

Analytical Techniques for Understanding Nixtamalized Corn Flour: Particle Size and Functionality Relationships in a Masa Flour Sample¹

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ABSTRACT

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Instant masa flour finds extensive use in the food industry for making tortillas, taco shells, tamales, corn chips, and tortilla chips, and as an ingredient in extruded snacks. Due to lack of standard techniques for measuring masa functionality, processors and end-users use masa flour particle-size distribution and rheological characteristics in an attempt to predict its end use. In this study, a commercial masa flour sample was characterized by fractionating on the basis of particle size. Physicochemical and functional properties of masa flour fractions were investigated to establish structure-composition and functionality relationships. It was observed that

Rapid Visco Analyser (RVA) pasting profiles of flour fractions and textural properties of dough prepared on rehydration were related to particle size, yet, upon regrinding, RVA profiles did not change as markedly as expected. Differences in RVA measurements of the sized fractions could not be explained on the basis of hydration rate or total starch content. It was concluded that masa dough textural and RVA characteristics may be influenced by the status of starch polymer structures formed during nixtamalization.

Convenient and functional nixtamalized corn or masa flour is extensively used to produce products such as corn tortillas, taco shells, tostadas, tamales, corn chips, and tortilla chips (Serna-Saldivar et al 1990) and extruded snacks. Masa flours are typically produced from whole kernel corn that has been cooked in a lime (calcium hydroxide) solution. After cooking, corn is washed to remove excess lime and loose pieces of pericarp. It is then stone-ground or hammer-milled and dried in large continuous tunnel driers or drying towers. Less commonly, whole kernel corn is rapidly dried in continuous dryers. The dried material is hammer-milled and sieved. Oversized particles are reground to the desired size. Different sieve fractions (particle sizes) are blended to obtain optimum particle-size distributions suitable for different applications (Gomez et al 1991). Other masa flour production techniques include further processing and blending of various dry-milled corn fractions.

Particle-size distribution is considered the most important criterion for masa flour applications (Bedolla and Rooney 1984, Gomez et al 1991). Tortillas require a fine particle size flour to develop flexibility and cohesiveness, whereas corn chips and tortilla chips require a coarse particle size formulation to promote crispiness in chips after frying (Montemayor and Rubio 1983). It is generally believed that smaller particles are responsible for most of the water uptake, cohesiveness, plasticity, and smoothness of masa (Gomez et al 1987). On the other hand, large particles are considered responsible for texture characteristics such as crispiness and blistering of fried products. Large particles disrupt the dough network, reduce blistering, and decrease oil uptake during frying (Gomez et al 1991). Pflugfelder et al (1988) concluded that masa cannot be considered a homogeneous dough with uniform composition and physical properties but instead is a complex mixture of fractions whose reactions and interactions determine the behavior of the dough during baking and frying.

Rehydrated masa flour dough functionality and textural attributes are governed by properties of the constituent particle-size fractions. Bedolla and Rooney (1984) characterized instant masa flour particle-size distribution by sieving flour in a series of number 60, 70, and

80 U.S. standard sieves and calculated a particle-size index (PSI). Using this fractionation process, Gomez et al (1991) studied particle-size distribution and functionality of commercial masa and observed differences in starch pasting, X-ray crystallinity, and apparent solubility of various fractions. Even though physicochemical and functional differences between masa particles varying in size have been observed, little information exists on how such differences translate into differences in masa texture and product characteristics.

To evaluate masa flour functionality, alkaline corn processors often characterize instant masa flour on the basis of particle size and rheological characteristics. The present research was designed to investigate the various analytical methods employed in characterizing the physicochemical and functional nature of masa flour. A commercial instant masa flour sample was sieve-fractionated into six fractions. Relationships between masa dough textural attributes (such as hardness, stickiness, gumminess, and cohesiveness) and other functional and compositional properties (such as starch crystallinity, polymer molecular weight characteristics, Rapid Visco Analyser [RVA] pasting properties, particle size, and proximate analysis) were investigated. Efforts were directed toward understanding the basic hypothesis behind processor and end-user particle-size distribution characterization and analysis of commercial masa flours.

MATERIALS AND METHODS

Masa Flour Fractionation

Instant masa flour produced in a commercial masa plant was obtained from a local store (Lincoln, NE) and stored frozen (-5°C) until thawed and equilibrated to room temperature for analysis. For fractionation, masa flour was sieved in a Strand shaker for 3 min as outlined by Bedolla and Rooney (1984). U.S. standard sieves no. 40 (425 μm), 60 (250 μm), 70 (212 μm), 100 (140 μm), 140 (52 μm), and 270 (45 μm) were used to obtain six flour fractions. Each fractionation trial was performed using 100 g of masa flour, and fraction yields as well as total recovery were calculated. The fractionation process was performed in triplicate. Means with standard deviations are reported.

Masa Flour and Fraction Characterization

The moisture content of masa flour and the various fractions was determined by Approved Method 44-15A (AACC 2000). Protein content ($\text{N} \times 6.25$) was calculated using the Kjeldahl procedure (Tecator, Inc., Herndon, VA) based on AACC Approved Method 46-12. Fat content was determined according to AOAC 960.39 using the Soxhlet procedure (HT 1043 and HT 1046, Tecator). Ash content was calculated by AACC Approved Method 08-01. Masa

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flour pH was determined by inserting a probe into a suspension of 1 g of sample in 5 mL of deionized distilled water.

Total starch content in the samples was determined by AACC Approved Method 76-13 using the amyloglucosidase/ α -amylase total starch kit (Megazyme International Ireland Limited, Wicklow, Ireland). The pasting characteristics of masa flour and fractions were evaluated using a Rapid Visco Analyser (RVA, Newport Scientific Pty Ltd., Warriewood, Australia). The amount of sample weighed in the RVA pan was calculated on a 3-g, 14% moisture basis. The temperature profile was set such that the suspension was first equilibrated to 50°C for 10 min, heated in 4 min 32 sec to 95°C, held at 95°C for 3 min 30 sec, and finally cooled to 50°C within 3 min 48 sec. The extended equilibration period of 10 min at 50°C was designed to ensure a more uniform and complete hydration of masa particles. RVA pasting parameters such as peak viscosity, final viscosity, setback, peak time, and peak temperature were recorded. All RVA analysis was conducted in duplicate and average values reported.

Differential scanning calorimetry (DSC) analysis of masa and fractions was conducted (Pyris 1, Perkin-Elmer, Norwalk, CT). The sample (\approx 15 mg) was accurately weighed into aluminum DSC pans. The samples were hydrated to \approx 80% moisture content and the aluminum pans firmly sealed. The pans were stored at room temperature (25°C) for \approx 12 hr to obtain uniform sample hydration. The pans were heated in the DSC (from 3C to 120°C at the rate of 5°C/min) to obtain the endotherms. DSC analysis of each sample was conducted in duplicate and mean values were reported. Peak onset (T_o) and melt (T_m) temperatures and enthalpy of melt (ΔH) were calculated by endotherm peak analysis.

High-performance size-exclusion chromatography (HPSEC) was used to characterize the masa starch polymers after solubilization in aqueous methyl sulfoxide following the procedure outlined by Mua and Jackson (1997). Molecular weight-average (M_w), z-average (M_z) and number-average (M_n) and polydispersity (M_w/M_n) of the starch polymers were calculated using multiangle laser light scattering (MALLS) coupled with HPSEC, also as described by Mua and Jackson (1997).

Texture Profile Analysis of Masa Fractions

Masa textural characteristics were evaluated using a texture analyzer (TAXT2i, Texture Technologies Corp, New York, and Stable Micro Systems, Surrey, England, UK) by slightly modifying the procedures developed by H. Almeida and L. W. Rooney as published in application notes describing techniques for measuring the texture of alkaline cooked corn products (Anonymous 1997). The quantity of flour fractions obtained on sieving was limited, so masa dough prepared by mixing 10 g of flour fraction and 11 mL of distilled water was used to form a plug (16 mm diameter and 40 mm long) for texture analysis. Texture profile analysis was conducted on fractions obtained over sieves no. 60, 70, 100, and 140, as well as unfractionated masa flour.

Statistical Analysis

Analysis of variance was used for textural profile analysis and DSC using NCSS statistical software (NCSS-2000; 1999 Visual

Statistical Systems, Kaysville, UT). Significant differences ($P < 0.05$) among means were established using the Tukey-Kramer multiple-comparison test. Correlation analysis (Pearson correlations) was used to establish relationships between masa textural and functional properties ($P < 0.05$). When correlations were calculated for particle size, the true screen size was used, not the U.S. Standard Sieve number.

RESULTS AND DISCUSSION

Particle-Size Distribution

It is generally assumed that attrition grinding or hammer milling, such as that employed for grinding masa flour, will result in a sigmoidal particle-size distribution uniformly distributed an average value. However, the commercial masa flour sample did not exhibit a sigmoidal particle-size distribution profile. Instead, it showed a bimodal distribution (Fig. 1). This suggests that the manufacturer blended various fractions to obtain this distribution. Sieve fractionation indicated that \approx 44.3% of the particles were 140–212 μ m. Of the particles $>212 \mu$ m, \approx 37.4% could be classified as coarse particles. Only \approx 0.5% of the material was $>425 \mu$ m and retained over the U.S. standard no. 40 sieve. Approximately 23.1% of the particles were $>250 \mu$ m but $<425 \mu$ m; 13.7% of the material was 212–250 μ m.

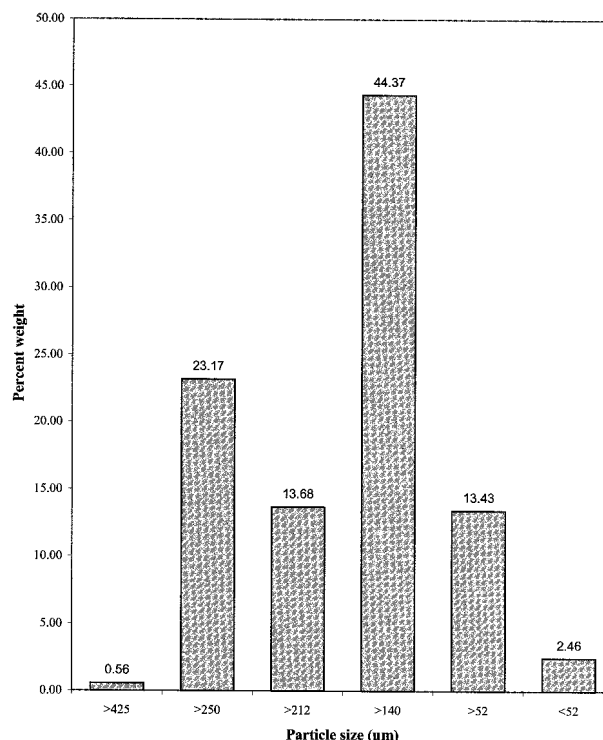


Fig. 1. Particle-size distribution of commercial instant masa flour.

TABLE I
Gross Composition and pH Level of Instant Masa Flour and Size Fractions^a

Sample	Total Protein (%)	Total Fat (%)	Ash (%)	Starch (%)	pH
Masa Flour ^b	8.60 (0.78)	4.59 (0.04)	1.39 (0.02)	71.4 (0.89)	6.55 (0.01)
No. 40 overs (425 μ m)	9.67 (0.81)	2.95 (0.02)	1.12 (0.02)	68.9 (0.05)	6.58 (0.01)
No. 60 overs (250 μ m)	9.43 (0.59)	3.33 (0.08)	1.06 (0.01)	72.0 (0.47)	6.57 (0.01)
No. 70 overs (212 μ m)	9.04 (0.69)	3.90 (0.16)	0.97 (0.14)	72.5 (2.10)	6.58 (0.02)
No. 100 overs (140 μ m)	8.12 (0.55)	4.72 (0.23)	1.48 (0.02)	72.8 (0.98)	6.58 (0.02)
No. 140 overs (52 μ m)	7.84 (0.48)	5.04 (0.05)	1.70 (0.01)	69.6 (1.44)	6.56 (0.02)
No. 270 overs (45 μ m)	8.06 (0.03)	5.43 (0.15)	1.83 (0.02)	69.3 (1.45)	6.55 (0.01)

^a Standard deviations in parentheses.

^b Unfractionated material.

Only a small portion (15.9%) was <140 μm . A trace amount of fine material that passed through the U.S. standard no. 140 (52 μm) sieve could also pass through the no. 270 (45 μm) sieve. Thus, all the material passing through the no. 140 sieve was assumed to be 45–52 μm .

Composition of Masa Flour and Fractions

Total protein ($N \times 6.25$), fat, ash, and percentage of total starch in the masa flour and its fractions are shown in Table I. Analysis of variance indicated that there were no significant differences in concentrations of total starch and ash between the various fractions. This suggests that during the nixtamalization process for production of this masa flour, ash and starches were uniformly distributed in the different particle-size fractions. Fat and protein contents were significantly different ($P = 0.0001$) between the fractions, the values correlated with particle size ($P < 0.007$, $r = -0.95$ and 0.92 , respectively). A significant inverse correlation was ob-

served between protein with fat and protein with ash contents of the fractions ($P < 0.02$, $r = -0.96$ and -0.88 , respectively).

Gomez et al (1991) determined that there were no compositional differences in protein, fat, ash, and starch contents of the coarse, intermediate, and fine masa particles, and concluded that the uniformity resulted from extensive mixing during hammer-milling, sieving, regrinding, and blending. We did not observe significant differences in total starch and ash contents within the fractions. However, significant differences in fat and protein contents were observed, probably because we obtained fractions with considerably more narrow particle-size distributions.

Pasting Characteristics

Various masa flour fractions exhibited significantly different RVA pasting characteristics (Fig. 2). The analysis of RVA profiles of fractions no. 40, 60, and 70 overs and unfractionated flour was complicated because a distinct breakdown was absent, and it was difficult to obtain a value for peak and trough viscosity. It was, however, obvious from the profiles that peak viscosity increased with decreasing particle size.

The fraction with 140–212 μm particle size exhibited the highest final viscosity. Final viscosity could not be significantly correlated with particle size or composition.

Gomez et al (1991) also observed significant differences in Brabender pasting characteristics within the coarse, intermediate, and fine fractions of nixtamalized corn flour obtained using no. 60 and 100 U.S. standard sieves. These researchers observed that starch concentration in the three fractions was almost identical due to uniform distribution of starch in ground masa, and inferred that differences in pasting behaviors of fractions were due to increased concentration of free starch granules in small particles. They suggested that the free starch granules were responsible for increased rapid viscosity during pasting. They concluded, however, that slower water diffusion into coarse particles and limited swelling of starch granules within the endosperm cells was responsible for the slow viscosity development of the coarse fractions in the amylograph.

To allow more uniform and complete hydration of masa particles, we equilibrated the RVA samples at 50°C for 10 min before starting the heating profile. Pasting differences within the fractions, however, were still evident. The rate of water diffusion was not a critical factor, and differences were probably due to factors other than extent and rate of fraction hydration.

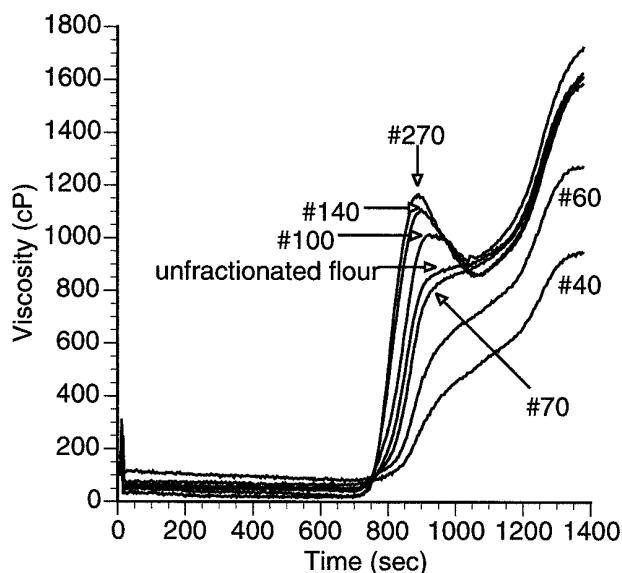


Fig. 2. RVA profiles of masa flour and particle size fractions (no. 40, >425 μm ; no. 60, 425–250 μm ; no. 70, fraction 250–212 μm ; no. 100, 212–140 μm ; no. 140, 140 and 52 μm ; no. 270 <52 μm).

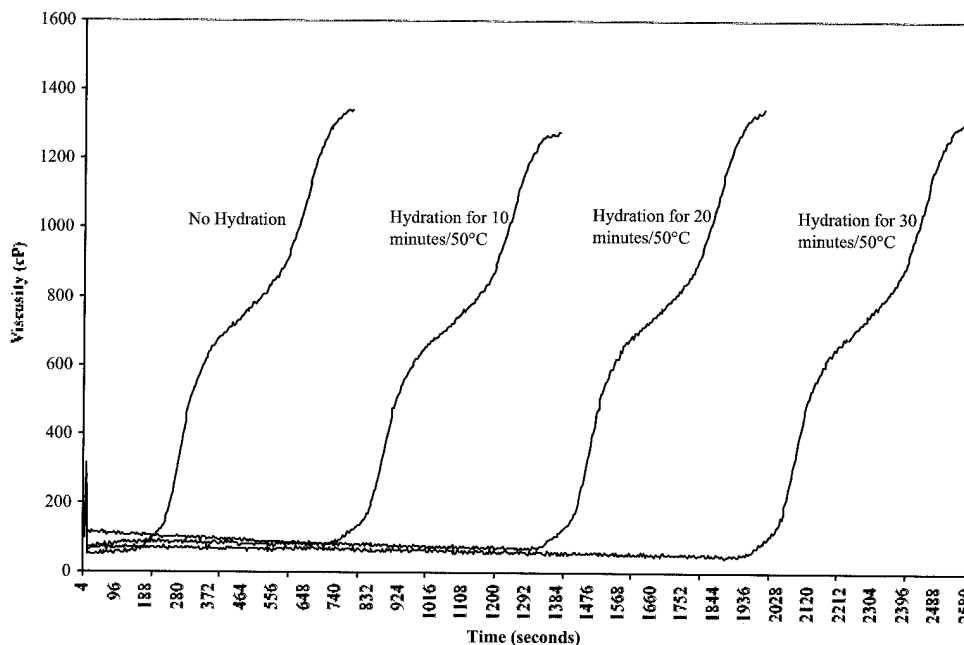


Fig. 3. Pasting properties of hydrated masa fraction (no. 60 overs).

To ensure that near complete particle hydration was achieved during 10 min and 50°C of equilibration and there were no unhydrated or partly hydrated particles remaining, additional RVA experiments were conducted. Pasting characteristics of the relatively large particle-size masa flour fraction collected over the no. 60 sieve (>250 µm) were measured after 50°C equilibration for 0, 20, and 30 min before initiating the heating profile (Fig. 3). No significant changes were observed on reducing or extending the equilibration period, suggesting that near-complete particle hydration was achieved even with no equilibration at 50°C, and differences in RVA profiles within the fractions cannot be attributed to degree of hydration. It is generally observed that RVA pasting profiles are influenced by starch concentration. Total starch content in masa fractions, however, did not show significant variability within the fractions (Table I) and pasting characteristics were significantly uncorrelated with starch content. Therefore, differences in masa fraction pasting characteristics cannot be attributed to starch concentration.

DSC Analysis

DSC analysis of commercial masa flour, as well as its fractions, exhibited typical gelatinization endotherms associated with melting of starch crystallites. Amylopectin retrogradation endotherms were not detected in any of the fractions. Although endotherms differed in shape, the enthalpy of gelatinization (ΔH) peak onset (T_o), and peak melt (T_m) temperatures did not show significant differences ($P > 0.05$) between the fractions (Table II). This indicates that differences in pasting characteristics of masa flour fractions cannot be attributed to differences in starch crystallinity and enthalpic properties.

Gomez et al (1991) investigated X-ray diffraction patterns of coarse, intermediate, and fine masa flour fractions and concluded that starch crystallinity decreased as particle size decreased. They attributed this decrease to mechanical damage imposed during grinding and regrinding, and concluded that starch in larger parti-

cles received less cooking and shear than starch in smaller masa particles. Our DSC data does not support this hypothesis because we could not observe significant differences in enthalpy or endotherm peak temperatures within the size fractions. It can also be argued, however, that the two techniques do not necessarily measure the same starch property or characteristic (Sahai and Jackson 1999). It appears that frequently documented crystallinity differences observed in the masa flour fractions using X-ray diffraction cannot be confirmed by enthalpic transitions observed in a DSC.

HPSEC Profiles

Various particle-size masa fractions exhibited uniquely different HPSEC profiles (Fig. 4). Presence of an intermediate polymer component with molecular weights between that of amylopectin and amylose was evident in several fractions, particularly those obtained over sieve no. 40, 70, 100, and 270. Differences in amylose and amylopectin apparent dimethyl sulfoxide (DMSO) solubility were also evident between the masa fractions.

Molar mass moments (M_w , M_z , and M_n) and starch polymers (amylose and amylopectin) polydispersity (M_w/M_n) are shown in Table III. The differences in starch polymer molar mass moments and (refractive-index and concentration) HPSEC profiles suggest that differences in the various fractions occur at the molecular level. Mua and Jackson (1997) indicated (using a model starch system) that molecular weight and amylose and amylopectin structures influence rheological changes during starch pasting. Differences in the HPSEC profiles of particle-size fractions could not be correlated to the fraction's nonstarch composition (protein, fat, and ash), suggesting that these constituents did not influence starch polymer structures in this system. The z -, weight-, and number-average molar mass of amylose were correlated with RVA final viscosity ($P < 0.04$, $r = 0.83$, 0.95 , and 0.85 , respectively), indicating that starch polymer structures may influence masa pasting.

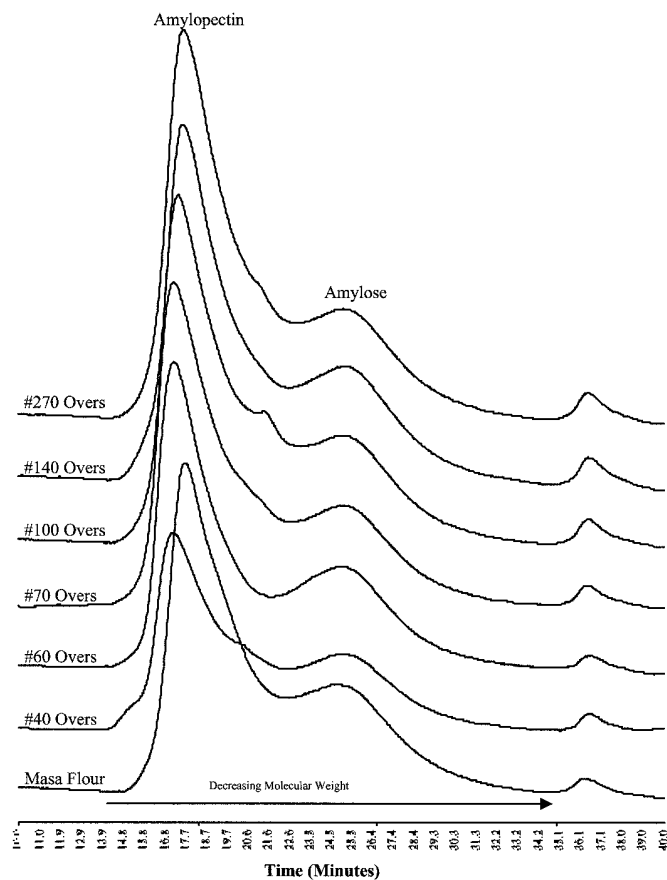


Fig. 4. HPSEC starch profiles of masa flour and fractions.

TABLE II
DSC Endotherms of Instant Masa Flour and Size Fractions^a

Sample	Onset (T_o , °C)	Peak (T_p , °C)	Enthalpy (ΔH , J/g)
Masa Flour ^b	67.16 (0.49)	75.33 (0.07)	3.51 (0.50)
No. 40 overs (425 µm)	68.89 (0.25)	78.20 (0.01)	3.17 (0.41)
No. 60 overs (250 µm)	66.84 (0.36)	75.43 (0.36)	3.22 (0.24)
No. 70 overs (212 µm)	68.11 (1.40)	77.11 (2.25)	3.19 (0.12)
No. 100 overs (140 µm)	67.52 (0.46)	75.74 (0.28)	3.43 (0.21)
No. 140 overs (52 µm)	67.12 (0.33)	76.56 (0.47)	3.39 (0.23)
No. 270 overs (45 µm)	68.49 (0.35)	76.55 (0.47)	2.86 (0.67)

^a Standard deviations in parentheses.

^b Unfractionated material.

TABLE III
Molar Mass Moments and Polydispersity of Amylopectin (AMP) and Amylose (AMY) Molecules in Masa Flour and Various Particle Size Fractions

Fraction	Molar Mass Moments (g/mol) ^a			Polydispersity
	M_n	M_w	M_z	(M_w/M_n)
Masa AMP	3.58×10^8	4.02×10^8	4.18×10^8	1.045
No. 40 AMP	5.19×10^8	6.54×10^8	7.86×10^8	1.258
No. 60 AMP	3.69×10^8	3.71×10^8	3.73×10^8	1.005
No. 70 AMP	4.59×10^8	5.01×10^8	5.40×10^8	1.009
No. 100 AMP	4.33×10^8	4.88×10^8	5.38×10^8	1.126
No. 140 AMP	4.76×10^8	5.06×10^8	5.38×10^8	1.064
No. 270 AMP	4.12×10^8	4.36×10^8	4.57×10^8	1.057
Masa AMY	7.94×10^7	7.96×10^7	7.97×10^7	1.002
No. 40 AMY	3.44×10^7	5.28×10^7	6.54×10^7	1.535
No. 60 AMY	2.35×10^7	5.58×10^7	8.49×10^7	2.377
No. 70 AMY	8.14×10^7	8.21×10^7	8.17×10^7	1.008
No. 100 AMY	8.01×10^7	9.20×10^7	1.10×10^8	1.148
No. 140 AMY	6.84×10^7	8.34×10^7	9.54×10^7	1.219
No. 270 AMY	7.68×10^7	8.11×10^7	8.64×10^7	1.056

^a M_n = number-average, M_w = weight-average, and M_z = z-average.

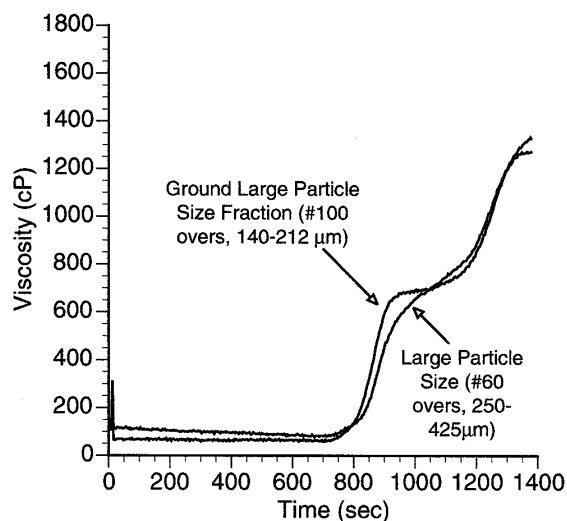


Fig. 5. RVA profile of reduced particle size masa fraction (no. 60 overs).

Dough Texture

Textural attributes of masa obtained from the texture profile analysis (TPA) masa test indicated significant differences in masa hardness, springiness, cohesiveness, gumminess, chewiness, and resilience between different size fractions ($P < 0.05$). TPA masa hardness was positively correlated ($P = 0.02$, $r = 0.97$), whereas springiness showed a significant negative correlation with particle size ($P = 0.03$, $r = -0.96$). This indicates that some masa textural attributes depend on particle size. Some masa TPA textural attributes were also significantly correlated with composition. Masa springiness was positively correlated with fat and protein content in the sample ($P < 0.04$, $r = 0.98$ and 0.96 , respectively). Masa hardness was negatively correlated to ash content ($P < 0.05$, $r = -0.95$), whereas masa adhesiveness was positively correlated to pH level ($P < 0.03$, $r = 0.97$). Masa cohesiveness also exhibited a positive correlation with masa fat ($P < 0.05$, $r = 0.95$). Masa textural characteristics (cohesiveness and adhesiveness) showed significant correlations with concentration of DMSO-soluble amylopectin and amylose estimated by HPLC. Masa cohesiveness was positively correlated with amylopectin ($P < 0.03$, $r = 0.97$), whereas adhesiveness was negatively correlated ($P < 0.01$, $r = -0.99$) with amylose concentration. It appears, from these relationships, that some textural properties of masa can be related to the concentrations of constituting masa flour starch polymers. Pflugfelder et al (1988) also suggested that free starch in masa was one of the primary determinants of texture, flavor, and keeping quality of masa products. Masa dough cohesiveness was also positively correlated with RVA final viscosity ($P < 0.05$, $r = 0.95$), indicating that RVA pasting characteristics of masa flour may reflect its textural behavior.

Structure-Functionality Relationships in Instant Masa Flour

It is apparent from the RVA profiles of various fractions that finer masa particles exhibit a distinct RVA peak and a noticeable breakdown. From this observation, it appears that particle size is an important attribute influencing RVA pasting measurements. For example, peak viscosity, final viscosity, and setback values were all correlated with particle size ($P < 0.02$, $r = -0.97$, -0.88 , and -0.90 , respectively). Thus, a reduction in particle size of coarser particles (by grinding) should be reflected in RVA measurements as an increase in peak viscosity, with development of a noticeable setback. To test this hypothesis, we subjected a relatively large particle-size fraction (sieve no. 60 overs, particle size $>250 \mu\text{m}$) to fine grinding in a Udy mill (attached with a $200\text{-}\mu\text{m}$ screen) and sifting through a no. 70 sieve to obtain particles $140\text{--}212 \mu\text{m}$. The pasting profile of the these ground particles was obtained. If RVA profiles were de-

pendent on masa particle size, reducing the particle size would, in fact, alter the RVA profile such that it would resemble that obtained from fractions with a similar particle size. However, RVA of the re-ground material exhibited pasting characteristics that were similar to the unground material (Fig. 5), suggesting that intrinsic properties of starch polymers composing the masa fraction have a greater impact on rheological properties than particle size. The slight change in shape observed after regrinding can be attributed to starch damage during grinding. This was also evident from the fact that granular birefringence observed under a polarizing microscope decreased slightly on grinding (data not shown).

The differences in pasting characteristics observed in the various size fractions could not be attributed to concentration of starch in the sample. The slightly changed RVA profile shape of the re-ground material appears to mimic the shape of similar particle size (no. 100 overs) fraction, although the two have widely different values for peak viscosity, breakdown, and setback. It appears that the shape of these RVA profiles depended on particle size. However, as indicated earlier with HPSEC data, factors such as composition and starch polymer characteristics likely influence the magnitude of viscosity development.

CONCLUSIONS

Nixtamalized masa flour is a mixture of particles that exhibit different functionality. To produce masa flour suitable for a specific food application, masa flour is usually separated based on particle size and blended to obtain the desired size distribution. Relationships between RVA pasting properties and textural attributes of rehydrated masa dough indicate that RVA can be used as a tool to evaluate masa flour. We conclude that when masa flour manufacturers alter masa size distributions through regrinding and blending they change masa flour functionality. This functional change, however, probably relates to differences in the intrinsic starch polymer characteristics of these fractions rather than absolute particle size. Further research on interactions between corn constituents during nixtamalization and an understanding of how to control starch characteristics to achieve desired masa quality and functionality would help to optimize nixtamalization processes and improve or standardize products made from instant masa flours.

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