Natural Fermentation of Lentils. Influence of Time, Flour Concentration, and Temperature on the Kinetics of Monosaccharides, Disaccharide, and α-Galactosides

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Lentil seeds (*Lens culinaris* var. Vulgaris cv. Magda 20) were left to ferment naturally at different initial lentil flour concentrations (79, 150, and 221 g/L) and different temperatures (28, 35, and 42 °C). During fermentation, samples were taken at 24 h intervals. Changes in pH, titratable acidity, monosaccharides (fructose and glucose), disaccharide (sucrose), and α-galactosides (raffinose, ciceritol, and stachyose) were investigated. A large decrease in pH was observed after 24 h of fermentation, which continued more gradually in every experiment for 96 h. This drop in pH was accompanied by a rapid increase of titratable acidity. During the preparation of the lentil flour suspension the initial concentration of the lentil flour−water suspension had an important influence on the levels of fructose, glucose, sucrose, and α-galactosides, while temperature had a minor effect. Once fermentation occured, it was observed that both flour concentration and temperature modified the soluble sugar content and that the higher the initial flour concentration the greater the sugar content and the higher the temperature the greater the decrease in soluble sugar content.

Keywords: *Natural fermentation; lentils; soluble sugars; flatulence-causing oligosaccharides; raffinose; ciceritol; stachyose*

INTRODUCTION

The shortage of and continuous rise in the cost of animal products, especially in developing countries, have prompted food technologists and nutritionists to search for alternative sources of food. This problem is likely to become even more important since the world population will increase to about 6.4 billion by the year 2000, with 90% of this increase occurring in the poorest countries. Since canned and frozen foods are unavailable or too expensive for hundreds of millions of the world's economically deprived and hungry population, fermentation remains one of the most practical methods of food preservation. It also often enhances the organoleptic and nutritional qualities of food. Lactic acid fermentation is used as a major method for processing and preserving vegetables, cereals, and legumes throughout the world and particularly in developing countries. It is a desirable method for processing and preserving food because of its low cost, low energy requirements, and high yield with acceptable and diversified flavors for human consumption.

Dry lentils are a good source of high-quality protein, vitamins, and a balanced range of minerals. They are also an excellent source of complex carbohydrates and dietary fiber (Adsule et al., 1989). In spite of the great importance of lentil seeds to local populations, the crop has been often relegated to marginal areas where it is grown without the benefit of fertilizers, herbicides, pesticides, or irrigation. The crop does have the advantage of all legumes in that it forms a symbiotic relationship with *Rhizobium* which fixes nitrogen from the atmosphere.

Research on improving the lentil crop has been minimal until the recent establishment of international agricultural research centers and, in particular, the International Center for Agricultural Research in the Dry Areas (ICARDA) in 1978. The mandate of ICARDA for lentil crop improvement resulted in a 72% increase in the world production of lentils during the 1980s (Oram and Agcaoili, 1994).

Lentil seeds are very acceptable in most countries and are not restricted by any religious code; they also provide one of the best means of combating malnutrition among people in developing countries (Savage, 1988). In addition, the protein fraction consists mainly of globulins and albumins, which makes it suitable for production of food for coeliac patients, and the absence of lactose makes it suitable for lactose-intolerant people.

A reduction in the consumption of legumes in general, and lentils in particular, in a number of countries has been related to their ability to cause flatulence, which results in serious discomfort in man. The enzyme α-(1−6)-galactosidase, which cleaves the galactose linkages, is not present in the human intestinal mucosa, and the unhydrolyzed sugars, therefore, are not absorbed by the intestinal wall. The unhydrolyzed oligosaccharides pass into the large intestine where they are fermented anaerobically to produce carbon dioxide, hydrogen, and traces of methane (Calloway et al., 1971). Fleming (1981) reported positive correlations between hydrogen production and stachyose and raffinose using expired hydrogen from rats as a measure of flatus

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Table 1. Experimental Conditions for the Selected Natural Fermentation Batches of Lentil

batch	temp (°C)	concn (g/L)	batch	temp (°C)	concn (g/L)
BH1	28	79	BH9	35	150
BH2	42	79	BH10	35	150
BH3	28	221	BH12	35	150
BH4	42	221			

formation. Nowak and Steinkraus (1988) reported that stachyose was the highest gas precursor in pea flour, while gas production from raffinose was negligible.

It has been reported that fermentation causes a general improvement in the nutritional value of legumes (Zamora and Fields, 1979a; Akpapunam and Achinewhu, 1985), brings about desirable changes in taste and texture, and may result in the breakdown of some of the antinutritional endogenous compounds. In general, fermentation of legumes leads to an improvement in protein quality and availability, increased palatability, reduced phytic acid and flatulence-causing oligosaccharides, increased levels of B vitamins, increased shelf life, and the removal of toxic compounds (Hesseltine, 1983; Bressani, 1983; Vidal-Valverde et al., 1993). Natural fermentation of lentils produces an increase in lentil PER from 0.79 in raw seeds to 1.22 in fermented material. In addition, it has been shown to significantly increase the availability of methionine and to decrease the phytic acid content by 45% after 3 days of fermentation, without change in the total phosphorus content (Shekib, 1988). Natural lactic acid fermentation increases the total amino acid content and improves the *in vitro* protein digestibility of lentils, and products prepared from fermented lentil flour have high consumer acceptability (Ragaee et al., 1986a,b). Nowak and Steinkraus (1988) reported that gas production was inhibited by fermentation. In a clinical trial, Jha and Verna (1980) observed that fermented pea flour caused no accumulation of gas in the abdominal region of patients.

We have shown previously that natural fermentation of lentils carried out for 96 h at 30 °C and a concentration of 100 g of lentil flour/L of water produced an increase in the riboflavin content and in the ratio of available starch to total starch. The trypsin inhibitor activity, however, was highly reduced, and the α-galactosides could no longer be detected (Vidal-Valverde et al., 1993). In the current study we have tried to optimize the conditions for natural fermentation (time, temperature, and concentration of flour) to remove antinutritional compounds, in particular, the effect on mono- and disaccharides and the α-galactosides.

MATERIALS AND METHODS

Fermentation. Lentil seeds (*Lens culinaris* var. Vulgaris cv. Magda 20, from Albacete, Spain), harvested in 1991, were finely ground in a ball mill and sieved, and the 0.050−0.250 mm was fraction collected. The flour was suspended aseptically in tap water at the concentrations and temperatures shown in Table 1, which were obtained from a 2^2 complete factorial design with three replicate centerpoints (Bayne and Rubin, 1986). The flour was allowed to ferment naturally for 4 days without aeration in a stirred fermentor (Infors ISF-100, Infors AG, Switzerland), using only the microorganisms present on the seeds. Zero time was assigned to when the flour was completely suspended under stirring at the controlled temperature. This was 10−40 min after the mixture was placed into the fermentor. Samples were collected daily and freeze-dried for later analysis.

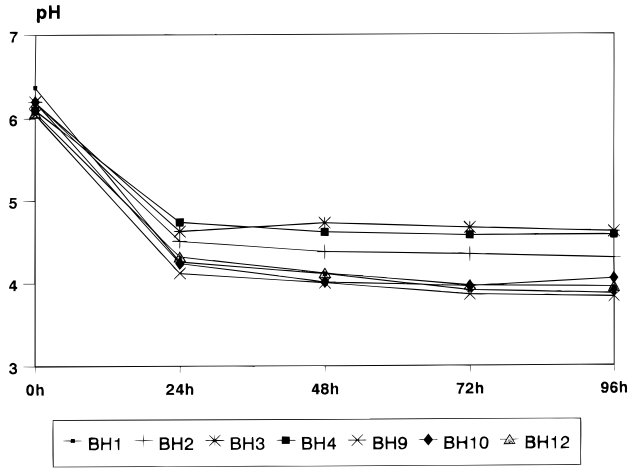

Figure 1. Changes in pH during natural fermentation of lentils.

pH and Titratable Acidity. Changes in the pH during the fermentation process were measured using an Ingold Model 465 pH meter, and titratable acidity, expressed as percentage of lactic acid, was determined by titration with 0.1 N NaOH.

Determination of Mono- and Disaccharides and α-Galactosides. Fifty milligrams of dry sample was suspended in 5 mL of 80% ethanol, boiled under reflux for 15 min, cooled, and then centrifuged at 5000 rpm. The residue was extracted twice more and then washed with distilled water until no carbohydrate was detected (Molisch's test; Pearson, 1975). The supernatants were combined and concentrated in a Büchner vortex evaporator, and the residue was used for chromatographic analysis using a HPAC-PAD system, as described by Frias et al. (1994a).

Statistical Analysis. Multifactor analysis of variance was applied to the data using Statgraphics Statistical Graphics System software ver. 5.

RESULTS

Figure 1 shows the pH values recorded throughout the natural fermentation of lentils at different temperatures and concentrations of flour. The pH fell sharply after 24 h from 6.4 to between 3.8 and 4.6. After this time, the pH of most of the fermenting samples fell gradually to pH 4 up to 72 h, and the pH values did not change significantly up to 96 h. The BH3 and BH4 treatments, however, in which the initial flour concentration was 221 g/L, showed a significant fall to pH 4.6 by 24 h but then showed little further changes up to 96 h. A similar but less significant effect was seen when the temperature of the fermentation was high (42 °C).

Table 2 shows changes of titratable acidity, expressed as grams of lactic acid per 100 g of dry matter. In each treatment, the titratable acidity increased rapidly for the first 24 h; this rate decreased up to 72 h and then increased slowly or became stabilized up to 96 h. At a similar flour concentration, high temperature (42 °C) gave higher titratable acidity (BH2, BH4) than low temperature (BH1, BH3). The three centerpoints maintained at 35 °C, (BH9, BH10, BH12) gave intermediate levels of acidity. Treatments with the lowest concentration (79 g/L) produced the highest titratable acidity (9.1−12.8 g of lactic acid/100 g of lentil dry weight), followed by the three centerpoints (9−10 g of lactic acid/100 g), with highest flour concentration (221 g/L) giving the lowest lactic acid values (6.6−6.8 g of lactic acid/100 g). The change in titratable acidity between 0 and 96 h was greater at the lowest flour concentration and the highest temperature (BH2).

Table 2. Titratable Acidity (Grams of Lactic Acid/100 g of Lentil Dry Weight) during Natural Fermentation of Lentils[a]

treatment	raw lentil	0 h	24 h	48 h	72 h	96 h
BH1 (79 g/L, 28 °C)	1.14 ± 0.01^{a}	1.17 ± 0.01^{ab}	5.43 ± 0.01	7.36 ± 0.01	8.35 ± 0.26	9.12 ± 0.01^{b}
BH2 (79 g/L, 42 °C)	1.14 ± 0.01^{a}	1.90 ± 0.01	9.70 ± 0.01	11.36 ± 0.01	13.14 ± 0.01	12.78 ± 0.01
BH9 (150 g/L, 35 °C)	1.14 ± 0.01^{a}	1.35 ± 0.01^{c}	6.23 ± 0.06^{b}	7.45 ± 0.06^{b}	9.17 ± 0.13	9.57 ± 0.01
BH10 (150 g/L, 35 °C)	1.14 ± 0.01^{a}	1.38 ± 0.01^{c}	6.63 ± 0.01	8.70 ± 0.01	$10.06 \pm 0.06_{1}$	$10.17 \pm 0.12_{1}$
BH12 (150 g/L, 35 °C)	1.14 ± 0.01^{a}	1.36 ± 0.01^{c}	6.15 ± 0.06^{b}	7.45 ± 0.01^{b}	8.72 ± 0.01	9.17 ± 0.01^{b}
BH3 (221 g/L, 28 °C)	1.14 ± 0.01^{a}	1.21 ± 0.04^{b}	4.00 ± 0.08	6.01 ± 0.01	6.76 ± 0.01	6.60 ± 0.01
BH4 (221 g/L, 42 °C)	1.14 ± 0.01^{a}	1.48 ± 0.01	5.25 ± 0.01	6.13 ± 0.01	5.99 ± 0.01	6.78 ± 0.01

[a] Values are the mean of two determinations ± standard error. Same superscript/subscript in the same column/row indicates no significant differences ($P \leq 0.05$).

Table 3. Changes in Fructose, Glucose, and Sucrose during Natural Fermentation of Lentils (Percent Dry Weight)[a]

batch	fructose	glucose	sucrose
raw lentil	0.08 ± 0.01	ND[b]	2.94 ± 0.03^{a}
BH1 (79 g/L, 28 °C)	0.15 ± 0.01	0.07 ± 0.01^{a}	2.43 ± 0.01^{b}
0 h	0.34 ± 0.01	0.18 ± 0.01	2.21 ± 0.01
24 h	0.46 ± 0.02	0.11 ± 0.01	0.19 ± 0.01
48 h	0.59 ± 0.02	0.03 ± 0.01	0.12 ± 0.01
72 h	0.88 ± 0.01	ND	ND
96 h			
BH2 (79 g/L, 42 °C)	0.16 ± 0.01	0.07 ± 0.01^{a}	2.46 ± 0.03^{b}
0 h	0.60 ± 0.01	0.33 ± 0.01	1.61 ± 0.01
24 h	1.07 ± 0.01	0.28 ± 0.01	0.12 ± 0.01
48 h	1.20 ± 0.02	0.05 ± 0.01	ND
72 h	1.32 ± 0.01	ND	ND
96 h			
BH3 (221 g/L, 28 °C)	0.45 ± 0.01	0.17 ± 0.01	3.97 ± 0.03^{g}
0 h	0.72 ± 0.01	0.52 ± 0.01	3.79 ± 0.03
24 h	1.41 ± 0.01	0.27 ± 0.01	0.23 ± 0.01
48 h	2.36 ± 0.02	0.09 ± 0.01	0.06 ± 0.01^{h}
72 h	2.39 ± 0.01	ND	ND
96 h			
BH4 (221 g/L, 42 °C)	0.50 ± 0.01	0.16 ± 0.01	3.99 ± 0.05^{g}
0 h	0.84 ± 0.01	0.64 ± 0.01	3.08 ± 0.03
24 h	1.76 ± 0.01	0.32 ± 0.02	0.14 ± 0.01
48 h	2.45 ± 0.01	0.11 ± 0.01	0.06 ± 0.01^{h}
72 h	2.54 ± 0.01	ND	ND
96 h			
BH9 (150 g/L, 35 °C)	0.30 ± 0.01^{a}	0.09 ± 0.01^{b}	2.84 ± 0.01^{c}
0 h	0.50 ± 0.01^{b}	0.40 ± 0.01	2.41 ± 0.01^{d}
24 h	0.89 ± 0.02^{c}	0.21 ± 0.01	0.21 ± 0.01^{e}
48 h	1.38 ± 0.01^{de}	0.07 ± 0.01^{e}	0.04 ± 0.01^{f}
72 h	1.45 ± 0.01^{g}	ND	ND
96 h			
BH10 (150 g/L, 35 °C)	0.29 ± 0.01^{a}	0.10 ± 0.01^{b}	2.91 ± 0.02^{a}
0 h	0.50 ± 0.01^{b}	0.39 ± 0.01^{c}	2.41 ± 0.01^{d}
24 h	0.86 ± 0.02	0.23 ± 0.01^{d}	0.19 ± 0.01^{e}
48 h	1.39 ± 0.01^{d}	0.08 ± 0.01^{e}	0.04 ± 0.01^{f}
72 h	1.45 ± 0.02^{g}	ND	ND
96 h			
BH12 (150 g/L, 35 °C)	0.29 ± 0.01^{a}	0.10 ± 0.01^{b}	2.84 ± 0.02^{c}
0 h	0.50 ± 0.01^{b}	0.38 ± 0.01^{c}	2.42 ± 0.02^{d}
24 h	0.88 ± 0.01^{c}	0.23 ± 0.01^{d}	0.21 ± 0.01^{e}
48 h	1.36 ± 0.01^{e}	0.07 ± 0.01^{e}	0.03 ± 0.01^{f}
72 h	1.45 ± 0.01^{g}	ND	ND
96 h			

[a] Values are the mean of five determinations ± standard deviation. The same superscript in the same column means no significant differences ($P \leq 0.05$). [b] Not detected.

Tables 3 and 4 show the changes in fructose, glucose, raffinose, ciceritol, stachyose, and total α-galactosides during fermentation. Fructose, sucrose, raffinose, ciceritol, and stachyose were all present in the dry flour, with the α-galactosides making up 68% of the total soluble carbohydrates. During fermentation the fructose content in each treatment increased significantly from the very low level found in the dry seed (0.1%). The increase occurred in proportion to the temperature and the flour concentration; the maximum fructose level was found at 221 g/L and 42 °C (BH4, 2.5%) and the minimum at 79 g/L and 28 °C (BH1, 0.9%).

On the other hand, glucose was undetectable in the dry lentils but was present at the beginning of fermentation at 0 h at levels ranging from 0.07% to 0.17%. The content of glucose at 0 h was related to the flour concentration but appeared not to be affected by temperature. In all treatments the maximum glucose level was recorded 24 h after fermentation had started. The highest glucose levels (0.5% and 0.6%) were found in BH3 and BH4 treatments (221 g/L). Temperature strongly affected the glucose content, the highest temperature (42 °C) producing the largest glucose content. The glucose contents of the treatments carried out at 150 g/L and 35 °C (BH9, BH10, BH12) were similar (0.4%). After 24 h, the glucose content started to gradually decrease, and it was not detectable after 96 h.

The flour concentration had a significant effect on the sucrose levels present in solution during the sample preparation prior to 0 h. Treatments containing 79 g/L (BH1, BH2) showed a decrease in sucrose content from 2.9% to 2.5%; at 150 g/L (BH9, BH10, BH12) the sucrose content decreased slightly, from 3% to 2.8−2.9%, while at 221 g/L (BH3, BH4) a notable increase in sucrose was obtained (4%). These changes during sample preparation were not affected by temperature. Once fermentation had started, however, temperature became more important in sucrose content. After 24 h of fermentation carried out at 42 °C (BH2, BH4), sucrose contents showed large decreases, reaching 1.6% and 2.4%, respectively, and treatments at 28 °C (BH1, BH3) showed decreases to 2.2% and 3.8%, respectively. The centerpoints (BH9, BH10, BH12) had intermediate sucrose levels (2.4%). By 48 h the sucrose content had decreased sharply in every treatment, the reductions being from 92% to 96% less than that found in the dry seed, and by 96 h sucrose could not be detected.

Sample preparation also affected the content of α-galactosides compared with the levels found in the dry seed. As with sucrose, the content of α-galactosides was dependent on the concentration of flour, while temperature had only minor effect. Treatment carried out at 79 g/L (BH1, BH2) produced lower α-galactoside contents at hour 0 than dry flour: raffinose decreased from 0.5% to 0.4%, ciceritol from 2.2% to 1.9%, and stachyose from 3.7% to 2.9%. At 150 g/L (BH9, BH10, BH12) there was a small increase in raffinose (0.6%) and a slight decrease in ciceritol (2.10−2.15%) and stachyose (3.5−3.6%). At 221 g/L (BH3, BH4) there was a pronounced increase in all of the α-galactosides: raffinose increased to 0.85%, ciceritol to 2.7%, and stachyose to 4.25% (Table 4).

The effect of the various treatments was similar for all of the α-galactoside sugars. After 24 h, there was a notable decrease in raffinose, ciceritol, and stachyose, the greatest decrease being observed at the highest temperature (42 °C; BH2, BH4). There was an interaction between flour concentration and temperature such

Table 4. Changes in α-Galactosides (Raffinose, Ciceritol, and Stachyose) during Natural Fermentation of Lentils (Percent Dry Weight)

batch	raffinose	ciceritol	stachyose	α-galactosides
raw lentil	0.52 ± 0.01	2.20 ± 0.02	3.74 ± 0.04	6.46 ± 0.02
BH1 (79 g/L, 28 °C)	0.37 ± 0.01[a]	1.87 ± 0.02[a]	2.92 ± 0.06[a]	5.17 ± 0.05[a]
0 h	0.14 ± 0.01	1.38 ± 0.03	2.40 ± 0.02	3.92 ± 0.06
24 h	ND[b]	0.16 ± 0.01	0.21 ± 0.01	0.37 ± 0.02
48 h	ND	ND	ND	ND
72 h	ND	ND	ND	ND
96 h				
BH2 (79 g/L, 42 °C)	0.39 ± 0.01[a]	1.89 ± 0.01[a]	2.91 ± 0.03[a]	5.19 ± 0.04[a]
0 h	ND	0.38 ± 0.01	1.13 ± 0.01	1.51 ± 0.01
24 h	ND	ND	ND	ND
48 h	ND	ND	ND	ND
72 h	ND	ND	ND	ND
96 h				
BH3 (221 g/L, 28 °C)	0.86 ± 0.03[c]	2.70 ± 0.02[d]	4.26 ± 0.01[c]	7.83 ± 0.01[c]
0 h	0.71 ± 0.01	2.04 ± 0.03	3.53 ± 0.02	6.29 ± 0.03
24 h	0.13 ± 0.01	0.43 ± 0.01	1.25 ± 0.01	1.82 ± 0.01
48 h	ND	ND	0.87 ± 0.01	0.87 ± 0.01
72 h	ND	ND	0.31 ± 0.01	0.31 ± 0.01
96 h				
BH4 (221 g/L, 42 °C)	0.85 ± 0.01[c]	2.71 ± 0.02[d]	4.24 ± 0.02[c]	7.80 ± 0.03[c]
0 h	0.22 ± 0.01	1.68 ± 0.02	3.05 ± 0.01	4.96 ± 0.01
24 h	ND	0.11 ± 0.01	0.43 ± 0.02	0.54 ± 0.02
48 h	ND	0.04 ± 0.01	0.21 ± 0.01[g]	0.26 ± 0.02
72 h	ND	ND	0.15 ± 0.01	0.15 ± 0.01
96 h				
BH9 (150 g/L, 35 °C)	0.57 ± 0.01[b]	2.10 ± 0.02[b]	3.50 ± 0.02[b]	6.16 ± 0.02[b]
0 h	0.45 ± 0.01[d]	1.76 ± 0.01[e]	2.90 ± 0.01[d]	5.10 ± 0.01[c]
24 h	0.04 ± 0.01[f]	0.35 ± 0.01[f]	0.54 ± 0.01[f]	0.93 ± 0.02[e]
48 h	ND	ND	0.20 ± 0.01[g]	0.20 ± 0.01[f]
72 h	ND	ND	0.07 ± 0.01[h]	0.07 ± 0.02[g]
96 h				
BH10 (150 g/L, 35 °C)	0.57 ± 0.02[b]	2.15 ± 0.04[c]	3.57 ± 0.08	6.29 ± 0.10
0 h	0.44 ± 0.02[de]	1.76 ± 0.02[e]	2.87 ± 0.02[de]	5.07 ± 0.03[cd]
24 h	0.04 ± 0.01[f]	0.37 ± 0.01[f]	0.52 ± 0.01[f]	0.93 ± 0.02[e]
48 h	ND	ND	0.21 ± 0.01[g]	0.21 ± 0.01[f]
72 h	ND	ND	0.06 ± 0.01[h]	0.06 ± 0.01[g]
96 h				
BH12 (150 g/L, 35 °C)	0.57 ± 0.02[b]	2.11 ± 0.02[bc]	3.50 ± 0.02[b]	6.18 ± 0.02[b]
0 h	0.43 ± 0.01[e]	1.75 ± 0.02[e]	2.86 ± 0.02[e]	5.03 ± 0.03[d]
24 h	0.04 ± 0.01[f]	0.35 ± 0.01[f]	0.52 ± 0.01[f]	0.91 ± 0.01[e]
48 h	ND	ND	0.20 ± 0.02[g]	0.20 ± 0.02[f]
72 h	ND	ND	0.06 ± 0.01[h]	0.06 ± 0.01[g]
96 h				

[a] Values are the mean of five determinations ± standard deviation. The same superscript in the same column means no significant differences ($P \leq 0.05$). [b] Not detected.

that at the lowest concentration and the highest temperature (BH2) raffinose was not detected and the lowest levels of ciceritol and stachyose were found (0.4% and 1.1%, respectively). The 150 g/L and 35 °C treatments (BH9, BH10, BH12) had intermediate α-galactoside contents (0.45% for raffinose, 2.15% for ciceritol, and 2.9% for stachyose).

After 48 h, raffinose could not be detected in any of the low flour concentration treatments (BH1, BH2) or in the high flour concentration and high temperature (BH4) treatment. For the rest of the batches, a significant decrease in raffinose content was observed. Similar effects were found for changes in ciceritol and stachyose.

By 72 h none of the treatments showed any raffinose or ciceritol, with the exception of a small amount of ciceritol in the highest flour concentration and temperature treatment (BH4, 0.04%). Stachyose, however, was completely absent only from treatments containing the lowest flour concentration (79 g/L; BH1, BH2). In the rest of the treatments, a significant decrease was observed even after 72 h and a small amount remained after 96 h (0.2−0.3%).

Taking the α-galactosides as a whole, it was found that at 0 h the two treatments containing the lowest flour concentration (79 g/L; BH1, BH2) behaved in a similar way, both showing a 20% reduction in α-galactosides. At 150 g/L (BH9, BH10, BH12) a smaller decrease of α-galactosides was observed (3−5% reduction), and at the highest concentration (221 g/L; BH3, BH4) there was a notable increase in content to 7.8%. During the preparation of the samples no significant differences were found between treatments for which the temperature differed at the same flour concentration. Temperature became more important once fermentation had started, the maximum decrease in the α-galactosides being at 42 °C, followed by 35 °C and then 28 °C.

DISCUSSION

Fermented food is a widely used source of good protein. Among the legumes, soybean is extensively used for this process (Salunkhe and Kadam, 1989), although other legumes such as *Phaseolus* beans and chickpea are also occasionally used for this application (Reddy and Salunkhe, 1989). Beuchat et al. (1985) proposed peanuts and cowpeas as acceptable substrates for the preparation of *nato*-like products. Ikenebomeh (1989) noted that natural fermentation at 37 °C improved the organoleptic quality of legume flour and that

this was better than flour fermented at 25 °C. It was noted, however, that the levels of reducing sugars and soluble nitrogen increased dramatically as a result of fermentation. In lentils, Ragaee et al. (1986a) reported that natural lentil fermentation for 4 days at 32 °C increased the available level of total amino acids and improved *in-vitro* protein digestibility. More recently, these authors (Ragaee et al., 1986b) observed good consumer acceptability for products formulated from fermented lentils. Mahaja and Chauhan (1988) noted that natural fermentation improved the HCl extractability of minerals including calcium, magnesium, and copper in pearl milled flour.

In the present study, raffinose, stachyose, and ciceritol, a pseudotrisaccharide α-galactoside detected in lentils (Bernabe at al., 1993), were observed in relatively high amounts in unfermented lentil flour. The observed values were within the range of data recorded in the literature (Reddy et al., 1984; Vidal-Valverde and Frias, 1992). Verbascose, however, was not detected in the variety of lentils used for this study, although it has been shown to be present in lentil varieties and to range from 0% to 1% of the dry seed weight (Frias et al., 1994b). Goel and Verma (1980) indicated that α-galactosides are the most abundant soluble carbohydrates in dry bean flour and legumes in general, with stachyose and raffinose being present at the highest concentration. In the current study, the α-galactosides represented 68% of the total soluble carbohydrate content.

Natural fermentation of lentils in which the pH decreases throughout is characteristic for lactic acid microorganisms. Ragaee et al. (1985) reported that lactic acid bacteria play a major role in natural fermentation of lentils, and pH decreases from 6.9 to 3.9 were observed after 4 days of natural fermentation. Similar changes in pH were observed in the present study. This decline in pH during fermentation is known to act as a bacteriostatic and preservative factor against bacteria associated with spoilage and against nondesirable and pathogenic microorganisms which cannot proliferate under these conditions (Wang and Hesseltine, 1981; Nout et al., 1989). Zamora and Fields (1979b) did not detect any toxic substances in fermented cowpeas and chickpeas as determined by an *in-vivo* test in chicken embryos.

Fermentation has been suggested as a technological procedure for partial or total removal of α-galactosides, compounds that are closely related with the occurrence of flatulence. Goel and Verma (1980) indicated that these oligosaccharides are broken down during fermentation to saccharides, with sucrose being one of the major products. Our results are in agreement with these authors since a decrease of α-galactosides was observed throughout the fermentation procedure. At the same time, an increase in the levels of fructose, glucose, and sucrose was observed from the beginning of the fermentation, although glucose and sucrose were not detected after 96 h of fermentation. Vidal-Valverde et al. (1993) observed the total elimination of sucrose and α-galactosides and an increase of fructose in lentils after 4 days of fermentation at 30 °C and a flour concentration of 100 g/L. In the present study we have found that when the concentration of lentil flour−water suspension is 79 g/L, the α-galactosides are totally removed after 48 h at 42 °C. These conditions also gave the highest fructose content (1.1%), while large amounts of glucose (0.3%) and sucrose were still present (0.1%). When fermentation was carried out at this concentration at 28 °C, the α-galactosides were removed after 72 h, with smaller amounts of fructose (0.6%) and sucrose (0.1%) and traces of glucose remaining. Reddy et al. (1980) reported that the lactic acid microorganisms involved in the natural fermentation of lentils exhibit α-galactosidase and invertase activity. These enzymes would hydrolyze the oligosaccharides and result in a gradual increase in fructose and the appearance of glucose. Odunfa (1983) studied the activity of the α-galactosidase and β-galactosidase enzymes during fermentation and found that the maximum activity occurred after 24 h at between 40 and 60 °C. This is in good agreement with our results which showed that there was a major decrease in the α-galactosides at 42 °C.

Effects have been observed at two stages during the natural fermentation of lentils. First, there is an effect of the sample preparation, which corresponds to changes between the composition of the raw material and the composition found at the beginning of the fermentation (0 h). During this time the fermentation temperature of the broth is reached and complete suspension of the flour obtained. The time taken to reach this stable temperature and suspension state is 10−40 min. During this time the initial concentration of the lentil flour−water suspension had an important influence on the levels of fructose, glucose, sucrose, and α-galactosides, while temperature had a minor effect. Second, the time over which fermentation occurs, which in the present study extended from 0 to 96 h, also has an effect. Both the flour concentration and the temperature, therefore, modified the soluble sugar content: the higher the initial flour concentration the greater the sugar content and the higher the temperature the greater the decrease in soluble sugar content.

Our results indicate that natural fermentation could be an alternative treatment for the removal of the flatulence-causing oligosaccharides. Lentil flour processed in this way, therefore, could have wide applications for human nutrition. In addition, we have shown that the initial concentration of lentil flour and fermentation temperature strongly affect the levels of the mono- and disaccharides and α-galactosides.

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