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Effects of polymerization changes in maize proteins during nixtamalization on the thermal and viscoelastic properties of masa in model systems

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ABSTRACT

Although nixtamalization is widely used in the food industry, a comprehensive understanding of the influence of proteins on the viscoelastic behavior and thermal properties in masa is still lacking. In this work, the effect of protein changes and its influence on the masa viscoelastic behavior were studied using model systems. Mixtures of lime-starch, zein-starch and lime-zein-starch were cooked at 90 °C from 20 to 150 min. Zein changes during cooking were analyzed by SDS-PAGE. Thermal transition temperatures and enthalpies were determined using differential scanning calorimetry (DSC). Dynamic oscillatory tests were undertaken on model system samples with 50% (w/v) moisture content, using a strain-controlled rheometer. SDS-PAGE showed that zein polymerizes during cooking. In the zein-starch model system, no visible protein bands were found after 30 min cooking; however, when lime was present, five bands were observed in all samples. Thermal transitions were observed around 55–62 °C for all model systems, probably corresponding to starch retrogradation. Rheological studies showed that protein exhibited higher influence in the gel strength by increasing the elastic character of the system. It was hypothesized that the combined effects of lime on starch, zein polymerization and the formation of calcium-zein bonds during cooking, yield a stronger and more elastic gel structure.

1. Introduction

Alkaline cooking of maize with lime (nixtamalization) is an important process in the preparation of tortillas, maize chips, taco shells, tamales, and other Mexican-style foods. During nixtamalization, maize is first cooked in the presence of lime, steeped, and then washed to produce nixtamal, which is then stone-ground to form soft, moist dough that is called masa. Although nixtamalization is widely used in the food industry, a comprehensive understanding of viscoelastic behavior and its thermal properties in masa is still lacking. Several reports are available on the effect of nixtamalization parameters on masa functionality, e.g. the influence of lime on maize starch and flour gelatinization (Gomez et al., 1992; Rodríguez et al., 1996), the physicochemical properties of nixtamalized maize and their intermediate products (Campus-Baypoli et al., 1999), and the rheological characteristics of starch in nixtamal (Méndez-Montealvo et al., 2006, 2008; Mondragón et al., 2006). Dynamic rheology is a sensible technique to study the changes from the processing and storage of food products, because applying small-amplitude would not alter the arrangement of the macromolecules inside the food matrix (Biliaderis, 1992). Starch in masa has been characterized (Gomez et al., 1992); however, the influence of maize proteins (mainly zeins) on physicochemical characteristics and rheological behavior of masa and starch as well as its byproducts (e.g. tortillas, tamales, totopos and other products from nixtamalized maize) is still not well understood.

The properties of starchy foods are affected by the type (or source) of starch, and food constituents. Food constituents such as proteins, fatty acids, and various additives affect the thermal behavior of starch, which has been documented by various studies conducted using predetermined mixtures of starch and other ingredients (Sayer et al., 2005; Zhang and Hamaker, 2003).

Batterman-Azcona and Hamaker (1998) proposed that maize proteins (zeins) must be released from protein bodies, complex together, and form viscoelastic polymers during the extrusion process. Hamaker et al. (1986) reported that solubility of sorghum

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and maize albumins, globulins and prolamins decreased after cooking the flours in water (neutral pH). Vivas et al. (1987) showed that alkaline processing reduced the solubility of proteins in salt solutions and alcohol, confirming that processing sorghum and maize into tortillas significantly affected protein solubility and structure. It has been proposed (Emmambux and Taylor, 2009; Ezeogu et al., 2005) that prolamin polymers of *M*r > 100 kDa were formed in sorghum and maize on cooking.

Starch in foods exposed to specific hydrothermal conditions, would not necessarily undergo the same structural and morphological changes as would isolate starch exposed to the same treatments. Because of inherent difficulties associated with starch isolation from food products, knowledge of the structural changes that take place from hydrothermal treatments and interactions with food constituents is limited.

The main objective of this study was to investigate the effect of changes in maize proteins during nixtamalization, on the viscoelastic behavior of masa by using model systems of lime-zein-starch, lime-starch and zein-starch.

2. Experimental

2.1. Materials

Maize starch was obtained from Cargill (Cerestar C* Gel 03420). Proteolytic enzyme, thermolysin (45 units/mg protein) from *Bacillus thermoproteolyticus rokko* [E.C.3.4.24.27], and zein were sourced from Sigma–Aldrich (St. Louis, MO). The alkaline treatment was done with commercial lime (calcium hydroxide: cal pirámide, Grupo Bertrán, Mexico City) commonly used in the tortilla industry. The rest of the reagents were analytical grade.

2.2. Maize starch protein removal

Maize starch was first defatted prior to protein removal. Starch was defatted with hexane and centrifuged (7500× g for 15 min), and this procedure was repeated three times (Emmambux and Taylor, 2009). Defatted starch was left in a hood overnight to remove the residual hexane and dried in an oven at 40 °C for 24 h. The protein in maize starch was removed using thermolysin by following the method of Mu-Foster and Wasserman (1998). Defatted maize starch (50 g) was mixed with 3300 units of thermolysin in 1000 mL of 5 mM calcium chloride in a water bath at 60 °C for 4 h with gentle hand-mixing at 30-min intervals. Afterwards, the mixture was cooled down to room temperature, filtered, washed with de-ionized water and this washing procedure was repeated five times. The protein-hydrolyzed starch was dried in an oven at 40 °C for 24 h and then sieved through a 150-μm sieve.

2.3. Preparation and cooking of model systems

The proportions of three components (lime:1 zein:10 starch:100) as well as cooking conditions were chosen to simulate the nixtamalization process. Due to its low water solubility, 2% (w/w) zein was first dissolved in 75% ethanol to ensure its complete dissolution and homogeneous distribution in the model systems. For the lime-zein-starch model; 9 g lime-starch mixture (0.1:10 w/w) was added to 50 g of 2% (w/w) zein in 75% ethanol to obtain a mixture of lime-zein-starch (0.1:1:10, w/w/w). For the lime-starch model, 10 g of a lime-starch mixture (0.1:10, w/w) was used. For the zein-starch model, 9 g of starch was added to 50 g of 2% (w/w) zein in 75% ethanol to obtain a mixture of zein-starch (1:10, w/w).

All mixtures were added with de-ionized water to a final weight of 200 g, placed in a 500 mL beaker covered with a weighing dish, and cooked in a water bath at 90 °C for 20, 30, 40, 90 or 150 min with constant stirring, with a 2.5-cm magnetic bar. Maize nixtamalization cooking time usually varies with the final products, i.e., standard cooking time 20–40 min is used to produce tortillas, and prolonged cooking time of 90–150 min for producing maize chips (Gomez et al., 1992). Immediately after cooking, samples were frozen and then dried using a freeze dryer (Freeze Dry System, Labconco, Kansas City, MO) at −43 °C and 59 × 10^{-3} Mbar for 48 h. Then samples were ground into flour using a cyclone sample mill (Cyclone Sample Mill, Udy Corporation, Fort Collins, CO) and sieved through a 150-μm sieve.

2.4. Protein analysis

The protein content of freeze-dried samples from zein-starch and lime-zein-starch model systems were determined using the Coomassie Plus1 protein assay reagent kit (Pierce Biotechnology) with BSA as standard (Bradford method) (BioRad Corporation, Hercules, CA).

The protein composition in model systems was analyzed using SDS-PAGE under reducing (heating and 4% 2-mercaptoethanol [2-ME]) and non-reducing (neither heating nor 2-ME) conditions. SDS-PAGE was done on a vertical gel system Mini-Protean 3 (Minigel, Bio-Rad Corporation, Hercules, CA) using a 15% acrylamide gel with a 4% stacking gel. Samples of freeze-dried flour of lime-zein-starch and zein-starch model systems of approximately 0.075 mg protein were added into 0.3 mL of buffer (0.01% bromophenol blue, 10% glycerol, 0.625 M Tris–HCl, 10% SDS) and reduced with 4% 2-ME to obtain a final concentration of 3.75 mg protein/mL. Under the reducing conditions, samples were placed in a boiling water bath for 5 min. For both reducing and non-reducing conditions, 20 μL of supernatant was loaded into sample wells along with 5 μL of standards with molecular mass ranging from 10 to 250 kDa (BioRad, Richmond, CA) to determine the molecular masses (M*r*) of polypeptide bands of the sample. Electrophoresis was performed at 200 V for approximately 30 min or when the running front reached the end of the gel. Gels were stained with Coomassie Brilliant Blue R-250 staining solution (BioRad, Richmond, CA) and then destained with 40% methanol and 10% glacial acetic acid. All gels were performed in triplicate. The molecular masses of proteins were estimated from the log–log plot of relative mobility versus molecular mass of protein standards.

2.5. Thermal analysis

The peak transition temperatures (*T*p) and enthalpies (Δ*H*) of all cooked model system samples were determined using a differential scanning calorimeter (DSC) (Pyris-1, Perkin–Elmer Corp., Norwalk, CT). DSC was calibrated with indium, and data were analyzed using the Pyris software. Approximately 100 mg of freeze-dried sample was mixed with de-ionized water to obtain a mixture of 50% moisture content and equilibrated at room temperature for 1 h. The moisture content for the thermal analysis was selected according to Sahai et al. (2001), who reported that the properly processed nixtamal and masa have moisture contents of 46–51%. Samples of approximately 8 mg of the equilibrated mixtures were placed in pre-weighed aluminum pans. The pans were hermetically sealed and scanned from 25 to 120 °C at 10 °C/min. All measurements were carried out in triplicate.

2.6. Viscoelastic behavior

The viscoelastic properties of all model systems (lime-starch, zein-starch and lime-zein-starch) subjected to different cooking times were studied with a stress controlled AR-2000 rheometer (TA Instruments, New Castle, DE), which was used in feedback on its