Nutritional Assessment of Raw, Heated, and Germinated Lentils

Gloria Urbano,*,† Maria Lopez-Jurado,† Javier Hernandez,† Mar Fernandez,† Maria-Carmen Moreu,† Juana Frias,‡ Concepcion Diaz-Pollan,‡ Marin Prodanov,‡ and Concepcion Vidal-Valverde‡

Departamento Fisiologia, Instituto de Nutricion, Facultad Farmacia, Universidad de Granada, Campus Universitario de Cartuja s/n, 18071 Granada, Spain, and Instituto Fermentaciones Industriales (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain

Studies of the nutritive value of raw, dry-heated, and germinated lentils were carried out by chemical and biological assays. A methionine-supplemented lentil assay was also carried out. The germination process led to the total elimination of α-galactosides and a considerable increase of thiamin, riboflavin, and niacin content of lentils. The total starch content and the trypsin inhibitor activity were reduced by dry-heating and germination processes. The lowest rat food intake ($P < 0.05$) was observed for germinated lentils. The highest daily weight increase and PER ($P < 0.05$) were obtained for rats fed methionine-supplemented lentils and dry-heated lentils. The ADC, nitrogen balance, and percentage R/A decreased when the lentils were processed. For the methionine-supplemented lentils, the nitrogen balance was higher than for the raw lentils, although no difference was observed for the ADC.

Keywords: *Lentils; germination; methionine supplementation; dry-heat treatment; nutritive utilization*

INTRODUCTION

Lentils, as other crop legumes, are highly nutritious and provide a good source of minerals. Containing about 25% protein, 56% carbohydrate, and 1.0% fat in seeds, lentils are one of the best and cheapest sources of vegetable protein (Adsule et al., 1989). Although lentils are considered to be one of the most nutritious pulses, they contain several antinutritional factors which could limit their consumption (Liener and Kakade, 1980).

It is widely accepted that simple and inexpensive processing techniques are an effective method of achieving desirable changes in the composition of seeds by the removal of undesirable components, which is essential to improve the nutritional quality of legumes and effectively utilize their full potential as human food. In this sense, processing techniques could improve the nutritive value of lentils for animal and human consumption.

Heat treatments generally inactivate heat-sensitive endogenous legume compounds such as protein inhibitors and volatile and off-flavor compounds. Germination procedures are receiving increasing attention because of the probability that flavor and nutritional qualities may be improved, particularly through the breakdown of certain heat-state antinutrients such as phytate and flatulence factors (Deshpande et al., 1984).

There exists some information about the effect of heat treatment and germination of lentils on the nutrient content of lentils (Savage, 1988; Vidal-Valverde and Frias, 1992; Vidal-Valverde et al., 1994), but it is very scarce on the implications of these processes on their nutritive values.

The objective of the present work is to obtain, by dry-heating and germination treatments, lentil flours with fewer antinutritive factors than the raw legume and to evaluate the processed lentils by chemical and biological assays.

MATERIALS AND METHODS

Samples. Lentils, *Lens culinaris* var. Vulgaris, were purchased at a local market. The seeds were submitted to the following processes.

Heating. Raw ground lentils were dry-heated under pressure at 120 °C, 1 atm for 15 min.

Germination. Seeds (25 g) were soaked in 125 mL of distilled water at room temperature and shaken every 30 min. After 6 h, the water was drained off and the seeds were transferred to a funnel and kept at 20 °C for 6 days; 12 h of light per day was provided. Every 24 h the seeds were moistened with distilled water and carefully shaken and drained. About 99% of the seeds germinated (5–7 cm), and sprout and seed residues were ground and freeze-dried.

Chemical Analysis. *Nitrogen* was determined according to the Kjeldahl method. The protein conversion factor was 5.7.

Amino Acids. The amino acid composition of the lentils was determined by high-performance liquid chromatography (Pico-Tag method) of acid-digested samples (except for tryptophan); cysteine and methionine were analyzed after performic acid oxidation.

Moisture contents of the raw and processed lentils were obtained by drying to constant weight in a vacuum oven (20 mmHg, 35 °C).

Ether extraction was performed by gravimetry of the ethyl ether extract.

Ash was measured by calcination at 500 °C to a constant weight.

Determination of Mono- and Disaccharides and α-Galactosides. The analyses of these compounds were carried out by HPLC according to the procedure given in a previous paper (Vidal-Valverde et al., 1992).

Starch Determination. Starch was analyzed using the procedure based on total enzyme digestion to glucose (Carpita and Kabanus, 1987) as modified by Bakhsh (1991). Glucose content was measured according to the method of Dalqvist (1964). The starch content was calculated by multiplying the resulting glucose concentration value by 0.90.

† Universidad de Granada.
‡ Instituto Fermentaciones Industriales.

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Table 1. Composition of Nitrogen and Fat of Raw, Heated, and Germinated Lentils (Grams per 100 g of Dry Matter)

	RL[a]	HL[a]	GL[a]
nitrogen	4.05	4.01	4.07
protein (N × 5.7)	23.1	22.9	23.2
fat	1.30	1.28	1.32

[a] RL, raw lentils; HL, heated lentils; GL, germinated lentils.

Thiamin and Riboflavin Determination. A single extraction procedure for both thiamin and riboflavin was carried. Three grams of powered samples was extracted by acid hydrolysis with 30 mL of 0.3 M HCl in an autoclave for 15 min at 121 °C. The pH of the solution was then adjusted to 4.0–4.5 with 2 mol/L sodium acetate. After cooling, extracts were incubated with 5 mL of a 15% aqueous solution of Taka–Diastase (Serva) for 3 h at 45 °C. Afterward, samples were filtered through Whatman No. 40 filter paper, filled to 100 mL, and filtered through a 0.2 μm pore size nylon filter membrane. Thiamin was quantified by HPLC. A postcolumn derivatization was carried out according to the procedure of Wimalasiri and Wills (1985). HPLC analyses were performed using a Waters Associates chromatograph (Waters Associates, Milford, CT), equipped with both M510 and M45 Model pumps, a Rheodyne M-7125 injector (Cocati, CA), a μBondapak C_{18} column (300 × 3.9 mm i.d.), a C_{18}/Porasil B Bondapak guard column (20 × 3.9 mm i.d.), and a Waters 470 scanning fluorescence detector set at 360 nm (excitation) and 435 nm (emission) wavelengths. The detector signal was recorded on a Maxima 820 chromatography workstation (Waters Associates). The mobile phase methanol/water/acetic acid (31/68.5/0.5), containing 5 mM sodium hexanesulfonate, was pumped at a flow rate 1.5 mL/min. The column temperature was 35 °C, and the injection volume was 50 μL.

Riboflavin was determined by HPLC according to the procedure given in a previous paper (Vidal-Valverde et al., 1993).

Available Niacin Determination. The analysis of available niacin was carried out according to the procedure of Vidal-Valverde and Reche (1991).

Trypsin inhibitor activity was determined according to the method of Kakade et al. (1974), and the extraction was performed according to the method of Valdebouze et al. (1980).

Biological Methods. *Diets.* Four diets have been studied: RL, raw lentils; ML, raw lentils plus 0.5% methionine; HL, dry-heated lentils; GL, germinated lentils.

Experimental Design. The influence of lentils, raw or submitted to different treatments, on the intake of diet and on the digestive and metabolic utilization of nitrogen was studied in rats fed for 13 days with the lentil diets. A total of 40 rats were divided into 4 groups of 10 animals each. Food intake, body weight, change in body weight, intake of nitrogen, and fecal and urinary excretion of N were determined in all rats.

Animals. The animals were 4-week old (recently weaned) Wistar albino rats with an initial body weight of 55 ± 5 g, reared by University of Granada Laboratory Animal Services. The animals were divided into groups of 10 rats each (5 male, 5 female), which were housed from day 0 of the experiment in individual metabolic cages designed for the separate collection of feces and urine; the cages were located in a well-ventilated, thermostatically controlled room (21 ± 2 °C) with 12 h light/dark period (light on at 9:00 a.m.). In all of the experiments, the Thomas–Mitchell biological technique was used (Mitchell, 1923). A period of 3 days was allowed for adaptation to the diet, followed by a 10 day experimental period when feces and urine were collected on alternate days. Food intake (the total amount consumed daily by each rat was determined by weighing the amounts of diet given, refused, and spilled) and body weight were recorded at the beginning and at the end of the experimental period, that is, on days 4 and 13 of all experiments. Throughout the experimental period all rats had free access to double-distilled water. The diet was consumed *ad libitum*.

Table 2. Amino Acid (AA) Composition of Raw, Heated, and Germinated Lentils (Grams per 16 g of Nitrogen)

amino acid	RL[a]	HL[a]	GL[a]
Asp	9.94	11.43	11.97
Glu	16.3	16.04	16.40
Ser	2.86	2.69	2.75
Gly	4.43	4.52	4.56
His	1.33	1.25	1.53
Thr	2.49	2.78	2.46
Ala	2.37	3.86	3.55
Arg	3.90	4.48	3.82
Pro	2.58	6.39	2.72
Tyr	1.13	0.65	1.11
Val	3.04	2.77	3.39
Ile	4.04	3.96	4.41
Leu	7.24	7.35	8.39
Phe	5.52	6.49	6.34
Lys	4.27	5.77	5.20
Met	1.10	0.98	1.30
Cys	1.46	1.40	1.41
total branched AA	14.32	14.08	16.19
total sulfur-containing AA	2.56	2.38	2.71
total aromatic AA	6.65	7.14	7.45

[a] RL, raw lentils; HL, heated lentils; GL, germinated lentils.

Biological Indices. The following indices and parameters were determined for each group: intake (expressed as dry matter), body weight, protein efficiency ratio (PER), apparent digestibility coefficient (ADC), nitrogen retention (nitrogen balance), and percent nitrogen retention/nitrogen absorption:

$$\mathrm{PER} = \frac{\text{weight gained (g per rat per day)}}{\text{protein intake (g per rat per day)}} \quad (1)$$

$$\mathrm{ADC} = \frac{I - F}{I} \times 100 \quad (2)$$

$$\text{balance} = I - (F + U) \quad (3)$$

$$\%\mathrm{R/A} = \frac{I - (F + U)}{I - F} \times 100 \quad (4)$$

In accordance with the formulas recommended by the FAO/OMS (1966), the factors used were I (nitrogen intake), F (fecal nitrogen), and U (urinary nitrogen). Body weight and protein intakes were expressed as grams per rat per day.

Statistical Methods. Multifactor analysis of variance was applied to the data using Statgraphic Statistical Graphics 2.1 System software (Statistical Graphics Corp., Rockville, MD) with an IBM Personal System/2 Model 20 computer (International Business Machines Corp., U.K.) with a PC.

RESULTS

Chemical Analysis. Table 1 summarizes the nitrogen, protein, and fat contents in raw and processed lentil diets. The protein and fat contents did not show any changes after heating and germination procedures.

The amino acid compositions of the raw, dry-heated, and germinated lentils are shown in Table 2. It was found that 6-day germination provided an increase in branched, sulfur, and aromatic amino acids, while dry-heated lentils were deficient in those amino acids (Table 3). The chemical score shows that most of the essential amino acids have low values. To improve the obtained results on the biological assay carried out with raw lentil seeds, this legume flour was supplemented with methionine, the first limited amino acid as shown in the chemical score, and ML diet was provided.

The contents of monosaccharides, disaccharides, and oligosaccharides of the raffinose family (α-galactosides)

Table 3. Chemical Score in Raw, Heated, and Germinated Lentils

amino acid	% RL[a]	% HL[a]	% GL[a]
Phe	95.24	111.90	109.22
Tyr	26.83	15.36	26.31
Ile	61.20	60.03	66.88
Leu	82.23	83.50	95.34
Lys	66.69	90.22	81.22
Met	35.45	31.71	41.97
Cys	60.79	58.42	58.79
Val	41.73	37.96	46.49

[a] RL, raw lentils; HL, heated lentils; GL, germinated lentils.

Table 4. Available Carbohydrate and α-Galactoside Content in Raw, Heated, and Germinated Lentils (Percent of Dry Matter)

	RL[a]	HL[a]	GL[a]
available carbohydrates			
fructose	0.10 ± 0.01[a]	0.13 ± 0.09[b]	1.06 ± 0.04[c]
glucose	ND[b]	ND	0.44 ± 0.03
sucrose	1.32 ± 0.05[a]	1.60 ± 0.07[b]	1.38 ± 0.06[c]
total starch	48.68 ± 0.83[a]	38.63 ± 0.63[b]	43.12 ± 0.93[c]
α-galactosides			
raffinose	0.21 ± 0.01[a]	0.21 ± 0.01[a]	ND
ciceritol	0.94 ± 0.03[a]	0.80 ± 0.06[b]	ND
stachyose	2.07 ± 0.07[a]	2.09 ± 0.20[a]	ND

[a] RL, raw lentils; HL, heated lentils; GL, germinated lentils. Values are the mean of six determinations ± standard deviation. The same superscript in the same row indicates no significant differences ($P \leq 0.05$). [b] ND, not detected.

in raw lentils and after dry-heating at 120 °C and 1 atm of pressure and germination treatments are collected in Table 4. In raw lentil seeds, stachyose was found in the highest content (2.1%) and sucrose and ciceritol were in lesser amounts (1.3 and 0.9%, respectively). Raffinose and fructose were presented in small amounts (0.2 and 0.1%, respectively). In lentil seed the presence of the monosaccharide glucose was not detected (Table 4). These data show that the α-galactoside compounds related to flatus production constitute 69% of the total soluble carbohydrates in the raw seed. When the lentil seeds were submitted to dry heat in an autoclave, a slight decrease in the total α-galactosides content was observed (3.7% of losses), because this treatment caused a significant decrease in the ciceritol content. This heat treatment brought about an increase of the total soluble carbohydrates, mainly due to the increase observed for sucrose after dry-heating, while no modification was observed for the disaccharide fructose. Germination for 6 days also modified the amount of the soluble carbohydrates, and the total elimination of the α-galactosides was observed. Fructose increased by 1% during germination, and this process did not modify significantly the sucrose content. However, while glucose was detected in the germinated lentil seeds in relatively high amount (0.1%), monosaccharide was not detected in the raw material.

The content of total starch in raw and processed lentils is also shown in Table 4. In raw lentil the total starch content was 49%, and as a consequence of heat treatment a 21% decrease of the total starch content was observed. A lesser reduction in the starch content (11%) was observed after germination treatment.

In Table 5 we have summarized the content of thiamin, riboflavin, and available niacin of raw and processed lentils. We observe that the pressure heating affected slightly by significantly ($P \leq 0.05$) the content of riboflavin and thiamin of raw lentils. The available niacin of lentils increased after heating, but this increase was not statistically significant ($P \leq 0.05$).

Table 5. Content in Thiamin, Riboflavin, and Available Niacin of Raw, Heated, and Germinated Lentils (Milligrams per 100 g of Dry Matter)

	RL[a]	HL[a]	GL[a]
thiamin	0.24 ± 0.002[a]	0.23 ± 0.005[b]	0.28 ± 0.03[c]
riboflavin	0.09 ± 0.004[a]	0.06 ± 0.005[b]	0.21 ± 0.007[c]
niacin	1.29 ± 0.032[a]	1.35 ± 0.026[a]	1.38 ± 0.034[b]

[a] RL, raw lentils; HL, heated lentils; GL, germinated lentils. Values are the mean of six determinations ± standard deviation. The same superscript in the same row indicates no significant differences ($P \leq 0.05$).

Table 6. Trypsin Inhibitor Activity of Raw, Autoclaved, and Germinated Lentils (TIU per Milligram of Dry Matter)

	RL[a]	HL[a]	GL[a]
TIA	4.65 ± 0.13[a]	1.97 ± 0.11[b]	3.65 ± 0.32[c]

[a] RL, raw lentils; HL, heated lentils; GL, germinated lentils. Values are the mean of six determinations ± standard deviation. The same superscript in the same row indicates no significant differences ($P \leq 0.05$).

Table 7. Food Intake and Weight Change in Rats

diet	daily wt,[a] g/rat	daily dry matter intake,[a] g/rat	daily protein intake,[a] g/rat	PER[b]
RL[c]	0.67 ± 0.2[a]	9.14 ± 0.5[a]	2.2 ± 0.1[a]	0.30 ± 0.07[a]
ML[c]	1.10 ± 0.2[b]	9.11 ± 0.8[a]	2.1 ± 0.2[a]	0.52 ± 0.1
HL[c]	0.96 ± 0.4[b]	9.11 ± 0.4[a]	2.1 ± 0.1[a]	0.46 ± 0.2
GL[c]	0.58 ± 0.2[a]	8.38 ± 0.5	2.1 ± 0.2[a]	0.35 ± 0.1[a]

[a] The same superscript in the same column indicates no significant differences ($P \leq 0.05$). Values are means ± SEM of 10 Wistar rats. [b] PER = wt gained (g per rat per day)/protein intake (g per rat per day). [c] RL, raw lentils; ML, methionine supplemented lentils; HL, heated lentils; GL, germinated lentils.

Table 8. Digestive Utilization

diet	daily nitrogen intake,[a] mg/rat	daily total fecal nitrogen,[a] mg/rat	daily absorbed nitrogen,[a] mg/rat	ADC[b]
RL[c]	354.2 ± 18.7[a]	86.43 ± 15.5[a]	267.7 ± 19.7[a]	75.57 ± 4.1[a]
ML[c]	340.0 ± 14.9[a]	75.20 ± 11.2[a]	265.0 ± 11.5[a]	77.75 ± 2.5[a]
HL[c]	336.6 ± 13.2[a]	95.78 ± 10.2[b]	240.7 ± 8.6[b]	71.53 ± 2.3[b]
GL[c]	341.0 ± 21.4[a]	95.22 ± 0.1[b]	245.8 ± 17.0[b]	72.09 ± 2.3[b]

[a] The same superscript in the same column indicates no significant differences ($P \leq 0.05$). Values are means ± SEM of 10 Wistar rats. [b] ADC = [(nitrogen intake − fecal nitrogen)/nitrogen intake] × 100. [c] RL, raw lentils; ML, methionine supplemented lentils; HL, heated lentils; GL, germinated lentils.

Germination produced a significant increase ($P \leq 0.05$) of thiamin, riboflavin, and niacin content.

The trypsin inhibitor activity (TIA) of the analyzed lentil samples is shown in Table 6. Heat treatment in dry conditions produced a 58% elimination of TIA in lentils. The effect of germination for 6 days on the TIA is also collected in Table 6. The TIA decreased 22% after germination.

Biological Analysis. The results obtained by biological analysis (Tables 7–9) indicate the daily food intake, expressed as grams per rat per day (Table 7), was not modified either when methionine addition was provided to the raw lentils diet (ML) or when the dry-heated was applied (HL) but decreased slightly when the germination procedure was performed on the raw lentil seeds, compared to that obtained from the raw lentils diet (RL).

Daily weight increases were significantly greater in rats fed with methionine-supplemented lentils (ML) and

Table 9. Metabolic Utilization

group	daily total nitrogen urinary,[a] mg/rat	balance[b]	%R/A[c]
RL[d]	216.4 ± 28.9	51.3 ± 6.6	19.1 ± 8.4
ML[d]	190.8 ± 5.6	75.1 ± 3.7	28.3 ± 1.9
HL[d]	207.0 ± 25.1	33.6 ± 24.3	13.9 ± 9.9
GL[d]	235.5 ± 22.8	10.3 ± 16.9	4.2 ± 7.1

[a] The same superscript in the same column indicates no significant differences ($P \leq 0.05$). Values are means ± SEM of 10 Wistar rats. [b] Balance = nitrogen intake − (fecal nitrogen + urinary nitrogen). [c] %R/A = [balance/(nitrogen intake − fecal nitrogen)] × 100. [d] RL, raw lentils; ML, methionine-supplemented lentils; HL, heated lentils; GL, germinated lentils.

dry-heated lentils (HL) in comparison with the other experimental diets. When body weight was calculated as grams increase per gram of protein consumed (PER), we found similar PER values for rats fed with raw lentils (RL) and germinated lentils (GL), and increases in body weight were significantly greater after feeding methionine-supplemented lentils (ML) and dry-heated lentils (HL), although values were greater for rats fed with ML.

The digestive utilization of nitrogen with the different diets is shown in Table 8. The methionine supplementation did not improve the absorption of nitrogen, accounted as apparent digestibility coefficient (ADC). These coefficients for dry-heated and germinated lentil diets were significantly lower than that for rats fed the raw lentils diet.

The metabolic nitrogen utilization for the different diets is shown in Table 9. It was found that the nitrogen balance was higher when rats were fed with methionine-supplemented lentils. Dry-heat treatment showed significantly lower values for nitrogen balance that were even lower for rats fed with germinated lentils. The same results were obtained on percentage of nitrogen retention/nitrogen absorption (%R/A).

DISCUSSION

The influence of lentil processing on carbohydrate content is summarized in Table 4. According to these results we observe that the carbohydrate fraction of lentil was affected by treatment. In the germination process the changes observed are considered to be consequences of the metabolic reaction undergone in the seeds during germination. The oligosaccharides of the raffinose family are secondary metabolites constructed during maturation of the seed, and the kinetics of their synthesis is typical of reverse substances (Kandler and Holf, 1980; Dey, 1980); the oligosaccharides play an important role in the viability of the seed in cold conditions (Castillo et al., 1990). During germination, these compounds are hydrolyzed and utilized as a source of energy, and it has been observed that the levels of the enzymes α-D-galactosidase and β-D-fructofuranosidase increased (Dey, 1980). At the same time, large amounts of fructose, glucose, sucrose, and galactose might be expected. Our results confirm also this hypothesis, where the monosaccharides glucose and fructose and the disaccharide sucrose increase significantly during germination. According to Dey (1980) these sugars that are liberated during germination are rapidly metabolized, and D-galactose, especially, is hardly detectable. This is in good agreement with our results for lentils, in which, under the conditions of germination employed, the oligosaccharides of the raffinose family were metabolized and either galactose or α-galactosyl sugars were detected. After 6 days of germination, a large amount of fructose and glucose, important monosaccharides during development of the seed, was detected.

Changes in low molecular weight carbohydrates during germination of legumes have been studied by many researchers. All of them agree that α-galactosides of the raffinose family decline rapidly during the first days of germination, while glucose and fructose increase. An increase in reducing sugars and a decrease in total sugars and starch were observed in germinated amphidiploids (black gram × mung bean) (Kataria et al., 1990) and in lima beans (Ologhobo and Fetuga, 1986). Vidal-Valverde and Frias (1992) observed that germination of lentils for 6 days produced the total elimination of α-galactosides and an increase of fructose and glucose, while sucrose slightly decreased. Trugo et al. (1990) noted that germination of black beans resulted in a progressive decrease in α-galactosides, with a 77% loss after the third day, which was efficient at reducing flatulence in humans. These results show germination may be an optional technique to reduce the content of α-galactosides of the raffinose family, compounds related to flatus production. After germination, we observed an 11% decrease in starch content. Several authors have found increased activity of α-amylase and β-amylase enzymes after germination (Subbulkshmi et al., 1976; Morad et al., 1980). Other authors also observed a decrease of total starch during germination of different legumes (Hsu, 1981; Jood et al., 1986; Ologobo and Fetuga, 1986; Ndzondzi-Bokouango et al., 1989). During germination of lentil seeds carried out in darkness for 6 days Vidal-Valverde and Frias (1992) observed a decreased of total starch; however, the ratio of digestible starch to total starch was considerably higher in the germinated than in the ungerminated lentil.

After heating treatment, the available carbohydrates decrease (Table 4). These results show that pressure heating could modify highly the structure of the starch granule. This behavior was also observed by Jood et al. (1986) in *Vicia faba* and by Ologobo and Fetuga (1988) in *Phaseolus lunatus*.

The decrease of thiamin and riboflavin content after heating treatment of lentils (Table 5) is lower than the values found in the literature for normal cooking of legumes (Savage, 1988; Augustin and Klein, 1989). The niacin content of lentils increased after heating treatment, which is due to the hydrolysis of bound forms of niacin (Chatuverdi and Gearvani, 1986; Carter and Carpenter, 1982; Vidal-Valverde and Reche, 1991).

The nutritional composition of sprouted legumes is extremely wide and contributes to a number of factors affecting the sprouting process such as time, temperature, and the presence or absence of light (Augustin and Klein, 1989). The germination process carried out in our laboratory increased the amount of thiamin, riboflavin, and niacin. These results are in accordance with those obtained by other authors (Hsu et al., 1981; Vanderstoep, 1981).

Trypsin inhibitor activity was affected by heating and germination procedures (Table 6). The effect of heating depends, according to the literature, on the conditions under which this process is carried out, such as intensity of heat and duration of the treatment. Griffiths (1984) observed that TIA in faba bean was stable in a range of temperatures between 60 and 100 °C, but the activity of this inhibitor disappeared after 10 min. Kozlowska

et al. (1990a,b) observed the elimination of this activity in faba beans after autoclaving. In lentils, Sohonie and Bhanderkar (1955) reported the total elimination of TIA using dry heat for 30 min, and Batra et al. (1986) found similar results when they autoclaved lentils at 121 °C for 20 min.

Many different results regarding the effect of germination on TIA of legumes have been reported in the literature, but there is little information about the effect of germination on TIA in lentils. El-Mahdy et al. (1985) observed a marked reduction in TIA after 24 h of germination and then the rate decreased, which may indicate that these compounds may be utilized in the first stage of germination as a source of energy. However, Batra et al. (1986) found that germination for 3 days decreased TIA only slightly, while 6 day germination lowered it substantially (21–54%). More recently, Weder and Link (1993) observed that 72 h of sprouting did not alter the total inhibitor content of lentils, and Vidal-Valverde et al. (1994) observed a 24 and 28% reductions in TIA after 6 days of germination in darkness of two varieties of lentils (*L. culinaris* var. Vulgaris and var. Variabilis, respectively). The results of the present work show a 22% reduction in TIA after this period, a difference that is between the ranges shown in the literature and also due to the characteristics of the lentil genotype. These results showed a substantial decrease of TIA after 6 days of germination, which could indicate that these compounds may be utilized during the germination of the seed as a energy source.

Chemical Analysis of Protein. The amino acid content in raw lentil seeds is not adequate to cover the requirements of growing Wistar rats; it is lacking in sulfur amino acids (Nestares et al., 1993) (Tables 2 and 3). The germination procedure brought about an increase of free amino acids, which is in agreement with information reported by other authors (Ndzondzi-Bokouango et al., 1989).

The proportion of essential amino acids was 42–45%, which is apparently high according to Santidrian (1987). However, the obained data did not reflect a good nutritional protein quality because the presence and proportion of amino acids were not appropriate.

The essential/nonessential amino acid ratios (E/N) were 0.73 for raw lentils, 0.84 for dry-heated lentils, and 0.74 for germinated lentils, data lower than those recommended to grow rats by Stucki and Harper (1962), who reported optimal growth after weaning at values for E/N of 4:1. These findings suggest that slower growth would be predicted in young rats fed raw and processed lentil diets than in those fed with a control diet (casein plus methionine), as was previously reported by Nestares et al. (1993). The higher E/N values were obtained for animals fed with dry-heated lentils and coincided with increased-weight animals, as is shown by comparison to animals fed the raw lentil diet. However, the highest increased weight was obtained for animals fed with methionine-supplemented lentils, which did not coincide with the highest E/N value (similar to that for rats fed with raw lentils). This item could be explained because the analytical methods used did not take into account amino acid imbalances or differences in their rates of absorption.

Food Intake. Modifications obtained on carbohydrates (ciceritol and starch decrease and increase on sucrose and fructose) when lentil seeds were dry-heated did not improve the food intake. Germination brought about the total removal of α-galactosides and a great increase of fructose and sucrose, and losses on the food intake were observed. This fact cannot be assigned to the amino acid imbalance as reported by Leung and Rogers (1969). Tyrosine, the amino acid connected to serotonin and catecholamine synthesis, which are related to intake regulation, did not change after germination, and the other amino acids increased compared to the raw and dry-heated lentil diets. Vidal-Valverde et al. (1994) noted a high increase of tannins and catechins after germination of lentils which could be linked to the decrease in the rat food intake because of the precipitation of saliva proteins, reducing the lubricant properties connected to dry feeling in the mouth and low food swallowing. Phenolic compounds are related to bitter taste, which could be a cause for low food intake (Griffiths and Mosely, 1980).

Protein Absorption Capacity. The results obtained for digestive protein utilization for raw lentil diets ranged between the data recorded in legumes (Patwardhan, 1962). The protein absorption capacity in lentils is assessed by different endogenous characteristics such as protein quality, trypsin inhibitor activity, and phenolic compound content. During the experimental conditions carried out in this work the methionine-supplemented lentils did not improve the ADC as was expected following the advice given by Heger et al. (1988), possibly due to the presence of multiple endogenous antinutritive compounds in lentil flour. Dry-heating at 120 °C for 15 min was not sufficient to remove the total content of these compounds. Nevertheless, a 58% decrease in TIA with heat treatment compared to the values for raw lentils and a significant decrease in ADC in rats fed with dry-heated lentil diets were observed due mainly to a fecal nitrogen loss (Longstaff and McNab, 1991). These results agree with those reported by Bressani and Elias (1974) and could be likely a consequence of heat treatment which yields, as has been demonstrated for soy, a depletion of the protein quality due to cystine and methionine decreases, amino acids which are deficient in lentils, to higher lentil protein resistance to the digestive enzymes, and/or to the increase observed in the tannin content after the heat process. Phenolic compounds may be linked to trypsin enzyme giving a protein–tannin complex and making the enzyme inactive to the protein hydrolysis. It has been also reported that tannins could interact with diet proteins interfering with their metabolic absorption and utilization (Mole, 1989). This fact is especially important for sulfur amino acids, the absorption of which could be curtailed by the presence of tannins. In faba beans Alzueta et al. (1992) noted a high endogenous nitrogen excretion, which could be similar to the fecal nitrogen loss observed in the present work.

The germination procedure also produced a decrease in ADC and an increase in total fecal loss similar to those observed for rats fed with heated lentil diet, although a 22% decrease in TIA was obtained after germination. It is possible that such TIA reduction was not sufficient to improve the protein absorption together with the negative effect caused by tannins, as has been discussed above.

Metabolic protein utilization has been studied as a nitrogen balance function, and it was found that this index showed a rise when rats were fed with methionine-supplemented lentil (ML) diet and a decrease when rats were fed with dry-heated lentil (HL) and germinated lentil (GL) diets in comparison with the values

obtained for raw lentil diets. It should be emphasized that the group of animals fed with the methionine-supplemented lentil diet had a food intake and protein digestible utilization similar to those of rats fed with raw lentil diet, but the obtained PER for the former was significantly superior to the PER for rats fed with raw lentils, which is in relation with the increase observed on the nitrogen balance and agrees with the results reported by Marquard and Campbell (1975), who noted growth and food intake/input body weight improved.

Nitrogen utilization as a growth parameter was proportionally better in the group of animals fed with HL, for which a significant increase of PER was observed compared to that for the group fed raw lentils, although the food intake was very close for both diets. It is difficult to explain why the highest rat growth was obtained when rats were fed with HL from the observed nitrogen balance.

The lentil germination procedure caused a rise of the essential amino acid content (Table 2), but a nitrogen retention decrease was observed, together with a %R/A decrease. In this case it was not possible to find an explanation to justify the PER values obtained from the nitrogen balance, mainly due to the poor protein quality of nitrogen that is lost in the urine and is not utilized for animal growth.

For both groups of animals fed with dry-heated lentil and germinated lentil diets the increased body weight observed may be due to the increased fat body accumulation. It has been suggested (Campbell and Dunkin, 1983; Coyer et al., 1987; McLeond, 1990) that the net efficiency of the energy utilization is affected by the diet protein quality and quantity in such a way that reducing the diet protein level and changing the amino acid composition may yield a decrease of partial energetic efficiency of protein decomposition and a rise in body fat accumulation.

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