a decrease of just 11% when extraction was performed at ambient temperature. This may be ascribable to the higher solubility of vitamin B_2 in ethanol at 50 °C. Extraction time was also observed not to have a significant effect on the vitamin B_2 content ($P > 0.05$).

The literature review has not disclosed any information on the effect of alcohol extraction on the content of these two vitamins in lentils.

In conclusion, ethanol extraction of raw lentils is an effective method of minimizing the content of the α-galactosides. Extraction at 50 °C for 2 h was the most effective extraction method in terms of the protein quality and vitamin B_1 content of the final product, although a decrease in the vitamin B_2 content higher than that observed at ambient temperature is produced. Furthermore, hot extraction offers the added advantage of inactivating heat labile antinutritional factors present in the lentils, such as trypsin inhibitor and lectin (*10*, *44*).

LITERATURE CITED

(1) Sathe, S. K.; Salunkhe, D. K. Technology of removal of unwanted components of dry legumes. In *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization*; Salunkhe, D. K., Kadam, S. S., Eds.; CRC: Boca Raton, FL, 1989; Vol. III, pp 249−270.

(2) Endres, J. G. Legume protein flour and concentrates. In *Legumes. Chemistry, Technology and Human Nutrition*; Matthews, R. H., Ed.; Dekker: New York, 1989; pp 139−160.

(3) Price, M. L.; Butler, L. G.; Featherston, W. R.; Rogler, J. C. Detoxification of high tannin sorghum grain. *Nutr. Rep. Int.* **1978**, *17*, 229−236.

(4) de Lumen, B. O.; Salamat, L. A. Trypsin inhibitor activity in winged bean (*Psophocarpus tetragonolobus*) and the possible role of tannin. *J. Agric. Food Chem.* **1980**, *28*, 533−536.

(5) Sathe, S. K.; Salunkhe, D. K. Investigations on winged bean (*Psophocarpus tetragonolobus* L.): proteins and antinutritional factors. *J. Food Sci.* **1981**, *46*, 1389−1393.

(6) Rackis, J. J.; Sessa, D. J.; Honig, D. H. Flavor problems of vegetable food proteins. *J. Am. Oil Chem. Soc.* **1979**, *56*, 262−271.

(7) Borchers, R. Raw soybean meal: improved protein quality after aqueous ethanol extraction. *J. Food Biochem.* **1981**, *5*, 181−183.

(8) Grant, G.; McKenzie, N. H.; Watt, W. B.; Stewart, J. C.; Dorward, P. M.; Pusztail, A. Nutritional evaluation of soya beans (*Glycine max*): Nitrogen balance and fractionation studies. *J. Sci. Food Agric.* **1986**, *37*, 1001−1010.

(9) Hancock, J. D.; Peo, E. R.; Lewis, A. J.; Chiba, L. I.; Crenshaw, J. D. Effects of alcohol extraction and heat treatment on the utilisation of soya bean protein by growing rats and pigs. *J. Sci. Food Agric.* **1990**, *52*, 193−205.

(10) Tolman, G. H. Effect of hot aqueous ethanol treatment on antinutritional factors, protein denaturation and functional properties in raw pea and pea protein isolate. *Anim. Feed Sci. Technol.* **1995**, *56*, 159−168.

(11) Frias, J.; Hedley, C. L.; Price, K. R.; Fenwich, G. R.; Vidal-Valverde, C. Improved method of oligosaccharide analysis for genetic studies of legume seeds. *J. Liq. Chromatogr.* **1994**, *17*, 2469−2483.

(12) Sanz, M. A.; Castillo, G.; Hernández, A. Isocratic high-performance liquid chromatographic method for quantitative determination of lysine, histidine and tyrosine in foods. *J. Chromatogr. A* **1996**, *719*, 195−201.

(13) Castillo, G.; Sanz, M. A.; Serrano, M. A.; Hernández, T.; Hernández, A. An isocratic high-performance liquid chromatographic method for determining the available lysine in foods. *J. Chromatogr. Sci.* **1997**, *35*, 423−429.

(14) Pedersen, B.; Eggum, B. O. Prediction of protein digestibility by an in vitro enzymatic pH-stat procedure. *Z. Tierphysiol. Tierernaehr. Futtermittelkd.* **1983**, *49*, 265−277.

(15) McDonough, F. E.; Sarwar, G.; Steinke, F. H.; Slump, P.; García, S.; Boisen, S. In vitro assay for protein digestibility. Interlaboratory study. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 622−625.

(16) Vidal-Valverde, C.; Frias, J.; Prodanov, M.; Tabera, J.; Ruiz, R.; Bacon, J. Effect of natural fermentation on carbohydrates, riboflavin and trypsin inhibitor activity of lentils. *Z. Lebensm. Unters. Forsch.* **1993**, *197*, 449−452.

(17) Sierra, I.; Vidal-Valverde, C. Effect of ripening stage on thiamin and riboflavin levels in lupin, pea and faba bean seeds. *Z. Lebensm. Unters. Forsch.* **1998**, *206*, 126−129.

(18) Bhatty, R. S. Relationship between physical and chemical characters and cooking quality in lentil. *J. Agric. Food Chem.* **1984**, *32*, 1161−1164.

(19) Hang, Y. D.; Steinkraus, K. H.; Hackler, L. R. Comparative studies on the nitrogen solubility of mung beans, pea beans and red kidney beans. *J. Food Sci.* **1970**, *35*, 318−320.

(20) Hang, Y. D.; Wilkens, W. F.; Hill, A. S.; Steinkraus, K. H.; Hackler, L. R. Enzymatic solubilization of nitrogenous constituents of mung beans. *J. Agric. Food Chem.* **1970**, *18*, 9−12.

(21) Hang, Y. D.; Wilkens, W. F.; Hill, A. S.; Steinkraus, K. H.; Hackler, L. R. Studies on the extraction of proteins from red kidney beans. 1. Improvement in filterability of aqueous bean extract. *Cereal Chem.* **1970**, *47*, 259−265.

(22) Fan, T. Y.; Sosulski, F. W. Dispersibility and isolation of proteins from legume flours. *Can. Inst. Food Sci. Technol. J.* **1974**, *7*, 256−259.

(23) McWatters, K. H.; Holmes, M. R. Concentration on nitrogen solubility and emulsification properties of soy flour. *J. Food Sci.* **1979**, *44*, 770−773.

(24) Dench, J. E. Extraction of nitrogenous material from winged bean (*Psophocarpus tetragonolobus* L.) flour and the preparation and properties of protein isolates. *J. Sci. Food Agric.* **1982**, *33*, 173−184.

(25) Hsu, D. L.; Leung, H. K.; Morad, M. M.; Finney, P. L.; Leung, C. T. Effect of germination on electrophoretic, functional, and bread-baking properties of yellow pea, lentil and faba bean protein isolates. *Cereal Chem.* **1982**, *59*, 344−350.

(26) Schein, C. H. Solubility as a function of protein structure and solvent components. *Biotechnology* **1990**, *8*, 308−316.

(27) Mattil, K. F. The functional requirements of proteins for foods. *J. Am. Oil Chem. Soc.* **1971**, *48*, 477−480.

(28) Reddy, N. R.; Pierson, M. D.; Sathe, S. K.; Salunkhe, D. K. Chemical nutritional and physiological aspects of dry bean carbohydrates: a review. *Food Chem.* **1984**, *13*, 25−68.

(29) Vidal-Valverde, C.; Frias, J. Changes in carbohydrates during germination of lentils. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 461−464.

(30) Vidal-Valverde, C.; Frias, J.; Valverde, S. Changes in the carbohydrate composition of legumes after soaking and cooking. *J. Am. Dietet. Assoc.* **1993**, *93*, 547−550.

(31) Frias, J.; Vidal-Valverde, C.; Bakhsh, A.; Arthur, A. E.; Hedley, C. An assessment of variation for nutritional and non-nutritional carbohydrates in lentil seeds (*Lens culinaris*). *Plant Breed.* **1994**, *113*, 170−173.

(32) Frias, J.; Vidal-Valverde, C.; Kozlowska, H.; Tabera, J.; Honke, J.; Hedley, C. L. Natural fermentation of lentils. Influence of time, flour concentration and temperature on the kinetics of monosaccharides, disaccharides and α-galactosides. *J. Agric. Food Chem.* **1996**, *44*, 579−584.

(33) Chatterjee, S. R.; Abrol, Y. P. Amino acid composition of new varieties of cereals and pulses and nutritional potential of cereal-pulse combinations. *J. Food Sci. Technol.* **1975**, *12*, 221−227.

(34) Bhatty, R. S.; Slinkard, A. E.; Sosulski, F. W. Chemical composition and protein characteristics of lentils. *Can. J. Plant Sci.* **1976**, *56*, 787−794.

(35) Risterucci, A. M.; Dumas, J. C.; Gast, M.; Frangne, R.; Adrian, J. Les products alimentaires du Nord Yémen céréales et légumineuses. 1. Composition en acides aminés. *Sci. Aliments* **1989**, *9*, 583−599.

(36) Kavas, A.; Sedef, E. N. Changes in nutritive value of lentils and mung beans during germination. *Chem. Mikrobiol. Technol. Lebensm.* **1992**, *14*, 3−9.

(37) Carbonero, M.; Cappelloni, M.; Nicoli, S.; Lucarini, M.; Carnovale, E. Solubility−digestibility relationship of legume proteins. *J. Agric. Food Chem.* **1997**, *45*, 3387−3394.

(38) Bhatty, R. S.; Christison, G. I. Composition and nutritional quality of pea (*Pisum sativum L.*), faba bean (*Vicia faba* L. ssp. *minor*) and lentil (*Lens culinaris* Medik) meals, protein concentrates and isolates. *Plant Foods Hum. Nutr.* **1984**, *34*, 41−51.

(39) Lusas, E. W.; Rhee, K. C. Applications of vegetable food proteins in traditional foods. In *Plant Proteins: Applications, Biological Effects and Chemistry*; Ory, R. L., Ed.; ACS Symposium Series 312; American Chemical Society: Washington, DC, 1986; pp 32−44.
(40) Petzke, K. J.; Ezeagu, I. E.; Proll, J.; Akinsoyinu, A. O.; Metges, C. C. Amino acid composition, available lysine content and in vitro protein digestibility of selected tropical crop seeds. *Plant Foods Hum. Nutr.* **1997**, *50*, 151−162.
(41) Booth, V. H. Problems in the determination of FDNB-available lysine. *J. Sci. Food Agric.* **1971**, *22*, 558−566.
(42) FAO/WHO/UNU. *Energy and Protein Requirements*; Report of the Joint FAO/WHO/UNU Expert Consultation; Technical Report Series 724; FAO, WHO and UNU: Geneva, Switzerland, 1985.
(43) Souci, S. W.; Fachmann, W.; Kraut, H. *Food Composition and Nutrition Tables 1986/1987*; Wissenschaftliche Verlagsgesellschaft mbH: Stuttgart, Germany, 1986.
(44) Mansour, E. H.; Dworschaark, E.; Lugesi, A.; Barna, E.; Gergely, A. Nutritive value of pumpkin (*Cucurbita pepo* kakai-35) seed products. *J. Sci. Food Agric.* **1993**, *61*, 73−78.

Received for review October 26, 2000. Revised manuscript received February 1, 2001. Accepted February 1, 2001. We thank the Comisión Interministerial de Ciencia y Tecnología (Projects ALI 91-1092-C02-01 and ALI 96-0480) for the financial support provided.

JF001293I