Effect of Temperature on Texture of Corn Tortilla With and Without Antistaling Agents

Francisco J. Bueso,[1,2] Ralph D. Waniska,[2] Rosana Moreira,[3] Koushik Seetharaman,[4] and Lloyd W. Rooney[2]

ABSTRACT

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Tortilla stiffening should occur between –23 to 57°C, showing a maximum rate near the midpoint of this range (17°C). Starch recrystallization below the glass transition temperature (T_g = –23°C) in corn tortillas is minimal due to lack of molecular mobility. The objective of this study was to determine the effect of storage temperature (–20 to 21°C) on the stiffening rate of corn tortillas with or without additives (carboxymethylcellulose [CMC] and maltogenic amylase). Tortilla pliability, stiffness, and energy dissipated obtained by stress relaxation, and amylopectin recrystallization determined by differential scanning calorimetry (DSC) showed a second-order polynomial relationship with temperature. Tortillas became stiff faster during refrigerated storage (3–10°C). Adding 0.25% CMC and 1,650 AU of amylase maintained tortilla softness and flexibility, both at room temperature and under refrigeration for at least three weeks.

When a tortilla is baked in the oven, masa transforms into an amorphous, rubbery material. A fresh tortilla, right out of the oven is a partially crystalline system due to retrograded amylose (Campas-Baypoli et al 2002). Apart from the amorphous phase (amylopectin), it contains crystal nuclei (retrograded amylose) in the rubbery matrix (continuous phase). According to Limanond et al (2001), below the glass transition temperature (T_g = –23°C) the amorphous phase is glassy, therefore, the composite material will show the same aging behavior as a purely amorphous polymer.

Unlike amorphous polymers, however, semicrystalline polymers age (recrystallize) at temperatures above the T_g. The magnitude of tortilla stiffening is a function of aging time and temperature. The retrogradation phenomenon has been described as a nonequilibrium polymer crystallization process in starch-water polymer melts (Levine and Slade 1990).

The rate of retrogradation of gelatinized waxy maize starch (Farhat et al 2000), white bread (Russell 1985), cakes (Guy et al 1983), and corn tortilla (Limanond et al 2001) show a second order "bell-shaped" dependence on storage temperature in a range between T_g and melting temperature (T_m) of the product. This behavior is in agreement with the general theory of crystallization, where the effect of temperature on the rate of crystallization is the result of its net effect on the nucleation and propagation rates (reviewed by Levine and Slade 1990).

Maximum rate of retrogradation was 25°C for cakes (Guy et al 1983), 13°C for corn tortillas (Limanond et al 2001), and 4°C for bread (Russell 1985). Compared with storage at room temperature, storage of starch gels containing 45–50% water (like corn tortillas) at low temperatures but still above the glass transition temperature ($T_g \approx$ –5°C), increase retrogradation, especially during the first days of storage (Gudmunsson 1994). Storage at freeze temperatures below T_g virtually inhibits recrystallization (Gudmunsson 1994). Higher temperatures (>32–40°C) effectively reduced retrogradation of wheat starch gels (Colwell et al 1969) and corn tortillas (Limanond et al 2001).

Limanond et al (2001) studied the crystallization rate of corn tortillas in the 6–35°C storage temperature range. The crystallization rate (k) estimated by the modified Avrami-nucleation model increased from 6 to 20°C and started decreasing from 20 to 35°C. The maximum crystallization was estimated at 12.3°C based on stiffness data from stress relaxation tests. In other words, tortillas staled at a slower rate when stored at room temperature (25°C) than when refrigerated (4°C).

MATERIALS AND METHODS

Experimental Design

Tortillas made with a combination of 1,650 AU of maltogenic amylase (Novamyl) and 0.25% carboxymethylcellulose (CMC) were evaluated in comparison with control tortillas (no additives) and tortillas containing 0.5% CMC under four storage temperatures (–20, 3, 10, and 21°C). A split-plot design with two replicates was used in the experiment. The main plots were the storage temperatures, and the treatments were designed as the subplots.

Raw Materials

Nixtamalized 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.

A bacterial maltogenic amylase (Novamyl, Innovative Cereal Systems, Wilsonville, OR) from *Bacillus subtilis* with a molecular weight of 69 kDa and an activity of 11,000 AU/g was evaluated. Optimum activity of the maltogenic amylase is reached at 40°C and pH 6.

Sodium carboxymethylcellulose (CMC) was the cellulose gum used in this study (Blanose 7HF, Aqualon, Wilmington, DE). The molecular mass determined by the producer is 4.35×10^5 g/mol, with a degree of substitution 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.

Tortilla Preparation and Stabilization

Tortillas were prepared in the Cereal Quality Laboratory pilot plant at Texas A&M University. Nixtamalized corn flour (NCF, 1 kg) was mixed with 5 g of potassium sorbate, 4 g of fumaric acid, CMC, and amylase 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 moisture was equilibrated in a polyethylene bag for 10 min before sheeting into 30-g disks, 15 cm in diameter (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 in low-density polyethylene bags (2-mm film thickness) at the designated temperature.

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[1] Currently at Escuela Agricola Panamericana, Tegucigalpa, Honduras. Corresponding author. Phone: 504-776-6140 ext 2205. E-mail: fbueso@zamorano.edu

[2] Dept. Soil and Crop Sciences, Texas A&M University, College Station, TX 77843.

[3] Dept. Biological and Agricultural Engineering, Texas A&M University, College Station, TX 77843.

[4] Dept. Food Science, Penn State University, University Park, PA 16802.

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Tortillas were stabilized with methanol for differential scanning calorimetry (DSC) testing. 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 with vacuum using filter paper (Whatman #2) to remove the excess methanol. Another rinse with 250 mL of methanol for 2 min followed by filtering was performed 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.

Moisture and pH

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 (a variation of Approved Method 44-15A). The pH of tortillas was determined using Approved Method 02-52 (AACC International 2000).

Texture Measurements

Tortillas were evaluated for subjective pliability and objective texture (stress relaxation) in triplicate at 20 min, 1, 7, and 21 days after baking. Frozen tortilla samples were microwaved for 10 sec to remove ice from the surface and then allowed to equilibrate to room temperature (21°C) for 1 hr before performing texture evaluations.

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 defined as 1 complete crumbling; 2 almost total crumbling; 3 a lot of cracking, no crumbling; 4 isolated cracks; and 5 completely pliable (no cracks).

Stress relaxation (Limanond et al 2001) and 1-D extensibility (Suhendro 1997) were performed on tortillas using a texture analyzer (model TA-XT2i, Texture Technology, 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/m^3) of tortillas during storage as a function of time and temperature.

A 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 system was set in the tension mode and the samples were tested at 3% strain levels (linear viscoleasticity region) for 180 sec. Pre- and post-test 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). Data were then transformed into compliance, stiffness, and energy dissipated using Matlab software (v.6.1, The Mathworks, Natick, MA).

Further transformation into stiffness (*Y*) was determined as

$$Y(t) = \frac{\sigma_{ij}}{\varepsilon_{ij}} = \frac{(1/V)\int_V \sigma_{22} dV}{(1/V)\int_V \varepsilon_{22} dV}$$

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).

Differential Scanning Calorimetry

Thermal analysis of methanol-stabilized corn tortilla samples was performed (model DSC-1, Perkin Elmer, Norwalk, CT). Starch-stabilized tortilla extract samples (4 mg) were rehydrated 20 min before heating with 8 mg of water and hermetically sealed in aluminum pans. The samples were then 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 measured in J/g), peak water, and amylopectin melting temperature (T_p measured in °C), tortilla midpoint glass transition temperature (T_g).

Statistical Analysis

Statistical analyses were performed using SAS products (v.8, SAS Institute, Cary, NC). Analysis of variance was performed using PROC GLM. Tukey's means separation test was performed with the MEANS statement and the Tukey option (α = 0.05%). Tukey's honest significant difference (HSD) was used for treatment comparisons.

RESULTS

Masa Quality

All three treatments produced masas with optimum cohesiveness and low stickiness. Masas were machinable, and tortilla yields were not significantly different among treatments.

Tortilla Quality

Tortilla moisture content and pH were not significantly different among treatments (Table I). The coefficient of variation (CV) was 1.22% for moisture content and 1.68% for pH.

Tortillas were individually packaged in polyethylene bags and frozen (–3°C) after 15 min of storage in a –20°C freezer. It took 2 hr for tortillas to reach –20°C in the freezer.

A second-order polynomial (bell-shaped) relationship between storage temperature and tortilla pliability (R^2 = 0.991) was visible for all treatments after just one day (Fig. 1). Loss of tortilla pliability under storage at freezing temperature (–20°C) was significantly higher than at room temperature (21°C) but not as high as under refrigeration (3–10°C). Tortillas without additives were least pliable when stored at 3°C, while tortillas with additives were least pliable at 10°C.

Pliability of tortillas after seven days of storage significantly decreased compared with tortillas evaluated after one day of storage at room and refrigeration temperatures (Fig. 1). Reductions in pliability for frozen tortillas were significant only for tortillas with <0.5% CMC. Again, storage at refrigeration temperatures produced tortillas with least pliability, treatment notwithstanding.

Freezing preserved tortilla pliability better than room and refrigeration temperatures after 21 days of storage, especially when 0.5% CMC was added. CMC may have provided improved freeze-thaw stability to tortilla. Refrigeration (3–10°C) caused the biggest losses in pliability.

A combination of 0.25% CMC and 1,650 AU of maltogenic amylase produced tortillas with higher pliability than control and tortillas with 0.5% CMC when stored at room temperature and under refrigeration (Fig. 1).

TABLE I
Effect of Maltogenic Amylase and Carboxymethylcellulose (CMC) on Moisture Content and pH of Fresh Tortillas

Treatment	Moisture Content (%)	pH
Control	47.5	4.80
0.5% CMC	47.5	4.89
0.25% CMC + 1,650 AU	47.4	4.90
HSD (0.05%)[a]	0.68	0.10

[a] Tukey's honest significant difference (HSD).

Tortilla Stiffness

Significant changes in tortilla stiffness due to storage temperature and additives were observed during 21 days (Fig. 2). The effect of amylase and CMC was not the same on tortilla stiffness at different storage temperatures. The model explained differences in stiffness among treatments well ($R^2 = 0.94$ overall and $R^2 = 0.91$ at 21 days). Precision of the stress relaxation method to estimate stiffness of tortillas was good (CV = 11.2% overall, CV = 16% at 21 days).

Fresh tortillas (20 min after baking) had similar stiffness regardless of the treatment. Most of the variation in tortilla stiffness was due to storage time. When tortilla stiffness was evaluated 21 days after storage most of the differences among treatments were caused by storage temperature and then by the additives.

A second-order polynomial model explained very well ($R^2 = 0.996$) tortilla stiffness dependence on storage temperature one day after baking (Fig. 2). Stiffness of the tortillas stored frozen (–20°C) was similar to that of tortillas stored at room temperature (21°C). Tortillas stored under refrigeration (3–10°C) were significantly stiffer than tortillas stored frozen or at room temperature. One-day-old tortillas with 0.25% CMC and 1,650 AU of maltogenic amylase were significantly less stiff than control tortillas at all storage temperatures, except for –20°C (Fig. 2).

Stiffness of tortillas increased significantly during the first week of storage regardless of temperature or treatment. Further stiffening was significant only when tortillas without additives or with 0.5% CMC were stored at 10°C (Fig. 2). This indicates that amylase was more effective in producing less stiffness than CMC, even at the storage temperature with the highest stiffening rate (10°C). Tortillas with no additives or with only 0.5% CMC were significantly less stiff when frozen 21 days than when stored refrigerated or at RT.

Enthalpy of Tortilla Amylopectin Retrogradation

For tortillas sampled 20 min after baking (and stabilized using methanol), DSC showed an endotherm at 57°C (data not shown). The endotherm is consistent with melting of recrystallized amylopectin. We are continuing our investigation of this observation.

Storage temperature significantly changed melting enthalpy of recrystallized amylopectin in tortillas (Fig. 3). Tortillas stored under refrigeration temperatures (3–10°C) for more than one week showed higher enthalpy values than tortillas stored frozen or at room temperature (Fig. 3). Amylase reduced the enthalpy during amylopectin melting of tortillas stored at freezing and at room temperature for 21 days compared with the control and 0.5% CMC tortillas. However, this reduction was not statistically significant under refrigeration temperatures.

Changes in Tortilla Viscous Component: Energy Dissipated

Energy dissipated (J/m^3) by tortillas decreased significantly during storage, and especially during the first 24 hr (1.22×10^{-4} J/m^3) compared with fresh tortillas (8.25×10^{-4} J/m^3) with no significant changes occurring afterward. A staled tortilla shows more solid behavior and less viscous properties than a fresh tortilla, therefore dissipating less energy when deformed.

Energy dissipated was a less precise indicator of changes in tortilla texture (CV = 76% overall, CV = 68% at 21 days) than stiffness and pliability. Model $R^2 = 0.73$ for the complete set of data and $R^2 = 0.41$ for measurements taken 21 days after baking.

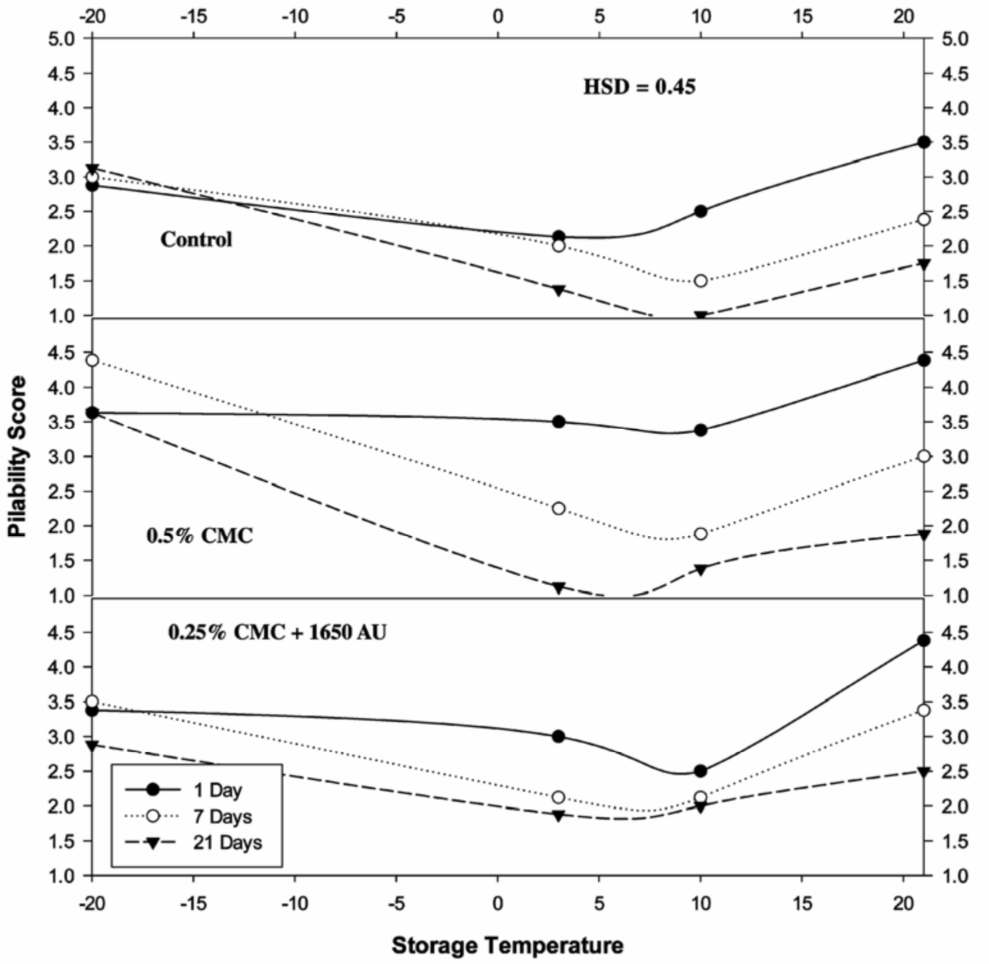

Fig. 1. Effect of storage temperature on pliability of tortillas with added carboxymethylcellulose (CMC) and maltogenic amylase after 1, 7, and 21 days.

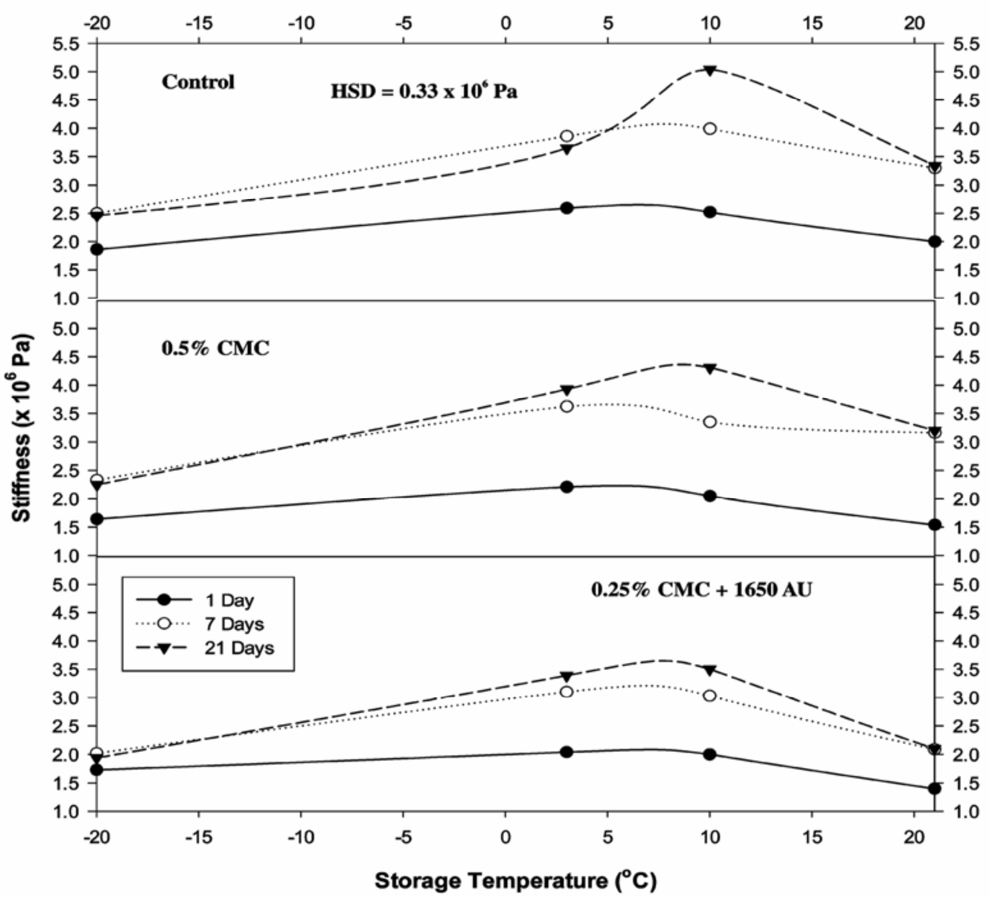

Fig. 2. Effect of storage temperature on final stiffness of tortillas with added carboxymethylcellulose (CMC) and maltogenic amylase after 1, 7, and 21 days.

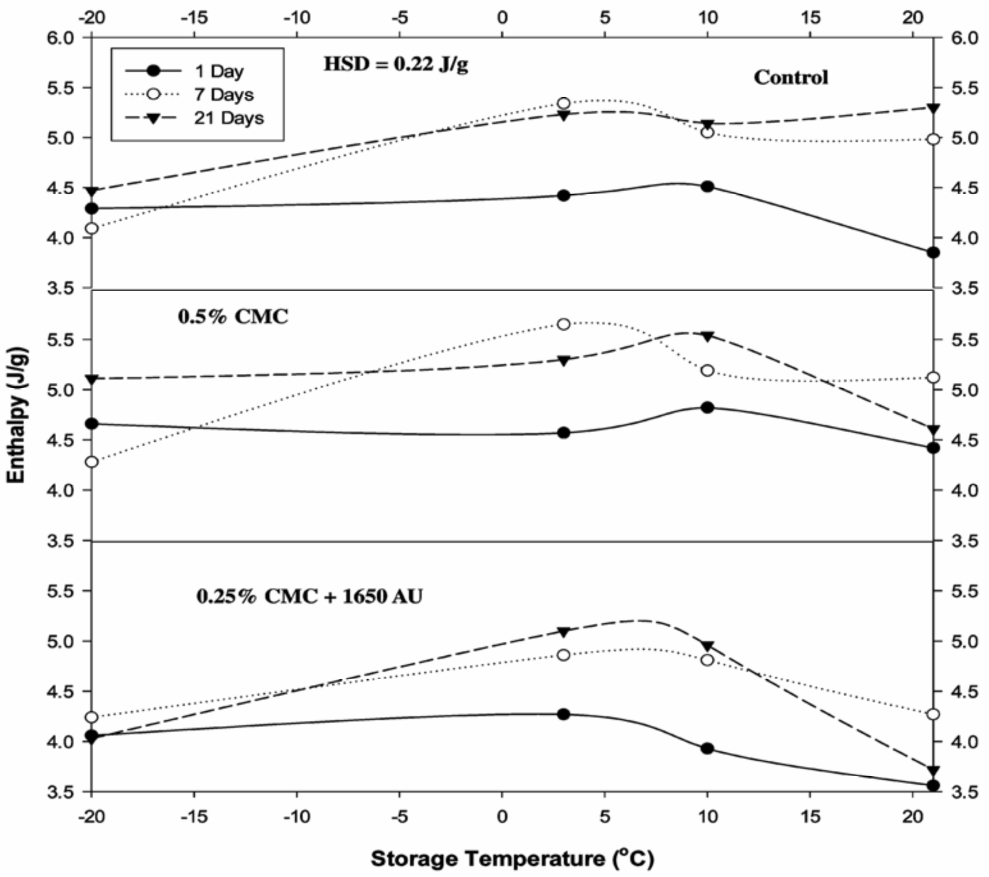

Fig. 3. Effect of storage temperature on amylopectin enthalpy of melting of tortillas with added carboxymethylcellulose (CMC) and maltogenic amylase after 1, 7, and 21 days.

Tortillas stored for one day dissipated less energy during the stress relaxation test when stored under refrigeration (3–10°C) than those stored frozen (–20°C) or at room temperature (Fig. 4). The combination of 0.25% CMC and 1,650 AU of amylase produced tortillas that dissipated significantly more energy than the control or 0.5% CMC under either refrigeration or room temperatures.

Tortillas with additives stored for 7–21 days at room temperature or under refrigeration did not have increased energy dissipation compared with the control (Fig. 4). Seven- and 21-day-old tortillas with amylase and CMC dissipated more energy than other treatments, when stored frozen.

DISCUSSION

Loss of flexibility of corn tortillas is a function not only of time but also of storage temperature. Tortilla pliability, stiffness, and energy dissipated obtained by stress relaxation and DSC showed a second-order polynomial (bell-shaped) dependence on storage temperature from –20 to 21°C. Results from this study support the theories of starch crystallization reviewed by Levine and Slade (1991) and applied by different scientists to study the retrogradation of gelatinization of waxy maize starch (Farhart et al 2000) and corn tortillas (Limanond et al 2001).

Corn masa and tortillas, according to these theories, are semicrystalline systems that, unlike amorphous polymers, age at temperatures above the T_g and below the T_m. Staling of tortillas during storage has been attributed to the nonequilibrium recrystallization of amylopectin (Fernandez et al 1999; Limanond et al 2001; Bueso et al 2004).

Limanond et al (2001) reported the T_g of corn tortillas was –23°C while DSC results of Campas-Baypoli et al (2002) indicate recrystallized amylopectin melts (T_m) at 45–64°C with a peak at 57°C. Our DSC results support these findings. Therefore, if tortillas age like a typical semicrystalline system, recrystallization should occur in the –23°C to 57°C range, showing a maximum rate around the middle of this range (17°C). Recrystallization below T_g (–23°C) would be minimal due to lack of molecular mobility (Struik 1978). Using linear regression, Limanond et al (2001) estimated the temperature where the maximum rate of starch retrogradation occurs in corn tortillas is 13°C. Crystal nucleation predominates over crystal growth at least over a 12-day storage period, thus shifting the maximum staling rate closer to T_g than to T_m.

The maximum loss of flexibility of tortillas occurs during refrigerated storage (3–10°C), as indicated by data from subjective pliability (Fig. 1), DSC (Fig. 3), and energy dissipated (Fig. 4). Control tortillas reached maximum amylopectin enthalpy when stored at 7°C for at least one week, even though a high correlation coefficient was observed between enthalpy and stiffness (Bueso et al 2004) where it was inferred that amylopectin retrogradation was maximum when tortillas were stored at 10°C. Tortilla pliability and energy dissipated were particularly sensitive indicators, showing a second-order polynomial tendency along the temperature

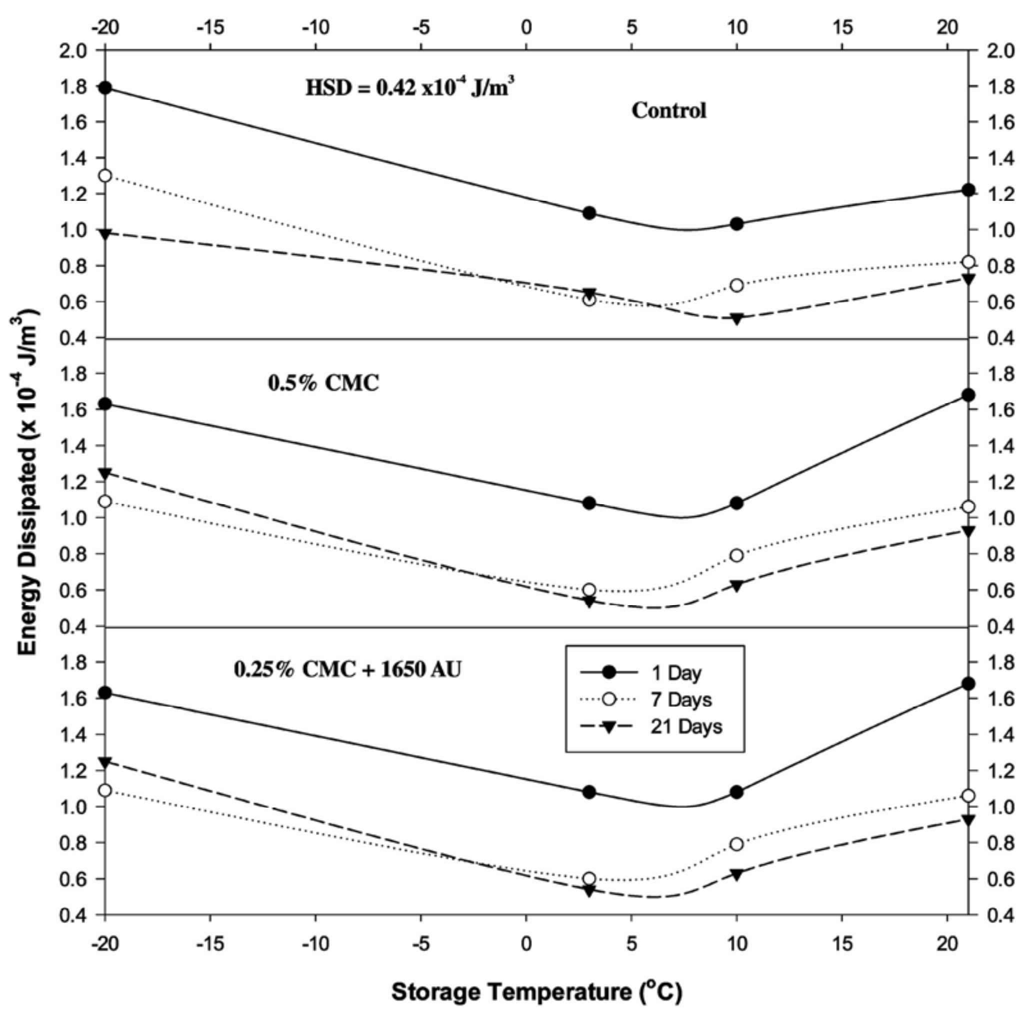

Fig. 4. Effect of storage temperature on energy dissipated of tortillas with added carboxymethylcellulose (CMC) and maltogenic amylase after 1, 7, and 21 days.

range as early as one day after baking but more clearly after seven days of storage (Figs. 1 and 4). Tortilla stiffness (estimated by stress relaxation) was a more precise indicator of texture changes during storage than subjective tests and energy dissipated (higher model fit and lower %CV). Stiffness data of tortillas stored for one day confirmed a higher degree of hardening in control tortillas stored under refrigeration. Stiffness data after one or three weeks of storage, however, clearly indicate that tortillas with or without additives stored at 10°C became stiffer than at any other temperature evaluated. These results support the findings of Limanond et al (2001) and the theory of crystallization of semicrystalline materials (Struik 1978).

Freezing might be the only option to limit stiffening in commercial tortillas without additives (antistaling agents). Freezing temperatures close to or below tortilla T_g limit the mobility of amylopectin and other amorphous molecules and therefore reduce their rate of crystallization. The combination of 0.25% and 1,650 AU made tortillas less stiff and more pliable than tortillas without additives or with 0.5% CMC, regardless of the storage temperature 21 days after baking (Figs. 1 and 2). This confirms the effectiveness of maltogenic amylase as an antistaling agent even at the temperature range where stiffening rate is highest (3–10°C). This means prolonging the shelf life of tortillas both in the supermarket (room temperature) and at home (refrigerated) requires additives that modify starch retrogradation.

CONCLUSIONS

Corn tortillas lost flexibility faster when refrigerated (3–10°C), with decreasing stiffening rates at higher or lower storage temperatures. Freezing tortillas retained fresh tortilla texture longer than at other temperatures. The best option to maintain tortilla softness and flexibility both at room temperature and under refrigeration for at least three weeks is to incorporate 0.25% CMC and 1,650 AU of amylase.

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