Effect of Lime Cooking of Grain Amaranth on Selected Chemical Components and on Its Protein Quality

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Samples of *Amaranthus cruentus* were cooked for 10 and 20 min at atmospheric pressure with 0, 0.2, 0.4, and 0.6% calcium hydroxide on the basis of sample weight. A raw sample served as control. Dry matter recovered ranged from 87.4 to 93.5%, but there was no relationship to lime level or cooking time. Protein and fat contents increased from 4 to 11% and from 3 to 13% in the cooked samples, respectively. Lysine content decreased about 10–12%, and no change was observed in tryptophan content upon cooking with lime. The fat acidity of the raw sample stored at ambient temperature for 146 days increased significantly. An increase was also observed in the cooked samples but was significantly less when 0.6% lime was used. Calcium content increased with respect to lime level, as well as Mg, but to a lower extent. The protein quality of amaranth was increased by cooking, either with or without lime, and protein digestibility was not affected.

Keywords: *Amaranth grain; lime cooking; chemical composition; effect on protein quality*

INTRODUCTION

Grain amaranth has received increasing attention in recent years due to a number of attractive features, such as its adaptability to diverse environments, its production of relatively high yields, its C4 photosynthetic metabolism, and its relatively good chemical composition, particularly in fat and protein content, with high levels of lysine, tryptophan, and methionine (Saunders and Becker, 1984; Teutonico and Knorr, 1985; National Academy of Sciences, 1984). The exceptionally well-balanced amino acid pattern in amaranth protein results in a high protein quality (Becker et al., 1981; Bressani et al., 1987a). Furthermore, due to its relatively high level of lysine, its supplementary effect to lysine-deficient cereal grains has been shown (Tovar and Carpenter, 1982; Pederson et al., 1987a,b; Bressani, 1989). In this respect, one possible application has been its use in mixtures with lime-treated maize for tortilla preparation (Tovar and Carpenter, 1982; Vargas-López et al., 1990). The problem is that both grains cannot be processed efficiently together since there is a significant difference in grain weight, with amaranth weighing about 0.9 mg/seed (Bressani et al., 1987b) and maize weighing around 0.27 g/seed (Bressani and Mertz, 1958). The lime-cooking process, or nixtamalization as it is often called, may result in losses of amaranth during the washing operation of the lime-cooked grain (Gómez et al., 1987). Therefore, the use of amaranth as a supplement to lime-cooked maize for tortilla products requires cooking amaranth separately from maize.

Recently, Vargas-López et al. (1990) evaluated the effect of temperature, calcium hydroxide concentration, and cooking time on the physicochemical properties of amaranth flour to be made into tortillas. They found temperature and cooking time to significantly affect water absorption index (WAI), color, and flow properties, while calcium hydroxide concentration affected only masa flow properties. Increasing T and cooking time increased pH, WAI, and color. These studies were conducted with cooking temperatures of 80–90 °C, lime concentration of 0.8–1.0 g/100 g of amaranth grain, and cooking times of 10–20 min. The same authors concluded that lime-cooked amaranth flour so prepared could be used for preparation of tortillas and similar products.

Lime cooking of maize has been reported to induce small losses in tryptophan and smaller ones in lysine content (Bressani et al., 1990; Ortega et al., 1986). However, the protein quality of lime-treated maize is equal to or slightly better than that of raw maize. Similar effects could take place in amaranth. Nevertheless, being a significantly smaller seed, cooking at an alkaline pH may induce higher losses in nutritional quality. The purpose of this research was to obtain more detailed chemical and nutritional information on lime cooking of amaranth grain and to learn if the process reduces the development of fat acidity, since amaranth grain oil has been reported to have a high acid value (García et al., 1987), which may cause problems with the acceptability of the ground seed.

MATERIALS AND METHODS

The studies were conducted with variety GUA-17 of *Amaranthus cruentus*. Portions of 1.0 kg of the grain, in duplicate, were used for sample preparation. The samples included a raw flour, prepared by grinding with a Raymond pulverizer with a 60-mesh screen; a sample processed by cooking in water at atmospheric pressure for 10 and 20 min without lime addition; and samples of an equal weight processed as indicated above but with additions of 0.2, 0.4, and 0.6% lime on the basis of grain weight. After cooking, the material was left to cool at room temperature (26 °C) and the pH was measured. Each preparation was then washed three times with water and weighed. The cooked grain was separated from the washing waters with a cheesecloth to minimize losses of grain. After that, it was placed in trays to dry to constant weight, with air at 60 °C. Once dried, it was weighed and ground as indicated above. This permitted estimation of weight losses due to processing.

All processed samples were analyzed for moisture, protein, fat, and fat acidity by methods of the AOAC (1984). The pH

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Table 1. Amaranth Dry Grain Recovered after Cooking by Nixtamalization and pH of Cooked Grain and of Flour

sample condition	lime level, %	dry wt recovered, g		pH[a]	
		10 min	20 min	10 min	20 min
raw		885.6 ± 0.5	885.6 ± 0.5	(6.56)	(6.56)
cooked	0	773.6 ± 31.9	784.5 ± 1.9	6.45 (6.65)	6.30 (6.75)
cooked	0.2	827.7 ± 2.3	808.4 ± 4.1	8.25 (6.70)	8.05 (6.81)
cooked	0.4	825.8 ± 4.2	795.9 ± 14.0	10.35 (6.99)	8.70 (7.16)
cooked	0.6	819.0 ± 10.9	790.8 ± 15.7	11.50 (7.35)	10.00 (7.40)

[a] Figure in parentheses is the pH of dry flour.

Table 2. Protein, Fat, Lysine, and Tryptophan Content (Dry Weight Basis)

sample condition	lime level, %	protein, %		fat, %		Lys, g/16 g of N		Trp, g/16 g of N	
		10 min	20 min	10 min	20 min	10 min	20 min	10 min	20 min
raw		16.4 ± 0.2	16.5 ± 0.2	7.1 ± 0.01	7.1 ± 0.01	4.90 ± 0.02	4.90 ± 0.02	1.15 ± 0.01	1.15 ± 0.01
cooked	0	18.0 ± 0.1	17.6 ± 0.2	8.0 ± 0.01	7.7 ± 0.01	4.12 ± 0.03	4.33 ± 0.16	1.28 ± 0.07	1.24 ± 0.01
cooked	0.2	17.1 ± 0.2	18.3 ± 0.5	7.4 ± 0.20	8.0 ± 0.20	4.59 ± 0.14	4.56 ± 0.06	1.26 ± 0.06	1.15 ± 0.06
cooked	0.4	17.1 ± 0	17.6 ± 0.6	7.7 ± 0.22	7.5 ± 0.22	4.49 ± 0.03	4.28 ± 0.03	1.20 ± 0.01	1.21 ± 0.04
cooked	0.6	17.5 ± 0.2	17.1 ± 0.4	7.8 ± 0.02	7.3 ± 0.02	4.39 ± 0.07	4.29 ± 0.05	1.20 ± 0.02	1.12 ± 0.05

Table 3. WAI and WSI of Amaranth Flour Cooked by Lime Cooking

sample condition	lime level, %	WAI		WSI	
		10 min, g of gel/g of dry flour	20 min, g of gel/g of dry flour	10 min, g of solids/100 g of dry flour	20 min, g of solids/100 g of dry flour
raw		2.29 ± 0	2.29 ± 0	6.82 ± 0	6.82 ± 0
cooked	0	2.66 ± 0.29	2.82 ± 0.05	4.74 ± 0	6.35 ± 0.54
cooked	0.2	2.56 ± 0.01	2.58 ± 0.07	5.26 ± 0.28	4.71 ± 0.03
cooked	0.4	2.43 ± 0.02	2.91 ± 0.01	5.48 ± 0.17	5.65 ± 0.56
cooked	0.6	2.56 ± 0.05	2.75 ± 0.15	4.93 ± 0.16	4.43 ± 0.26

of the flour was obtained by suspending 5 g in 15 cm^3 of distilled water, and the pH was measured after 15 min. Minerals were measured by atomic absorption. Reactive lysine was measured according to the technique of Hurrell et al. (1979) and tryptophan as suggested by Villegas et al. (1982). The samples were also analyzed for WAI and by the water solubility index (WSI). WAI is an indirect measure of the degree of starch gelatinization induced upon cooking, and it is expressed as the weight of gel obtained per gram of dry sample. WSI represents the amount of solids in the water solution from the WAI analysis and is expressed as percentage of dry solids by methods of Anderson et al. (1969).

For the biological evaluation, the net protein ratio (NPR) method was applied (The United Nations University, 1980) using diets in which amaranth provided 10% protein. These diets were supplemented with 5% refined cottonseed oil, 1% cod liver oil, 4% mineral mixture (Hegsted et al., 1941), and 5 mL of a complete vitamin solution per 100 g of diet (Manna and Hauge, 1953). Cornstarch was used to adjust to 100%. A casein diet providing 10% protein was also prepared as well as a nitrogen-free diet. During the last 7 days of the 14-day assay, quantitative fecal collections were made for protein digestibility. These samples were also used to determine fecal carbohydrate content (López de Romaña et al., 1980) according to the formula

$$\text{fecal CHO (g)} = [\text{total fecal energy} - (\text{N\%} \times 6.25 \times 5.65) - (\text{g of fat} \times 9.4)]/4.15$$

The values 5.65, 9.4, and 4.15 are accepted calorie conversion factors for protein, fat, and carbohydrate, respectively.

Each experimental group was made up of eight rats, 22–23 days of age, assigned to individual cages. Room temperature was 23 °C with a 12-h light cycle. Water and diets were available at all times. The fat acidity (AOAC, 1984) of the samples was established at 0, 62, 102, and 146 days, on samples stored at room temperature (23 °C).

All data are expressed as a mean value ± SD. Results were analyzed by the two-way analysis of variance (ANOVA). A probability value (P) of 0.05 or less was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 shows the results of the dry weight recovered when grain amaranth was processed by lime cooking.

Table 4. Fat Acidity (Milligrams of KOH/100 g) of Amaranth Grain Flours Cooked with Lime after Different Storage Times

sample condition	lime level, %	cooking time	
		10 min	20 min
0 Days of Storage			
raw		114.9 ± 0	114.9 ± 0
cooked	0	79.4 ± 6.5	81.9 ± 6.5
cooked	0.2	80.9 ± 2.9	82.5 ± 2.5
cooked	0.4	79.4 ± 0.6	64.9 ± 6.0
cooked	0.6	64.4 ± 1.6	55.0 ± 4.9
62 Days of Storage			
raw		235.2 ± 0	235.2 ± 0
cooked	0	123.4 ± 13.9	113.5 ± 0.1
cooked	0.2	94.6 ± 3.6	94.1 ± 3.3
cooked	0.4	97.0 ± 1.6	88.6 ± 1.7
cooked	0.6	79.8 ± 0.1	69.0 ± 1.5
102 Days of Storage			
raw		261.8 ± 3.0	261.5 ± 3.4
cooked	0	142.7 ± 27.9	144.0 ± 22.8
cooked	0.2	101.0 ± 3.5	104.7 ± 1.5
cooked	0.4	101.9 ± 4.0	105.3 ± 5.2
cooked	0.6	79.1 ± 0.1	71.2 ± 5.2
146 Days of Storage			
raw		295.1 ± 0	295.1 ± 0
cooked	0	174.1 ± 26.7	172.9 ± 18.9
cooked	0.2	122.4 ± 8.8	122.4 ± 11.5
cooked	0.4	125.4 ± 1.0	126.1 ± 3.9
cooked	0.6	89.6 ± 3.2	88.3 ± 7.5

The pH of the material after cooking increased from 6.4 to 10.7 as lime concentration increased. The flours show pH values from 6.6 to 7.4. This same range was observed by Vargas-López et al. (1990) on lime cooking of amaranth and by Gómez et al. (1987) for maize. The losses of grain solids were higher when cooking without lime at both cooking times (12.6 and 11.4% for 10 and 20 min, respectively). Higher grain recoveries were observed when cooking was done with 0.2% lime (93.5 and 91.3%), and these decreased slightly as lime concentration increased to 0.6% at both cooking times (92.5 and 89.3%). Losses ranged from 6.5 to 12.6%, which

Table 5. Mineral Content of Lime-Cooked Amaranth Flour

sample condition	lime level, %	phosphorus, mg/100 g		calcium, mg/100 g		magnesium, mg/100 g		iron, mg/100 g	
		10 min	20 min	10 min	20 min	10 min	20 min	10 min	20 min
raw		602.1 ± 0	602.1 ± 0	300.0 ± 0	300.0 ± 0	277.5 ± 0	277.5 ± 0	19.0 ± 0	19.0 ± 0
cooked	0	655.7 ± 26.0	642.3 ± 13.5	237.5 ± 12.5	212.5 ± 12.5	321.2 ± 16.2	318.7 ± 8.8	15.5 ± 1.5	21.5 ± 3.5
cooked	0.2	667.6 ± 15.0	695.8 ± 19.5	375.0 ± 25.0	387.5 ± 87.5	313.7 ± 16.1	333.7 ± 3.7	22.5 ± 3.5	16.5 ± 2.5
cooked	0.4	669.1 ± 13.0	642.3 ± 40.2	350.0 ± 25.0	512.5 ± 12.5	311.2 ± 4.3	322.5 ± 5.0	21.5 ± 6.5	17.0 ± 0
cooked	0.6	669.1 ± 13.0	655.7 ± 26.7	450.0 ± 25.0	425.0 ± 25.4	318.7 ± 16.3	300.0 ± 47.5	16.0 ± 3.0	16.0 ± 1.0

Table 6. Protein Quality Expressed as Net Protein Ratio of Lime-Cooked Amaranth Flours

sample condition	lime level, %	food intake, g		wt gain, g		NPR[a]	
		10 min	20 min	10 min	20 min	10 min	20 min
raw		131 ± 15.2a	131 ± 15.2a	33 ± 7.3a	33 ± 7.3a	2.97 ± 0.44h	2.97 ± 0.44h
cooked	0	201 ± 15.5b	190 ± 20.2b	65 ± 9.9bc	59 ± 7.8bcde	3.82 ± 0.85abcdef	3.88 ± 0.38abcde
cooked	0.2	196 ± 22.4b	199 ± 17.7b	61 ± 8.3bcde	60 ± 7.2bcde	3.90 ± 0.31abcd	3.74 ± 0.34bcdefg
cooked	0.4	184 ± 16.3b	213 ± 8.7b	50 ± 5.6de	70 ± 0.6b	3.52 ± 0.14cdefg	4.18 ± 0.39a
cooked	0.6	197 ± 22.8b	201 ± 13.5b	59 ± 11.6bcde	62 ± 9.4bcd	3.93 ± 0.25abc	3.95 ± 0.33ab
casein		167 ± 18.4b		46 ± 7.4de		3.64 ± 0.35cdefg	

[a] Net protein ratio = (wt gain of rats in test diet + wt loss of rats on nitrogen-free diet)/protein intake.

Table 7. Fecal Carbohydrate and Apparent Protein Digestibility of Lime-Cooked Amaranth Flours

sample condition	lime level, %	fecal carbohydrates, g		apparent protein digestibility,[a] %	
		10 min	20 min	10 min	20 min
raw		3.46 ± 1.11abcde		79.2 ± 1.1a	
cooked	0	4.70 ± 0.73ab	4.51 ± 0.74abcd	76.1 ± 1.3abcdef	77.5 ± 0.8abc
cooked	0.2	3.52 ± 1.17abcde	3.12 ± 0.80abcde	77.5 ± 1.5abc	73.9 ± 5.7bcdfg
cooked	0.4	2.67 ± 0.71e	3.89 ± 1.19abcde	72.1 ± 3.4efg	70.3 ± 4.8g
cooked	0.6	4.66 ± 1.34abc	4.91 ± 1.38a	72.4 ± 2.1abcd	77.2 ± 1.2abcde
casein				92.9 ± 0.5h	

[a] Apparent protein digestibility [(nitrogen intake from diet − fecal nitrogen output)/nitrogen intake from diet × 100.

are lower than those for lime cooking of maize, where losses of up to 17% have been reported (Bressani, 1990). The analysis of variance showed significant differences ($P < 0.05$) due to the treatments and between raw and cooked amaranth. Furthermore, there was a quadratic effect due to lime level, which suggested optimum level was between 0.2 and 0.4%, while cooking time at boiling T should be less than 20 min.

Table 2 summarizes the protein, lysine, and tryptophan content of the samples, as well as the fat content. Lime cooking induced a small increase in total protein content; no significant changes were observed with increasing levels of lime. The same seems to be true for total crude fat. These changes may be due to small losses in solids from the grain, such as starch and sugars. Lime cooking of maize has been reported not to affect protein content but a decrease in hexane-soluble substances has been reported. Available lysine content appeared to decrease some 10.4−12.4%; however, tryptophan was not affected. Various workers have shown minimal losses of lysine during the lime-cooking process. Tryptophan content has also been shown to decrease about 10−15% upon lime cooking of maize (Ortega et al., 1986; Bressani, 1990).

The WAI and WSI values of the samples are shown in Table 3. A small increase and a small decrease in WAI and WSI, respectively, were observed upon lime cooking. WAI values of 2.7 g of gel/g of dry flour were obtained in the present study as compared to values of 2.7−7.6 g of the study of Vargas-López et al. (1990). Gómez et al. (1987) reported lower values for maize flour cooked with lime. Imeri et al. (1987) observed values between 3.5 and 4.2 on *Amaranthus caudatus*, and Mendoza and Bressani (1987) values of 3.4−3.5 on extrusion-cooked samples.

As shown in Table 4, lime cooking decreased fat acidity, and the decrease was associated with lime level. After 62 days in storage at 23−34 °C, fat acidity doubled for the raw flour, but it increased around 1.2 times for the lime-processed samples. This is an interesting observation for the lime-cooking process, which tends to extend the shelf life of maize processed by nixtamalization. At 102 days, fat acidity increased further in the raw sample and only slightly in the lime-processed sample. At 146 days, the fat acidities were 295.1 mg of KOH/100 g for the raw sample and 89.6 and 88.3 mg of KOH/100 g for the lime-cooked samples (0.6% lime) and 10 and 20 min of cooking, respectively.

Table 5 shows the levels for various minerals in amaranth grain processed with different levels of lime. Calcium content increased with respect to calcium hydroxide level of addition during cooking up to 0.4%. Similar observations have been made when maize is cooked by the nixtamalization process (Bressani et al., 1989; Ortega et al., 1986). A small increase also occurred in P and Mg content upon cooking with increasing levels of lime. No change, however, was observed with respect to iron content. The levels of Cu, Mn, and Zn were 10, 30, and 40 mg/100 g and not influenced by lime level or cooking time.

Table 6 summarizes the protein quality expressed as net protein ratio of the various samples. Cooking increased average weight gain over the raw value, but lime cooking did not affect significantly weight gain over that of cooking without lime. The same is true for NPR. This observation has been reported before for water cooking, in which significant increases in weight gain and NPR have been demonstrated (Bressani et al., 1987a,b). No explanation has been given for the beneficial effect of a heat treatment to amaranth which improves diet intake, weight gain, and consequently, protein quality. The levels of the common antiphysiological substances in amaranth grain are too low to account for such an effect.

Another possibility would be a greater bioavailability of carbohydrates upon heat treatment. In this respect, López and Bressani (1987) showed an increase in metabolizable energy in extruded amaranth over that of the raw seed, while Acar et al. (1988) found no change with respect to the raw seed for autoclaved cooking of the grain. In the present study the fecal carbohydrate content of the rats fed the different lime-cooked amaranth samples was determined. The results are shown in Table 7, as well as those of protein digestibility. Analysis of variance showed significant differences ($P < 0.05$) due to treatments and a quadratic effect for the cooking × lime interaction. At both 10 and 20 min of cooking time a decrease in fecal carbohydrate with respect to the whole raw seed was observed when 0.4% lime (10 min of cooking) and 0.2% lime (20 min of cooking) were used, as was an increase again when 0.6% lime was used. Except for the raw amaranth diet which contained 14% maize starch, all other diets contained 24%. Analysis of variance of protein digestibility showed significant differences ($P < 0.05$) due to treatments and a quadratic effect for the cooking time × lime level interaction. Protein digestibility decreases up to 0.4% lime at both cooking times, but an increase was observed at both cooking times with 0.6% lime addition. These results indicate that lime cooking of grain amaranth does not affect the quality of its protein even at a level of addition of 0.6%; however, it cannot be assumed that higher levels will not affect digestibility and protein quality.

The results of this study have shown that various benefits can be derived from applying the nixtamalization process to amaranth grain. It does not decrease the bioutilization of the amino acids of the protein as suggested by the relatively high NPR; it increases the shelf life of the flour, which is of significance in its utilization alone or in food products, and it increases the calcium content, improving the P/Ca ratio. Furthermore, its flavor resembles that of lime-treated maize. Future studies should analyze in more detail the functional properties of lime-cooked amaranth flour for food product development. In our opinion lime cooking is a good alternative in amaranth grain processing.

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