

Combining Maltogenic Amylase with CMC or Wheat Gluten to Prevent Amylopectin Recrystallization and Delay Corn Tortilla Staling

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ABSTRACT

Cereal Chem. 81(5):654–659

Antistaling properties of a bacterial maltogenic amylase, sodium carboxymethylcellulose (CMC), and vital wheat gluten on quality of corn tortillas were evaluated during 14 days of storage. Amylopectin recrystallization was the driving force behind the staling of corn tortillas. Increasing levels of recrystallized amylopectin measured by differential scanning calorimetry (DSC) correlated significantly with increased tortilla stiffness ($r = 0.43$) and reduction in tortilla pliability ($r = -0.42$) during storage. Maltogenic amylase (275–1,650 activity units) made tortillas less stiff but did not preserve pliability and extensibility as effectively as CMC (0.25–0.5%). The combination of 825 MANU of malto-

genic amylase (to interfere with intragranular amylopectin recrystallization) and 0.25% CMC (to create a more flexible intergranular matrix than retrograded amylose and amylopectin) produced less stiff, equally flexible, and less chewy tortillas than did 0.5% CMC. Vital wheat gluten was not as effective as CMC in preserving tortilla flexibility or as good as the maltogenic amylase in reducing stiffness. Further research is required to optimize the addition of maltogenic amylases in continuous processing lines that use fresh masa instead of nixtamalized corn flour (NCF) and to determine how these amylases interfere with amylopectin recrystallization.

Tortilla staling is defined as a gradual decrease in rollability and pliability, a gradual increase in firmness, and a more friable and brittle structure (Friend et al 1992). The quality of a corn tortilla changes dramatically within the first 24 hr and then shows smaller changes for the remainder of its shelf life (Fernandez et al 1999; Limanond et al 2001). Most researchers (Suhendro 1997; Miranda 1999; Fernandez et al 1999) attribute changes in firmness of tortillas mainly to the recrystallization of the starch components especially the amylopectin fractions, as it happens with bread (Schoch and French 1947; Kulp and Ponte 1981).

Maltogenic amylases remove oligosaccharides with DP 2–7 from amylopectin and amylose. This shortening of amylopectin chain by enzymes reduce retrogradation tendencies of amylopectin (Boyle and Hebeda 1990). A thermostable maltogenic α -amylase from *Bacillus licheniformis* has been genetically engineered to exhibit maximal activity at $\approx 80^\circ\text{C}$ but with a level of 60% activity at room temperature (Fitter et al 2001). This type of enzyme would be adequate for the tortilla system because the rest period of masa, if any, and baking time are very short (10 and 1 min, respectively) compared with bread. Therefore, the enzyme should hydrolyze amylopectin during the rest period at a higher rate than regular enzymes and could be inactivated before the tortilla comes out of the oven to avoid product gumminess. Miranda (1999) used Novamyl 1,500 MG at levels of 0.04% (600 maltogenic amylase novo units [MANU]/kg of nixtamalized corn flour) in corn tortillas. Tortillas stored under refrigeration were more rollable and pliable than the control. Suhendro (1997) suggested the need for additives that create a new viscoelastic network to compensate for the weakened structure affected by the enzymes.

Sodium carboxymethylcellulose (CMC) is currently the most popular antistaling agent used in commercial tortillas for maintaining rollability and extensibility during storage. It is believed that CMC does not retard starch retrogradation of tortillas during storage. Instead, it creates a more flexible structure in the tortilla (Quintero-Fuentes 1999). When added in aqueous solutions at concentrations of 0.25% or higher CMC chains overlap causing

the formation of an amorphous network structure (Florjancic et al 2002). The recommended level for corn tortillas varies from 0.25–0.5% (Serna Saldívar et al 1990). However, tortillas with 0.5% CMC tend to have a rubbery texture regarded as undesirable by some consumers (Quintero-Fuentes 1999).

Wheat gluten has been evaluated as an antistaling agent in corn tortillas. Yau et al (1994), after testing a series of additives to dry masa, reported that a mixture of 0.5% CMC, 2% gluten, and 3% sorbitol extended storage stability of tortillas to 12 days; compared with a three-day shelf life of the control and seven days for tortillas with 0.5% added CMC. Apparently, gluten modified the structure of masa and baked corn tortillas and had a synergistic interaction with CMC and starch. Gluten incorporation $>2\%$ increased the number and size of burned spots in corn tortillas.

The objective of this study was to determine the combination of CMC, amylase, and vital wheat gluten that provides the softest, more flexible, and less rubbery tortilla texture, and the longest shelf-stability, and the role of these additives in preventing amylopectin recrystallization.

MATERIALS AND METHODS

Experimental Design

Three concentrations of Novamyl maltogenic amylase (0, 825, and 1,650 MANU/kg of NCF) provided by Innovative Cereal Systems (ICS), three levels of CMC (0, 0.25, and 0.5%), and three levels of vital wheat gluten (0, 1, and 2%) were evaluated. Using a central composite design, only 12 of the 27 possible treatment combinations were included in the experiment (Table I). The experiment was repeated three times (processing days).

Data were analyzed using response surface methodology (RSM) to generate second-order regression models using the RSREG procedure (v. 8, SAS Institute, Cary, NC). Response surface plots were produced using Microsoft Excel 2000 software. Plots show the combined effect of two of the additives on a particular tortilla trait, assuming the effect of the third additive is zero. Tukey's means separation test was performed with the MEANS statement and the Tukey option ($\alpha = 0.05$). Tukey's honestly significant difference (HSD) was used for treatment comparisons in graphs and tables.

Raw Materials

Nixtamalized (alkaline-cooked) corn flour (NCF) Tortilla #4 with no additives (Minsa, Red Oak, IA) was used to make tortillas. Antimolding agent potassium sorbate (ADM Arkady, Olathe, KS) and fumaric acid powder (Balchem Co. Slate Hill, NY) were added at 0.5 and 0.4% (based on NCF weight), respectively.

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A bacterial maltogenic amylase (Novamyl, provided by ICS, Wilsonville, OR) from *Bacillus subtilis* with MW 69 kDa, and an activity of 11,000 MANU/g was evaluated. MANU is defined as grams of enzyme that cleave 1 μmol of maltotriose/min at 60°C and pH 5.0 from a 5% starch solution. Novamyl amylase optimum activity is reached at 40°C and pH 6.

CMC used in this study (Blanose 7HF cellulose gum) is a commercial product of Aqualon. The molecular mass determined by the supplier is 4.35×10^5 g/mol, with degree of substitution (DS) of 0.65–0.90, pH of 6.5–8.5, sodium fraction of 7–8.9%, and an average viscosity of 2,500 mPa.sec at a 1% concentration.

Vital wheat gluten (Midwest Grain Products, Atchinson, KS) was also evaluated as an antistaling agent. Supplier-determined values were pH 5.5, protein 81.6% (db), and ash 1% (db).

Tortilla Preparation and Stabilization

Tortillas were prepared in the Cereal Quality Laboratory pilot plant at Texas A&M University. NCF (1 kg) was mixed with 5 g of potassium sorbate, 4 g of fumaric acid, CMC, maltogenic amylase, and vital wheat gluten for 5 min at low speed in a 20-qt mixer (model A-200, Hobart, Troy, OH). Distilled water (1.2 kg/kg of NCF) was added, and masa was formed with a hook for 30 sec at low speed and 90 sec at medium speed.

Masa was equilibrated in a polyethylene bag for 10 min before sheeting to disks 15 cm diameter, 30 g each (model CH4-STM, Superior Food Machinery, Pico Rivera, CA). Tortillas were baked in a gas-fired three-tier oven (320°C top, 270°C middle, and 220°C bottom) (model C-0440, Superior Food Machinery) for 60 sec, cooled, and stored folded in polyethylene bags at 21°C.

Tortillas were stabilized with methanol for DSC testing following the procedure used by Seetharaman et al (2002). A sample of 100 g of tortilla was mixed with 250 mL of methanol in a blender and ground for 2 min at maximum speed. The ground extract was filtered using vacuum with filter paper (Whatman #2) to remove the excess methanol. Another rinse with 250 mL of methanol for 2 min was followed by filtering before drying the stabilized extract at 50°C for 3 hr in a forced-air oven. Stabilized tortilla extracts were stored at –40°C until DSC testing.

Tortilla Yield, Moisture, and pH

Tortilla yield was recorded as kg of acceptable tortillas/kg of NCF. Moisture content was determined by grinding the tortilla in a coffee grinder (model KS M2, Braun, Lynnfield, MA) for 45 sec, and drying 4 g of ground sample to constant weight in a forced-air oven at 105°C for 48 hr (Approved Method 44-15A). Tortilla pH was determined using Approved Method 02-52 (AACC 2000).

Texture Measurements

Subjective and objective measurements of tortilla texture were evaluated 20 min, 1, 7, and 14 days after baking. Squeezing a tortilla in the palm of one hand, holding it for 2 sec, and then releasing it was done to evaluate pliability. The five-point scale was identified as 1, complete crumbling; 2, almost total crumbling; 3, a lot of cracking, no crumbling; 4, isolated cracks; and 5, completely pliable (Suhendro 1997).

Stress relaxation (Limanond et al 2001) and 1-D extensibility (Suhendro 1997) were performed on tortillas using a texture analyzer (model TA-XT2i, Texture Tech. Corp., Scarsdale, NY).

Stress Relaxation

The stress relaxation method developed by Guo (1998) and modified by Limanond et al (2001) was used to determine the changes on final stiffness (Pa) and energy dissipated (μJ/m³) by tortillas during storage as a function of time and temperature.

A uniform tortilla strip (70 × 35 mm) taken from the center of a baked tortilla was clamped between two grips. The distance between the two arms was set to 21.8 mm. The texture analyzer was set in the tension mode and the samples were tested at 3% strain levels (linear viscoelasticity region) for 180 sec. Pretest and posttest speed was 0.5 mm/sec. Test speed was 0.1 mm/sec.

The stress relaxation data (force as a function of time) were transformed into relaxation modulus (*E*) and then fitted to a generalized Maxwell model with seven parameters using a modification of the Matlab program developed by Spadaro (1996) and Guo et al (1999) (Mathworks, Natick, MA). Data were then transformed into compliance, stiffness, and energy dissipated using Matlab software v. 6.1. Further transformation into stiffness (*Y*) was determined as

$$Y(t) = \sigma_{ij} = (1/V) \int_V \sigma_{22} dV, \quad \epsilon_{ij} = (1/V) \int_V \epsilon_{22} dV$$

where σ_{ij} is the homogenized stress; ϵ_{ij} is the homogenized strain; *V* is the volume of the tortilla sample; σ_{22} and ϵ_{22} are the normal stress and strain acting in the plane perpendicular to x_2 in the direction of x_2 , respectively. Stiffness is the ratio of homogeneous stress to the homogeneous strain, which may be referred to as the modulus of elasticity or Young's modulus. This parameter indicates the hardness of materials. The higher value corresponds to a harder (firmer) material (more solid-like). Data is reported as average of triplicates.

1-D Extensibility

A tortilla strip (70 × 35 mm) was held between two tensile grips, with one end attached to the analyzer platform and the

TABLE I
Effect of Sodium Carboxymethylcellulose (CMC), Maltogenic Amylase (MANU), and Wheat Gluten Combinations on Tortilla pH, Moisture, and Yield

Treatment Combination			Moisture (%)	pH	Yield (kg/kg of NCF) ^b
CMC (%)	Amylase (MANU) ^a	Gluten (%)			
0	0	0	48.5	4.84	0.93
0	0	2	47.1	4.88	0.99
0	825	1	47.3	4.84	1.03
0	1,650	0	47.6	4.85	0.94
0.25	0	1	47.7	4.85	1.02
0.25	825	0	47.7	4.91	1.15
0.25	825	1	48.1	4.87	1.04
0.25	825	2	46.7	4.84	1.09
0.25	1,650	1	47.7	4.83	1.09
0.5	0	0	47.3	4.89	1.05
0.5	825	1	47.4	4.91	1.10
0.5	1,650	2	47.3	4.94	1.04
			0.6 ^c	0.16 ^c	0.10 ^c

^a MANU, maltogenic activity novo units.

^b NCF, nixtamalized corn flour.

^c HSD, Tukey's honest significant difference ($\alpha = 0.05$) for means separation.

other end attached to the analyzer arm (Suhendro 1997). The distance between the tensile grips was calibrated at 21.8 mm. During the test, the tortilla was pulled until it broke apart. The extensibility method was run using Texture Expert software in tension mode with the return to start option. The maximum force (N) and distance (mm) required to break apart the tortilla was calculated. Data are reported as the average of quintuplicates.

Differential Scanning Calorimetry

DSC thermal analysis of methanol-stabilized samples of corn tortillas was performed after 20 min (fresh) and 14 days of storage (model DSC-1, Perkin Elmer, Norwalk, CT). Previously starch-stabilized tortilla extract samples (4 mg) were rehydrated 20 min before heating with 8 mg of water and hermetically sealed in aluminum pans. Each sample was heated at a rate of 10°C/min from -40 to 100°C.

The parameters evaluated were ΔH (enthalpy of water and amylopectin crystal fusion in J/g), peak water and amylopectin melting temperature (T_p in °C), and tortilla midpoint glass transition temperature (T_g).

RESULTS

Masa Quality

All treatment combinations produced masas with optimum cohesiveness and without excessive stickiness. Therefore, masas were sheetable.

Tortilla Moisture, pH, and Yield

Differences in tortilla moisture content among treatments, although statistically significant, were within the acceptable range (Table I). Tortilla pH was similar for all treatments, with an overall mean value of pH 4.83 and CV 7.2%.

As previously reported (Serna-Saldivar et al 1990), only CMC significantly increased tortilla yield (Table I) compared with the control. This increase in yield was caused by improved masa machinability that produced more acceptable tortillas because moisture content of tortillas with CMC was actually lower than that of control tortillas. Tortilla yields in this study were also lower than the industry standard due to tortillas folding and cracking in the oven belts. Addition of 1,650 MANU of maltogenic amylase did not significantly reduce tortilla yield compared with the control, as seen when higher levels of amylase were evaluated.

Changes in Tortilla Texture During Storage

Tortillas from all treatments received the highest score (5) for pliability 20 min after baking. However, after 14 days of storage, significant differences in tortilla pliability were observed among treatments (Fig. 1). Combinations of 550–1,100 MANU of maltogenic amylase and 1% gluten produced tortillas with pliability statistically similar to tortillas with 0.5% CMC, suggesting a synergy between the softening effect of the maltogenic amylase and the flexible matrix-building effect of gluten. However, only combinations of 0.5% CMC plus 550–1,100 MANU of maltogenic amylase produced tortillas with a pliability score >2 and significantly higher than 0.5% CMC alone.

The stress relaxation test did not detect significant differences in tortilla stiffness among treatments 20 min after baking. The second-order regression model explained differences in tortilla stiffness well ($R^2 = 0.85$) and consistently (CV 9.7%). CMC, maltogenic amylase, and gluten significantly changed stiffness of tortillas after 14 days (Fig. 2) compared with the control. CMC significantly increased tortilla stiffness when added alone at levels >0.25% compared with the control. According to the response surface model, 275 MANU of maltogenic amylase was enough to significantly decrease stiffness of tortillas after 14 days. Tortilla

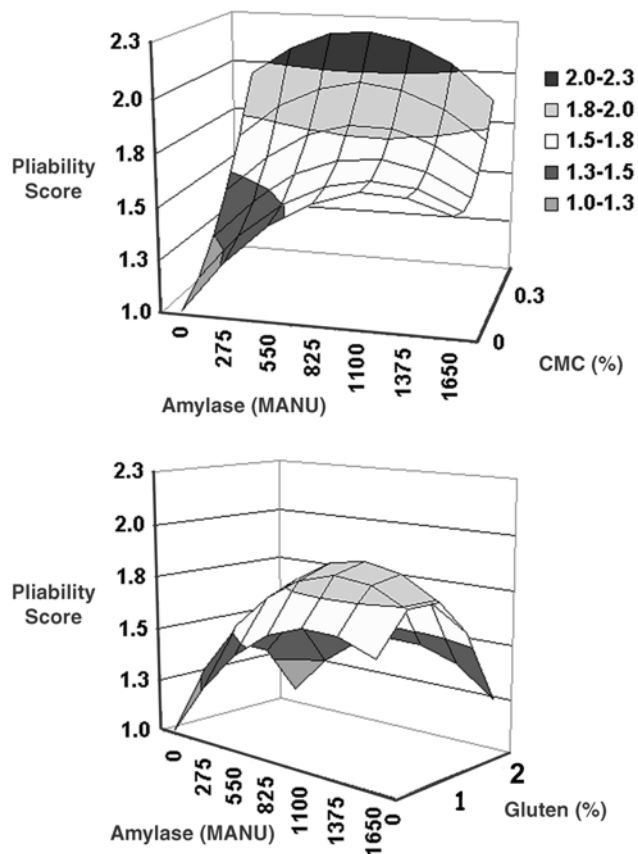


Fig. 1. Pliability of tortillas stored 14 days containing maltogenic amylase, CMC, and vital wheat gluten. HSD = 0.25.

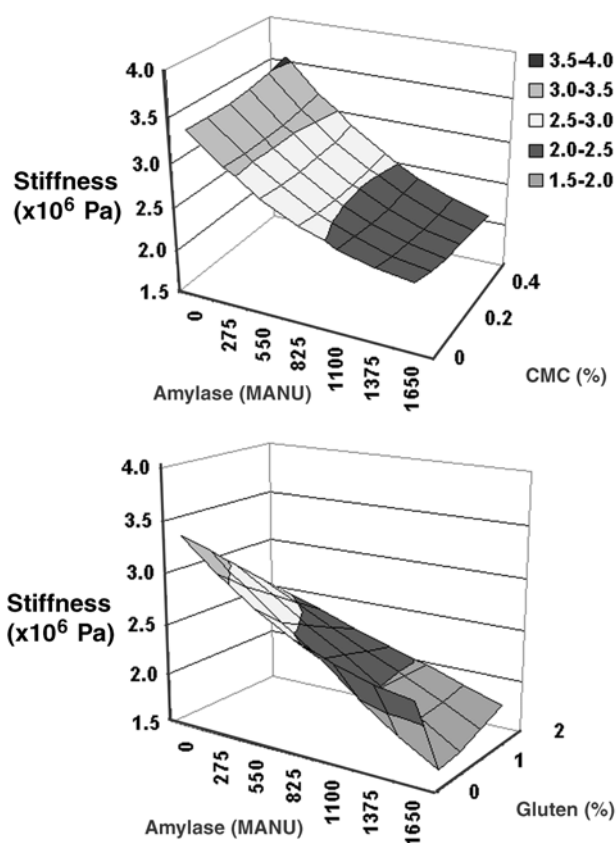


Fig. 2. Final stiffness of tortillas stored 14 days containing maltogenic amylase, CMC, and vital wheat gluten. HSD = 0.21 × 10⁻⁶ Pa.

stiffness was not affected by CMC, only by the level of amylase used. Vital wheat gluten ($\geq 1\%$) significantly decreased tortilla stiffness after 14 days of storage compared with the control, especially at the lower levels of amylase. However, the reduction in tortilla stiffness by amylase was significantly more dramatic than that of gluten.

In fresh tortillas (20 min after baking), no native endothermic peak was detected by DSC in the 45–65°C range for any treatment. Melting of recrystallized amylopectin usually occurs within this temperature range in starch-based products (Campas-Baypoli et al 2002). DSC detected an endothermic melting peak in methanol-stabilized tortilla extracts after 14 days of storage. Onset of amylopectin melting occurred at 50.3°C, peaked at 57.4°C, and ended at 65.9°C. CMC, amylase, and gluten did not alter these temperatures significantly in tortillas after 14 days of storage.

The endothermic amylopectin melting peak (ΔH , J/g) for tortillas after 14 days of storage was significantly reduced only by maltogenic amylase (Fig. 3). The presence of CMC and or gluten did not affect amylase activity and the enthalpy value. Therefore, only amylase affected DSC results compared with the control.

In fresh tortillas (20 min after baking), only CMC ($\geq 0.25\%$) significantly increased rupture distance, making tortillas more extensible than the control. Significant reductions in rupture distance in tortillas were observed during storage: 8.8 mm after 20 min (fresh) and 1.72 mm after 14 days. Again, CMC ($\geq 0.25\%$) was the only additive that made tortillas significantly more extensible than the control after 14 days of storage (Fig. 4). Both maltogenic amylase (≥ 550 MANU) and gluten ($\geq 1\%$) produced tortillas with significantly lower extensibility than that of the control when added alone. Amylase and gluten made tortillas softer but also less extensible.

DISCUSSION

For all treatments in this study, 18 min passed from the start of dry mixing until tortilla baking was completed. Hydration is the starting critical moment for additive activation and tortilla structure formation. Maltogenic amylase (MW 69 kDa), which is a much smaller molecule than CMC, wheat gluten, and NCF particles (Gomez 1990; Florajancic et al 2002), was probably dispersed in the continuous matrix formed by hydrated NCF particles, CMC, and wheat gluten. Maltogenic amylase ($\leq 1,650$ MANU) did not affect handling properties of masa. Dextrinization of starch was limited, there was no excessive water absorption, and masa stickiness was avoided. The relatively low proportion of enzyme-susceptible starch (30% in NCF vs. 55% in fresh masa) available (Gomez et al 1991), and the short time allowed for enzyme activity (17 min) before inactivation during baking limited amylase activity in the masa. Therefore, undesirable tortilla gumminess, crumbliness, and sweet taste were avoided. Lower levels of amylases should be required when using fresh masa instead of NCF due to its higher level of enzyme-susceptible starch.

At least 0.25% CMC was necessary to significantly improve masa machinability and consequently increase tortilla yields. CMC 0.25–0.5% is added commercially to improve reconstituted dry masa cohesiveness and to increase machinability and tortilla yields (Serna-Saldivar 1996). Given that CMC has a higher affinity for water than the rest of the masa components, it is reasonable to believe that its linear molecules will tend to form an entangled amorphous matrix around hydrated NCF particles, therefore increasing the cohesiveness and flexibility of masa compared with the control and improving handling properties. Subjective masa texture observations and tortilla yield data (Table I) support this theory.

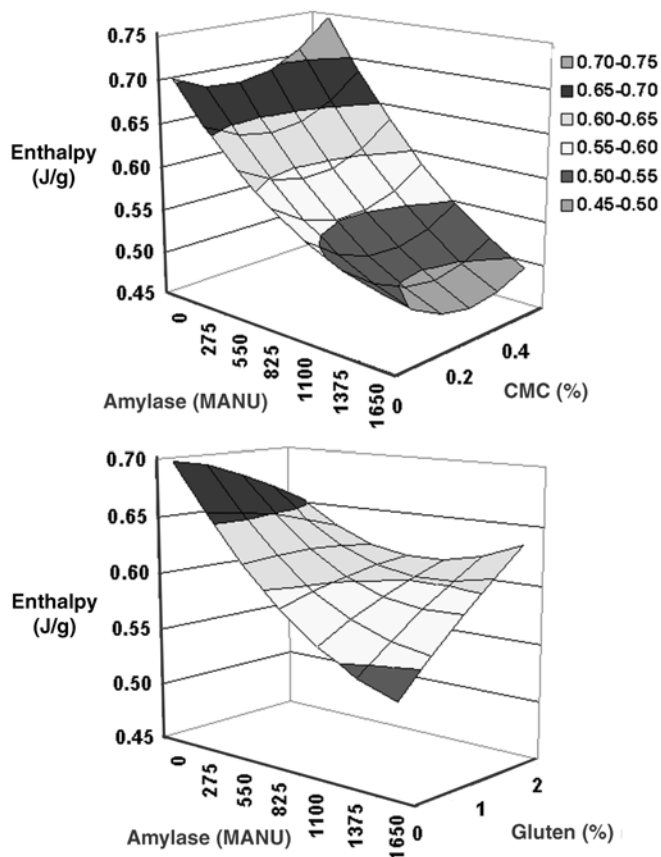


Fig. 3. Effect on enthalpy of amylopectin melting of starch residues from tortillas stored 14 days. HSD = 0.15 J/g.

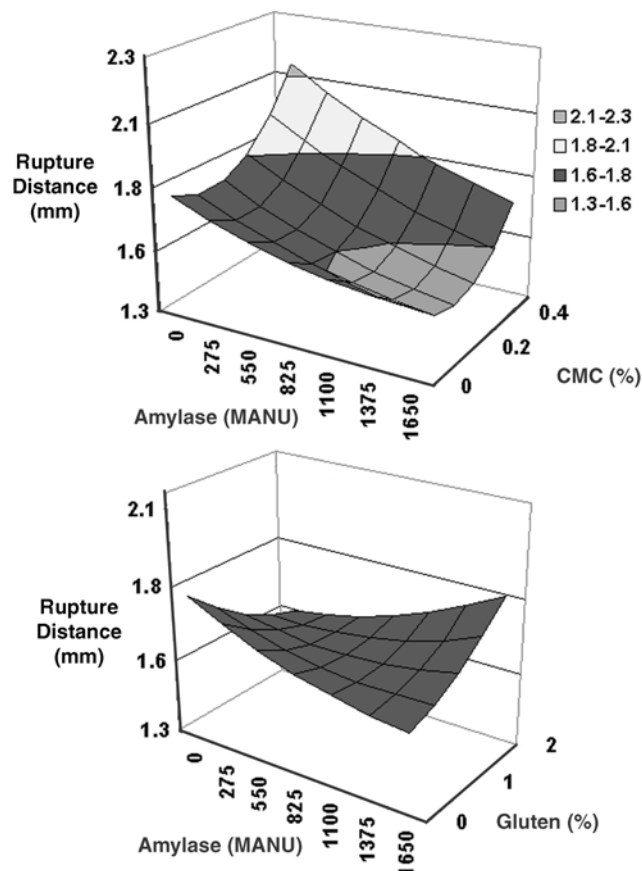


Fig. 4. Rupture distance of tortillas stored 14 days containing maltogenic amylase, CMC, and vital wheat gluten. HSD = 0.21 mm.

Hydrated vital wheat gluten will also tend to form a cross-linked and entangled amorphous matrix between NCF particles, similar to that of the gluten network in bread dough. However, 2% wheat gluten apparently was not enough to generate a matrix as cohesive and flexible as CMC in the masa. Lower hydration capacity of vital gluten compared with CMC and shorter NCF mixing time compared with breadmaking might also have confined matrix development to certain clusters in masa, rendering gluten unable to improve masa machinability as CMC did.

Baking tortillas took ≈ 60 sec. During this period and the subsequent cooling stage, the tridimensional structure of the tortilla was set. Gelatinization of starch occurred, amylopectin crystallinity disappeared, and double helices of amylose leached from the granule to form a flexible, amorphous, insoluble network on retrogradation in the intergranular aqueous continuous phase (Gomez et al 1992; Fernandez et al 1999).

Limited hydrolysis of amylose and amylopectin by 1,650 MANU of maltogenic amylase did not significantly weaken fresh tortilla structure to the point of reducing extensibility (lower rupture distance) compared with the control. Gomez et al (1992) and Fernandez et al (1999) proposed retrograded (cross-linked) amylose gel as the glue that binds and holds the fresh tortilla together. If that model is true, then 1,650 MANU of maltogenic amylase did not significantly hydrolyze the amylose matrix during masa reconstitution and baking, and its antistaling properties were not related to its activity on the amylose matrix.

Addition of 0.25% CMC made fresh tortillas more elastic and cohesive than the control, therefore requiring more distance to rupture. Wheat gluten did not change fresh tortilla texture significantly, suggesting a lower ability to form a flexible matrix than CMC. Furthermore, addition of vital wheat gluten should be limited to no more than 1% because higher levels introduced a noticeable wheat flavor and produced a higher number of brown spots on the tortilla surface. This confirms the findings of Yau et al (1994) and Miranda (1999).

A tortilla with acceptable texture should have rollability and pliability scores of at least 4 and requires no more than 4 N or 7 mm of extension to break. Stiffness values should be lower than 0.5×10^6 Pa and energy dissipated at least 1×10^{-3} J/m³. These values correspond to tortillas without additives stored for 4 hr at room temperature (21°C).

DSC analyses of fresh tortillas (20 min after baking) were unable to detect an endothermic melting peak corresponding to native or retrograded amylopectin within the 45–70°C temperature range in any sample (data not shown). DSC results suggest that amylopectin in tortillas lost its crystallinity during baking and was in an amorphous state 20 min after baking. No significant amylopectin recrystallization (retrogradation) had occurred 20 min after baking and tortillas were perfectly pliable. This supports the theory that tortilla staling, just like bread staling, is a process dominated by the nonequilibrium recrystallization of amylopectin (Levine and Slade 1991). The appearance of a detectable endothermic melting peak at 57°C in all tortilla samples stored for 14 days further confirms this theory. Control tortillas stored for 14 days were significantly less pliable than tortillas with CMC and maltogenic amylase (Fig. 1).

As little as 275 MANU of maltogenic amylase were enough to reduce the enthalpy of amylopectin melting (Fig. 3) and produce softer tortillas (lower stiffness) than the control after 14 days of storage (Fig. 2). It can be concluded that the antistaling properties of maltogenic amylase rely on preventing the intragranular recrystallization of amylopectin in tortillas during storage. Boyle and Hebeda (1990) proposed that the reduction in length of amylopectin outer branches by removal of maltooligosaccharides (DP 2–7) during mixing and baking was the mode of action of antistaling maltogenic amylases in bread. A higher proportion of short outer branches of amylopectin (DP 6–11) have been associated with reduced retrogradation in maize starches (Shi and Seib 1995).

CMC and gluten did not reduce amylopectin enthalpy compared with the control in tortillas stored 14 days, confirming that antistaling properties do not rely on preventing amylopectin recrystallization inside or outside the starch granule. As suggested in previous studies (Gomez et al 1991; Suhendro 1997; Miranda 1999; Quintero-Fuentes 1999), CMC and gluten most likely delay staling by creating a more flexible matrix than amylose alone in the tortilla intergranular space.

A combination of 0.25% CMC and 275–825 MANU of maltogenic amylase produced tortillas with better texture after 14 days of storage at 21°C. Tortillas were softer than the control; and less chewy and equally flexible than tortillas with 0.5% CMC. Tortilla pliability (Fig. 1) and rupture distance data (Fig. 4) support the need for a two-way approach in delaying tortilla staling by using combinations of amylase (to reduce amylopectin recrystallization) and an amorphous, matrix-forming additive (CMC) to counteract the collateral damage caused by the hydrolytic activity of amylase on the intergranular amylose matrix and provide a more flexible “tortilla skeleton”.

Reductions in tortilla stiffness and extensibility by addition of $\geq 1\%$ gluten might be caused by limited interference in the formation of the amylose network during baking at the intergranular spaces. Similar effects have been seen by addition of 5% native soy flour to corn tortillas (unpublished data). When matrix-forming molecules are added in insufficient amounts to become the predominant continuous phase, they will be dispersed into clusters that will interfere with the amylose matrix at random points. Tortillas with these additives (gluten or soy flour) will be softer (shorter structure) but loss of extensibility during storage will not be reduced. Wheat gluten might be useful as a softening agent at levels $\leq 1\%$ in commercial tortillas as long as the cost is lower than adding 275 MANU of a maltogenic amylase per kg of NCF.

CONCLUSIONS

The results from this study give a clearer picture of the mechanisms that lead to corn tortilla staling and how tortilla components and additives accelerate or delay this process. Addition of 275–1,650 MANU of maltogenic amylase effectively reduced amylopectin retrogradation but was not able to maintain tortilla flexibility. Even though amylopectin recrystallization is the main force behind tortilla staling, it does not mean that interfering with this process is the only way to maintain tortilla softness and flexibility.

Delaying tortilla staling requires a two-pronged approach: interfering with amylopectin recrystallization inside gelatinized starch granules and creating a more flexible intergranular matrix than recrystallized amylose provides (with CMC, β -glucans, pentosans, or soybean proteins). The combination of 825 MANU of maltogenic amylase and 0.25% CMC appeared to do that. Tortillas with this combination of additives were softer, equally flexible, and less chewy than tortillas with only 0.5% CMC. This combination of additives makes stored tortillas resemble more closely the original texture of a fresh tortilla without additives than those with only 0.5% CMC. Further studies should evaluate combinations of maltogenic amylases (both fungal and bacterial) with other matrix-forming additives that may be able to substitute CMC totally or partially (β -glucans, pentosans, etc.) at a lower cost, and determine the mode of action of maltogenic amylases in preventing amylopectin recrystallization.

ACKNOWLEDGMENTS

This research was partially funded by the Tortilla Industry Association.

LITERATURE CITED

- Boyle, P. J., and Hebeda, R. E. 1990. Antistaling enzyme for baked goods. *Food Technol.* 44:129.

- Campas-Baypoli, O. N., Rosas-Burgos, E. C., Torres-Chavez, P. I., Ramirez-Wong, B., and Serna-Saldivar, S. O. 2002. Physicochemical changes of starch in maize tortillas during storage at room and refrigeration temperatures. *Starch* 54:358-363.
- Fernandez de Castro, D. A., Waniska, R. D., and Rooney, L. W. 1999. Changes in starch properties of corn tortillas during storage. *Starch* 51:136-140.
- Fitter, J., Herrmann, R., Dencher, N., and Blume, A. 2001. Activity and stability of a thermostable alpha-amylase compared to its mesophilic homologue: Mechanisms of thermal adaptation. *Biochemistry* 40:10723-10731.
- Florjancic, U., Zupancic, A., and Zumer, M. 2002. Rheological characterization of aqueous polysaccharide mixtures undergoing shear. *Chem. Biochem. Eng.* 16:105-118.
- Friend, C. P., Waniska, R. D., and Rooney, L. W. 1992. Effects of hydrocolloids on processing and qualities of wheat tortillas. *Cereal Chem.* 70:252-255.
- Gómez, M. H., Lee, J. K., McDonough, C. M., Waniska R. D., and Rooney, L. W. 1992. Corn starch changes during tortilla and tortilla chip processing. *Cereal Chem.* 69:275-279.
- Gómez, M. H., Waniska R. D., and Rooney, L. W. 1991. Starch characterization of nixtamalized corn flour. *Cereal Chem.* 68:578-582.
- Gómez, M. H., Waniska R. D., and Rooney, L. W. 1990. Effects of nixtamalization and grinding conditions on starch in masa. *Starch* 42:475.
- Guo, Z. 1998. Prediction of corn tortilla textural quality using stress relaxation methods. MS thesis. Texas A&M University: College Station, TX.
- Guo, X. H., Castell-Perez, M. E., and Moreira, R. 1999. Characterization of masa and low-moisture corn tortilla using stress relaxation methods. *J. Texture Stud.* 30:197-215.
- Kulp, K., and Ponte, J. G. 1981. Staling of white pan bread: Fundamental causes. *Crit. Rev. Food Sci. Nutr.* 15:11.
- Levine, H., and Slade, L. 1991. A food polymer science approach to structure-property relationships in aqueous food systems: Non-equilibrium behavior of carbohydrate-water systems. Pages 29-101 in: *Water Relationships in Foods. Advances in the 1980s and Trends for the 1990s.* H. Levine and L. Slade, eds. Plenum Press: New York.
- Limanond, B., Castell-Perez, M. E., and Moreira, R. 2001. Modeling the kinetics of corn tortilla staling using stress relaxation data. *J. Food Eng.* 53:237-247.
- Miranda-Lopez, R. 1999. Effects of some anti-staling additives, pH and storage on the staling of corn tortillas. PhD dissertation. Texas A&M University: College Station, TX.
- Quintero-Fuentes, X. 1999. Characterization of corn and sorghum tortillas during storage. PhD dissertation. Texas A&M University: College Station, TX.
- Schoch, T. J., and French, D. 1947. Studies on bread staling. I. The role of starch. *Cereal Chem.* 24:231-249.
- Seetharaman, K., Chinappa, N., Waniska, R. D., and White, P. 2002. Changes in textural, pasting and thermal properties of wheat buns and tortillas during storage. *J. Cereal Sci.* 35:215-223.
- Serna-Saldivar, S. O. 1996. *Química, almacenamiento e industrialización de los cereales.* Alhambra Editor: México.
- Serna-Saldivar, S. O., Gómez, M. H., and Rooney, L. W. 1990. Technology, chemistry and nutritional value of alkaline-cooked corn products. Page 243 in: *Advances in Cereal Science and Technology.* Vol. X. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Spadaro, V. S. 1996. Biomechanical characterization of meat texture. PhD dissertation. Texas A&M University: College Station, TX.
- Suhendro, E. 1997. Instrumental methods for the evaluation of corn tortilla texture. PhD dissertation. Texas A&M University: College Station, TX.
- Yau, J., Waniska, R. D., and Rooney, L. W. 1994. Effects of food additives on storage stability of corn tortillas. *Cereal Foods World* 39:396-402.

[Received October 23, 2003. Accepted March 3, 2004.]