Evaluation of Lime Heat Treatment on Some Physicochemical Properties of Amaranth Flour by Response Surface Methodology

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ABSTRACT

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This study was conducted to evaluate the effect of temperature, calcium hydroxide concentration, and cooking time on physicochemical properties of amaranth flour to be made into tortillas. Response surface methodology was utilized for this evaluation. Changes in pH, water absorption index, color, and flow properties were assessed after selected levels of temperature, calcium hydroxide concentration, and cooking time were set at 80 and 90°C, 0.8 and 1.0 g of $Ca(OH)_2$/100 g of amaranth, and 10 and 20 min, respectively. Regression models obtained for each physicochemical property accounted for 81.02–94.40% of the total variation. The effect of temperature and cooking time was significant for all measured parameters of experimental flours, whereas that of calcium hydroxide concentration in the tested range was only significant on masa flow properties. Values of pH, water absorption index, and color increased with increasing temperature and cooking time. In relation to flow properties, the power law model was found to correlate accurately the effects of shear stress and shear rate on fluid consistency and flow behavior indices. Lime-cooked amaranth flour may be used for preparation of tortillas and similar products.

Amaranth was a major food source for ancient civilizations in Mexico and Central and South America. However, it fell into disuse and present consumption is almost negligible. Today there is a resurgence of interest in amaranth because of its high agronomic and food potentials. To a certain degree the plant is tolerant of drought, high temperatures, and pests. Amaranth grows in soils of lower quality than most commercial crops. It produces good yields of a grain that contains relatively high levels of protein (15–18%) and starch (48–62%) (Paredes-López et al 1988a, and E. Espitia, *unpublished data*, INIFAP 1988).

The outstanding nutritional and physicochemical properties of amaranth proteins are well documented (Mendoza and Bressani 1987, Pedersen et al 1987, Paredes-López et al 1988b); they contain high levels of lysine and adequate amounts of tryptophan and sulfur-containing amino acids, whereas in most cereal grains lysine and tryptophan are found in low proportions as are sulfur-containing amino acids in legumes. Several studies have been made with the aim of increasing consumption of amaranth grain. It was popped and milled to prepare blends of nixtamalized maize flour for tortilla making (Sánchez-Marroquín 1980, Tovar and Carpenter 1982, Sánchez-Marroquín and Maya 1985). Blends of amaranth and wheat flours were prepared for baking applications (Lorenz 1981). Amaranth grain has been germinated to be used in various food formulations (Paredes-López et al 1988a). However, no studies have been published on the alkaline cooking of amaranth grain to prepare tortillas and maize tortilla-based products. Preliminary studies carried out in our laboratory suggested that amaranth can be cooked with lime to produce flour that possesses the basic functional properties to prepare tortillas (Vargas-López et al, *unpublished*). This type of processing might eventually lead to consumption of amaranth on a large scale, taking advantage of its relatively high nutritional value. Thus, the experiments reported here were designed to assess the effects of temperature, calcium hydroxide concentration, and cooking time on some of the physicochemical properties of amaranth flour using response surface methodology (RSM).

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MATERIALS AND METHODS

Flour Preparation

Samples of *Amaranthus hypochondriacus,* Mercado type, were harvested in the experimental station of INIFAP, Chapingo, Mexico. This is a high-yielding cultivar with some desirable agronomic traits (e.g., plants with a short vegetative period, susceptible to mechanical harvesting). The lime-cooked flour was prepared by cooking 500 g of amaranth in 1.5 L of distilled water containing 0.8 or 1.0% $Ca(OH)_2$ (amaranth weight basis). The mixture was heated on a hot plate at 80 or 90°C during 10 or 20 min (Table I), steeped for about 30 min at room temperature, and then the cooking liquor was decanted off. The alkaline-cooked grain was washed twice with 2 L of distilled water, dried in an oven (Ríos-Rocha, Mexico City) with forced air at 60°C for 6 hr. Finally it was ground in a Udy cyclone sample mill (Udy Corp. Boulder, CO) with a 1-mm mesh screen and kept at 4°C until used.

Determinations of Moisture, pH, Water Absorption Index and Hunter Color

Moisture was determined by AACC method 44-19 (AACC 1983). The pH was measured at 25°C after preparing a slurry with 10 g (db) of flour and 100 ml of boiled, distilled water, which was agitated every 5 min for a total of 20 min; then pH was read (method 02-52, AACC 1983). Water absorption index (WAI) was assessed as described by Anderson et al (1969). Surface color of samples was measured using a Hunter-Lab model D25-2 color difference meter (Hunter Associates Inc., Reston, VA). *L*, *a*, and *b* color values were recorded as compared to a white standard with the following values: $L = 91.2$, $a = -1.0$, and $b = -1.7$. Total color difference (ΔE) and chromaticity difference (ΔC) were calculated from previous color parameters (Clydesdale 1976). In all cases, each value represents the mean of two determinations.

Masa Viscoelastic Test

Masa (dough) viscoelastic properties were determined utilizing a Haake Rotovisco RV3 (Haake Buchler Instruments Inc., Saddle Brook, NJ), equipped with a cone-plate fixture. Masa was prepared with 10 g of experimental flour (14% mb) plus 12.5 ml of distilled water at 70°C and mixed gently in a bowl for 3 min. A 0.5-g sample of masa was immediately weighed, then carefully placed into the cone-plate fixture of the instrument and allowed to rest for 1 min. Temperature of rotator and plate were adjusted to 25°C. Rotator speed was raised from 0 to 128 rpm at a rate of $50/\text{min}^2$. Torque was registered on recording paper. Each measurement was duplicated using fresh masa samples. Fluid consistency index (k) and flow behavior index (n) were calculated from the Ostwald-De Waele model, also known as the power law model (Otun and Crawshaw 1986), which relates shear stress (τ) and shear rate ($\dot{\gamma}$) for non-Newtonian food materials:

$$\tau = k\dot{\gamma}^n \qquad (1)$$

Logarithmic transformation of equation (1) yields:

$$\ln(\tau) = \ln(k) + n \ln(\dot{\gamma})$$

After obtaining τ and $\dot{\gamma}$, linear regression of ln (τ) and ln ($\dot{\gamma}$) was computed. The intercept and slope values estimated ln (k) and n, respectively.

TABLE I
Treatments Applied for Alkaline Cooking of Amaranth

Treatment	Temperature (°C)	$Ca(OH)_2$ (g/100 g of amaranth)	Time (min)
1	80	0.8	10
2	80	0.8	20
3	90	0.8	10
4	90	0.8	20
5	90	1.0	10
6	90	1.0	20
7	80	1.0	10
8	80	1.0	20

Statistical Analysis

The ranges and interval of experimental parameters for RSM analysis followed the 2^3 factorial design of Myers (1976), with two replications completely randomized. This experimental design was used to evaluate the dependent variables (pH, WAI, ΔE, ΔC, k, and n) in terms of temperature (T), calcium hydroxide concentration (CH) and cooking time (CT). Ranges of the independent variables are shown in Table I. Preliminary experiments (Vargas-López et al, *unpublished*) indicated that these variables had measurable effects on the final product (masa) and that levels selected were reasonable. Data analysis and graphic plotting were done with the Statgraphics program (STSC 1985). Linear models were used to create three-dimensional response surfaces. In RSM, independent variables are located along the x- and y-axes, and the dependent or response variable is on the z-axis.

RESULTS AND DISCUSSION

The effect of lime heat treatment variables on the physicochemical properties of amaranth flour are summarized in Table II. Analysis of variance for each response variable and for full regression were calculated. The contribution of each factor was evaluated by its contribution to the coefficient of determination R^2, the higher R^2 values indicating stronger dependence of the response measured. The independent parameters T and CT were selected for representation of response surface for all physicochemical properties evaluated in this study in view of their significant ($P \leq 0.01$) contribution to the linear regression model. CH in the evaluated range (0.8 and 1.0 g $Ca[OH]_2/100$ g) only had a significant effect on masa flow values at the 99% confidence level.

TABLE II
Effect of Lime Heat Treatment Variables on Physicochemical Properties of Amaranth Flour

Independent Variables	Percent Contribution to R^2					
	pH	Water Absorption Index	Total Color Difference	Chromaticity Difference	Fluid Consistency Index	Flow Behavior Index
Temperature	55.61**[a]	25.83**	39.85**	43.85**	30.95**	23.67**
Calcium hydroxide	0.70	0.10	0.03	0.70	13.78**	19.92**
Cooking time	12.89**	51.97**	33.93**	36.75**	31.86**	30.59**
Temperature ×						
cooking time	0.70	3.63	1.96	3.24	0.00	5.20*
calcium hydroxide	9.22	1.71	12.26*	5.44	15.19**	5.78*
Calcium hydroxide ×						
cooking time	1.90	0.80	0.03	0.08	2.33	9.24**
R^2 for full regression	81.02**	84.04**	88.06**	90.06**	94.11**	94.40**

[a] ** $P \leq 0.01$, * $P \leq 0.05$. Values without asterisks were not significantly correlated.

pH, WAI, and Color

Some changes were observed in the pH of flour suspensions. Statistical analyses of data indicated a strong dependence of pH values on T and CT, whereas the dependence on CH was not significant in the range tested (Table II). The pH value was lowest at low T (80°C) and low CT (10 min) and highest at higher T (90°C) and higher CT (20 min) (Fig. 1A). In general, increases of processing T and CT resulted in pH increases, which means that both processing parameters affected the pH of experimental amaranth flours. The effect of T accounted for the greatest variation in pH measurements (55.61%), and CT was the second most important factor (12.89%). All the interactions were nonsignificant. Unexpectedly, CH had no significant contribution (0.70%) on the regression model. This anomalous result might be due to the close range tested for CH and to an undifferentiated effect of T and CH. However, this aspect requires further investigation. Gomez et al (1987) reported that pH of rehydrated nixtamalized maize flours varies from 5.2 to 10.5, with pH 7.0–9.0 the most common values. Interestingly, most pH values for amaranth flour suspensions fall within the latter range.

The linear regression model for pH was:

$$YpH = 7.156 + 0.169T + 0.081CT$$

($P \leq 0.01$, $R^2 = 0.81$).

The effect of the alkaline cooking variables on WAI is presented in Table II. The WAI response variable was strongly related to CT (51.97%), followed by T (25.83%). As with pH results, CH and all the interactions were also nonsignificant. A regression model predicting this reponse accounted for 84.04% of total variation ($P \leq 0.01$). The response surface developed for WAI

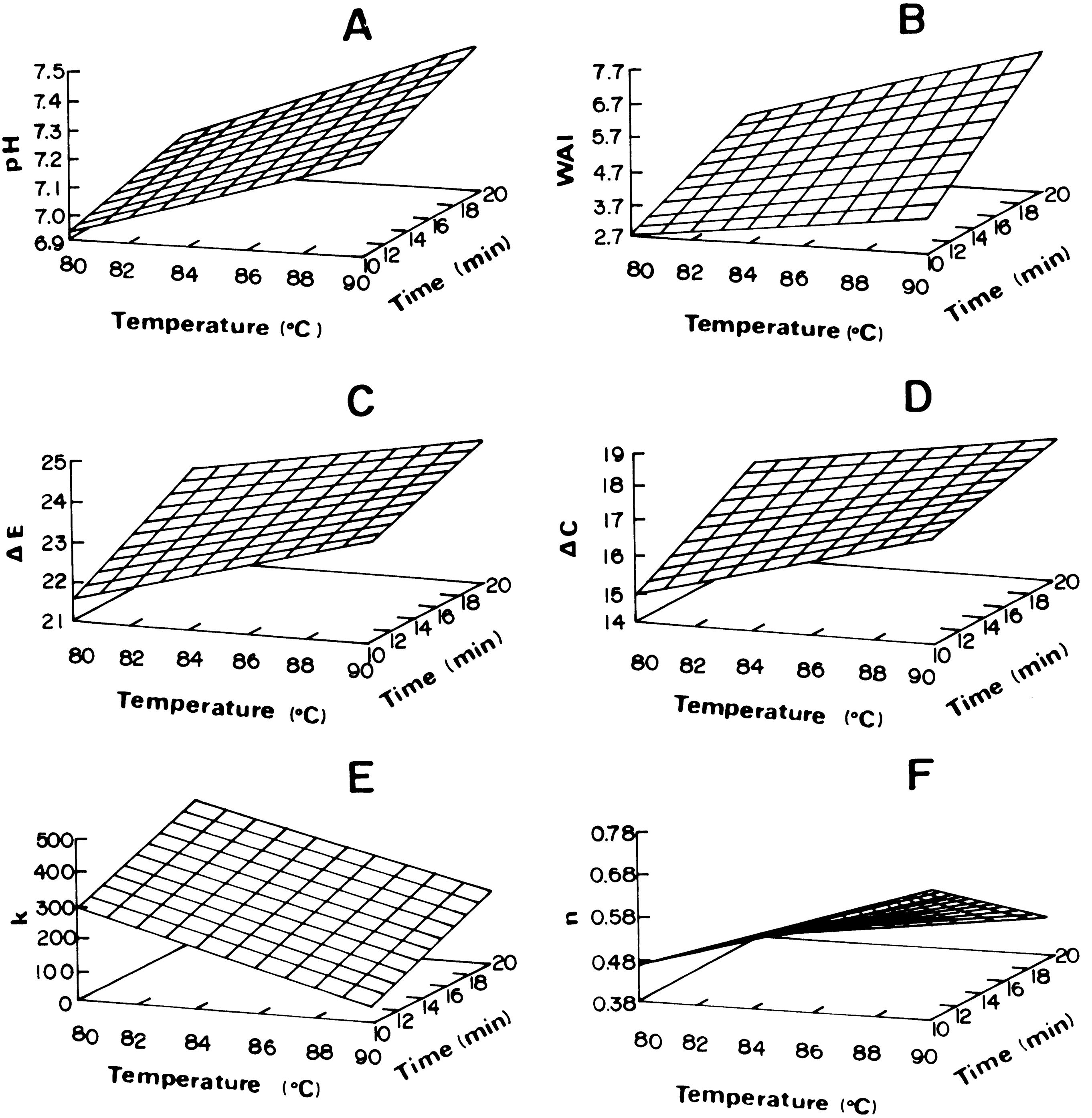

Fig. 1. Response surface diagrams illustrating the effect of temperature and cooking time on: **A,** pH; **B,** water absorption index (WAI); **C,** total color difference (ΔE); **D,** chromaticity difference (ΔC); **E,** fluid consistency index (*k*); and **F,** flow behavior index (*n*).

is illustrated in Figure 1B. It indicated that increases of T and CT resulted in increases of WAI. WAI values for alkaline-cooked amaranth flours ranged from 2.7 to 7.6 g of gel per gram of dry flour, which were higher than those reported for nixtamalized maize flours (Gomez et al 1987). Increases of WAI for treated samples might be related to flour starch damage (Barba et al 1989). WAI may predict tortilla yields in weight, the higher WAI value indicating the bigger tortilla yield (Ramírez-Wong 1989). Anderson (1982) reported that cooking T increased WAI for several grains. Results of this study are in general agreement with those findings. Bedolla (1983) found that WAI for masa flou was positively correlated with pH. This correlation was also found for nixtamalized amaranth flour ($r = 0.800$, $P \leq 0.05$).

The linear regression model for WAI was:

$$\mathrm{YWAI} = 4.431 + 0.844\mathrm{T} + 1.206\mathrm{CT}$$

($P \leq 0.01$, $R^2 = 0.84$).

Analysis of variance showed that Hunter color values were primarily a function of T and CT (Table II). The interaction T × CH for ΔE was the next significant variable ($P \leq 0.05$) with a contribution to R^2 of 12.26%. All other interactions were nonsignificant. Hunter lightness (L) decreased with higher process temperature and cooking time (data not shown). In these conditions, color of alkaline-cooked flour became slightly brownish yellow. The reaction of amaranthin pigment, which is present in amaranth seeds (Sánchez-Marroquín 1980), with other flour components is the likely cause of color changes of treated flours. Higher ΔE values for amaranth flours processed at 90°C for 20 min were found, as opposed to those samples prepared at 80°C for 10 min (Fig. 1C). However, color differences between experimental flours were small ($\Delta E < 4$). Interestingly, ΔC (Fig. 1D) was found to be positively correlated ($r = 0.993$, $P \leq 0.01$) with ΔE.

The linear regression models for ΔE and ΔC were:

$$\mathrm{Y}\Delta\mathrm{E} = 23.219 + 0.781\mathrm{T} + 0.719\mathrm{CT} - 0.431\,(\mathrm{T} \times \mathrm{CH})$$

($P \leq 0.01$, $R^2 = 0.88$),

and

$$\mathrm{Y}\Delta\mathrm{C} = 16.738 + 0.888\mathrm{T} + 0.812\mathrm{CT}$$

($P \leq 0.01$, $R^2 = 0.90$).

Masa Viscoelastic Properties

Rheological parameters of lime-cooked amaranth masa were dependent on process CT, T, and CH, listed in decreasing order of contribution to the variation of flow index values at the significance level of $P \leq 0.01$ (Table II). Some interactions were also significant. The power law model adequately fitted the flow properties of masa within the tested range of $\dot{\gamma}$. Considerable decreases in k values were observed as process T was increased, attaining the lowest level at high T (90°C) and low CT (10 min) (Fig. 1E). Figure 1F shows that n values were greater at high T and minimum CT. Changes in rheological behavior of amaranth masa at the various processing conditions might especially be due to free starch and dissolved solids. Pflugfelder et al (1988) suggested that these components are the primary determinants of the texture of maize masa. The flow behavior of amaranth masa showed typical patterns given by pseudoplastic materials. Similar patterns have been reported for maize masa (Cervone and Harper 1978). Assessment of indices k and n is required to characterize the flow behavior of pseudoplastic materials (Chhinnan et al 1985). These indices are subject to change by various thermal, chemical, and mechanical actions (Otun and Crawshaw 1986). Hence, results obtained in this study should be considered as relative values expressing the comparative effects of processing variables on the consistency of amaranth masa. Regression analysis for both indices showed that linear terms contributed overwhelmingly to the corresponding model. Also, coefficients of determination (R^2) obtained were high (0.94).

The linear regression models for parameters k and n were

$$\begin{aligned}\mathrm{Y}k = {} & 243.064 - 111.304\mathrm{T} + 112.928\,\mathrm{CH} \\ & + 74.269\mathrm{CT} - 77.978\,(\mathrm{T} \times \mathrm{CH})\end{aligned}$$

($P \leq 0.01$, $R^2 = 0.94$),

and

$$\begin{aligned}\mathrm{Y}n = {} & 0.502 + 0.078\mathrm{T} - 0.089\mathrm{CH} - 0.072\mathrm{CT} \\ & - 0.036\,(\mathrm{T} \times \mathrm{CT}) + 0.038\,(\mathrm{T} \times \mathrm{CH}) \\ & - 0.049\,(\mathrm{CH} \times \mathrm{CT})\end{aligned}$$

($P \leq 0.01$, $R^2 = 0.94$).

Food Uses of Nixtamalized Amaranth Flour

Previous experiments carried out in our laboratory suggest that lime-cooked amaranth flour may be successfully used to replace maize, totally or partially, for tortilla preparation. This amaranth flour has also some basic functional properties to be used for snack production. The higher nutritional value of amaranth compared with maize (Tovar and Carpenter 1982, Mendoza and Bressani 1987) is an important advantage for most of the population in developing countries. However, it should be emphasized that further physicochemical, functional, and nutritional studies on nixtamalized amaranth flour need to be performed.

CONCLUSION

Changes of pH, WAI, color, and flow indices k and n of alkaline-cooked amaranth flour were highly dependent upon the process parameters T and CT. At the tested range of 0.8–1.0 g of $\mathrm{Ca(OH)_2}/100$ g of amaranth, CH was only significant ($P \leq 0.01$) on masa flow properties. Values of pH for all treated flours remained within the reported range for nixtamalized maize flours. WAI increased by increasing T and CT, which suggests that better product yields in weight might be obtained by using lime-cooked amaranth flour. Slight, but significant ($P \leq 0.01$), increases were observed for Hunter color values ΔE and ΔC. These color changes did not appear to be large enough to impair food uses of these flours. However, sensory evaluation studies need to be carried out. Viscosity studies showed that the power law can be used to correlate accurately the effects of τ and $\dot{\gamma}$ on indices k and n. These indices can be used as indicators of fluid performance. Further studies are needed to explore in detail the use of alkaline-cooked amaranth masa for preparation of tortillas and similar products.

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