Comparison of flour starch properties in half-sib families of *opaque-2* maize (*Zea mays* L.) from Argentina

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ABSTRACT

Since the discovery of the *o2* mutation in maize, many studies have reported the characterization of the protein quality of *opaque-2* genotypes. However, few have reported the properties of their starch. The objective of this study was to characterize flour starch properties of twelve half-sib families of *opaque-2* maize from Argentina. Chemical composition and thermal and pasting properties of whole grain flour were determined. Non-opaque genotypes were used as control. Starch content of *opaque-2* genotypes did not show significant differences compared to non-opaque genotypes; yet, amylose content was significantly lower. A high variability in pasting and thermal properties was observed in genotypes. Opaque samples showed a significantly higher peak viscosity and a lower pasting temperature as compared with non-opaque samples, probably

due to larger and less compacted starch granules in the floury endosperm. The higher the gelatinization enthalpy of *opaque-2* genotypes was the lower the amylose content in relation to non-opaque varieties. Two retrogradation endotherms were observed in DSC analysis: one corresponding to amylopectin crystallization and the other to melting of amylose-lipid complex. Both enthalpies were considered total starch retrogradation (ΔH_{RT}). A wide range of variation was obtained in ΔH_{RT} in *opaque-2* genotypes, but no significant differences between opaque and non-opaque genotypes were observed. The differences in starch properties found in this study would make it possible to identify *opaque-2* families with particular characteristics for the development of starchy food items adapted to specific processing traits.

KEYWORDS

Pasting properties, gelatinization, retrogradation, amylose, *opaque-2* maize

INTRODUCTION

Maize is the major crop grown in the world. This cereal as raw material and its transformation products or technologies for production are central elements in negotiations between countries worldwide (MAIZAR 2011). The mature corn kernel is composed of over 70% of starch. Most of this starch (80% to 90%) is found in the endosperm, which comprises about 80% of the total kernel dry weight (Boyer and Hannah 2001). Starch is found as discrete granules with different shapes, sizes, and composition, depending on the corn genotype. The physicochemical and functional properties of starch are linked to the structure and morphology of the granules. Small changes in the amylose-amylopectin proportion alter their functional behavior and influence the nutritional characteristics of starchy foods (Akerberg et al. 1998; Bird et al. 2006). Both gelatinization and gelation of starch are basic processes to obtain processed products from rich

matrices in this biopolymer, whose conditions determine the quality of the final product. There are a lot of corn kernel types that vary in structure and composition (Robutti 2010). By using genetic variation, the composition of the kernel can be changed for both quantity and quality of starch, protein, and oil throughout kernel development (Boyer and Hannah 2001).

Currently, we can see a trend towards the production of materials with well-defined quality to meet the growing demand of this cereal. These characteristics are directly associated with the end use of the product and justify their identity-preserved marketing as opposed to the bulk production that turns it into a commodity (Robutti 2010). Argentina has a great diversity of maize germplasm due to its wide variation in latitude and altitude (Seetharaman et al. 2001). The largest grown area in the country is covered by hybrid varieties. The annual purchase of hybrid seeds represents a high fixed cost, therefore, only producers with substantial economic resources and technologies may remain in the production system. On the other hand, in areas where small farmers do not have economic access to hybrid seeds, the open-pollinated varieties prove to be a good choice, since producers can keep their own seeds for sowing the following year, thereby reducing their dependence on seed external sources (CIMMYT 1999). In addition, the genetic variability of this kind of varieties makes their adaptation to different weather and soil conditions easy.

Many researchers have suggested that, to achieve major gains per cycle, the assessing of progeny through individual breeding methods is essential. Modified ear-to-row method (Lonnquist 1964) based on the among-and-within selection of half-sib families, is an effective method of recurrent selection in maize (Carena and Cross 2003; Hyrkas and Carena 2005). The half-sib selection is in fact based on general combining ability since the entire population serves as a tester for pollination of female plants. Success of selection is assured by the presence of superior

genotypes, which in turn depends on sample size and heritability of selected traits (Borojevic 1990).

Some corn genotypes are studied worldwide as their grains have beneficial nutritional properties for human consumption. The *opaque-2* maize has the recessive mutant gene (*o2*) (Mertz et al. 1964) which restricts the zein synthesis and increases the other protein fractions such as albumin, globulin and glutelin. As a result, a thinner protein matrix with different amino acid distribution is generated, resulting in duplication of lysine and tryptophan. The expression of this gene allows higher nutritional value than that in normal corn (Gibbon and Larkins 2005).

The maize endosperm is composed of an opaque floury region rich in starch, located near the center of the kernel, and of another hard vitreous region rich in protein, located near the periphery of the kernel (Hoseney 1998). *Opaque-2* corn contains a higher proportion of floury endosperm, with a dull appearance due to the loose packing of the starch granules (Joshi et al. 1980). Since the discovery of *o2* mutation, many research studies have been conducted to develop varieties (QPM) that improve consumers' nutrition, especially in developing countries (Vivek et al. 2008). Many studies have characterized the protein quality of this type of germplasm (Gibbon and Larkins 2005; Landry et al. 2004; Landry et al. 2005; Vivek et al. 2008; Mendoza-Elos et al. 2006). However, few studies have reported the characteristics and functional properties of starch, which determine interesting possibilities for industrial applications of this type of maize. Therefore, the objective of this study was to characterize and compare the flour starch properties of half-sib families of *opaque-2* maize, grown in Argentina in two crop years.

MATERIALS AND METHODS

Genetic Material

An *opaque-2* maize germplasm provided by International Maize and Wheat Improvement Center (CIMMYT), México, was used as starting material (POr). An adaptation seeding was performed (planting date December 2011) at the experimental field of Facultad de Ciencias Agropecuarias of the Universidad Nacional de Córdoba, Argentina (31°28' 49.42" S and 64°00' 36.04" W). Twelve main cobs of different plants with good sanitary features were selected (half-sib families) from the original population (POr) and individually harvested (June 2012). After harvest, the cobs were individually threshed and the opaque grains of each cob were selected according to Vivek et al. (2008). Through this methodology, a light table is used to select the grains that possess the *o2* gene in recessive homozygous state (*o2o2*), using the degree of opacity as indirect measure or secondary characteristic of this genotype. Approximately ninety opaque kernels of each cob were stored at 4°C until planting; the rest were milled in the laboratory to obtain the whole grain flour. In December 2012, the seeds of each cob were planted in individual rows (Lonnquist 1964) under a randomized complete block design with three replications. The experimental plots were composed of twelve rows (row-family) of five meters long, with a spacing of 0.70 m between rows. At flowering, the female plants of each row in each replication were artificially pollinated with a pollen mix of male plants of the same row, in order to obtain individual progenies (half-sib progenies). In June 2013, the cobs of the best plants within each progeny were harvested (grain moisture at harvest in both years was around 18%) and the threshed grains were selected following the methodology described above (Vivek et al. 2008). Grains were then stored and milled under the same conditions as those of the 2012 harvest. The field trials in both crop years (December 2011 - June 2012 and December 2012 - June 2013) were conducted in the same experimental field (same location) but in different plots uncultivated for three-year period. Plots were equally prepared before planting. The assays were carried out both years under dry conditions without nitrogen fertilization, in order to compare both crops under

equal experimental conditions. Supplementary Table I shows the average monthly temperature and precipitation for both crop years.

The opaque grains selected from genotypes harvested in both crop years (twelve half-sib families grown in 2012, and their individual progenies obtained in 2013) were milled in a blade mill (Fbr Decalab) without predrying. Whole grain flour was obtained (moisture around 12 to 15%) and stored until chemical analysis. The original population (POr) used as starting material and two non-opaque genotypes (a white corn synthetic variety, BL, and an open-pollinated popcorn variety, PS) were used as controls for comparison.

Chemical Composition

The moisture, protein (N x 6.25), lipid and ash contents of whole grain flour were determined according to AACC International Approved Methods 44-19.01, 46-12.01, 30-25.01 and 08-01.01, respectively. Resistant starch (RS) content was determined using a resistant starch kit (Megazyme International Ireland Ltd., Wicklow, Ireland) according to AACC International Approved Method 32-40.01 and AOAC International Method 2002.02. Before such determination, the whole grain flour (100 mg) of each genotype was defatted, suspended in 1.6 mL of distilled water, and cooked in a boiling water bath for 12.30 min in order to allow starch gelatinization by simulating a cooking process. Then, samples were incubated with pancreatic α-amylase and amyloglucosidase (AMG) in a shaking water bath for 16 h at 37°C to solubilize and hydrolyze non-resistant starch to D-glucose by the combined action of the two enzymes. Subsequently, the non-resistant starch (NRS) was separated by centrifugation; the RS-containing pellet was purified with ethanol, solubilized with 2M KOH, and hydrolyzed to glucose with AMG. Reagent glucose oxidase/peroxidase was used to quantify D-glucose by spectrophotometer. RS plus NRS (digestible starch) was considered total starch content and expressed as g/100 g flour (db).

Amylose content was measured with amylose/amylopectin kit (Megazyme International Ireland Ltd., Wicklow, Ireland) according to the procedure described by Gibson et al. (1997). Briefly, whole grain flour (25 mg) of each genotype was completely dispersed by heating in dimethyl sulphoxide (DMSO). Lipids were then removed in ethanol (96%); amylopectin was precipitated by the addition of Con A and removed by centrifugation. Amylose and total starch were enzymatically hydrolyzed to D-glucose with the addition of amyloglucosidase (200 U) plus fungal α-amylase (500 U). Reagent glucose oxidase/peroxidase was used to quantify the amylose concentration in the starch sample by spectrophotometer at 510 nm. The results were expressed as g of amylose/100 g of starch (db). All determinations were measured at least in duplicate.

Pasting Properties

A Rapid Visco Analyzer (Model RVA-4500, Perten Instruments, Inc) was used to measure changes in viscosity by heating of whole grain flour of each genotype, using the RVA general pasting method. Three grams of sample (db) were transferred to the RVA vessel and 25.0 mL distilled water was added. The suspension was stirred at 160 rpm while heated to 50°C. The slurry was held at 50°C for 1 min, and then heated to 95°C at a heating rate of 9.4°C/min and a stirring rate of 960 rpm. Then, it was held at 95°C for 2.5 min and finally cooled to 50°C at a cooling rate of 11.8°C/min. Pasting temperature (PT), peak viscosity (PV), final viscosity (FV), trough viscosity (TV), breakdown (BD), and setback (SB) were obtained from the pasting curves. All determinations were analyzed at least in duplicate and the results were expressed in cP.

Thermal Properties

A Differential Scanning Calorimeter (DSC 823, Mettler-Toledo, Switzerland) was used to measure thermal properties of starch from whole grain flour. Four mg samples (db) of each

genotype were weighed in aluminum pans and 12 µL (ratio of 1:3) of distilled water were added. Before the heating process, pans were sealed and stored for 24 h at room temperature. The samples were heated from 25 to 120°C at a rate of 5°C/min. The DSC calibration was performed with indium, and an empty pan was used as reference. Gelatinization parameters were obtained: onset temperature (T_{oG}), peak temperature (T_{pG}), endset temperature (T_{eG}) and gelatinization enthalpy (ΔH_G). Gelatinization temperature range (R_G) was calculated as T_{eG} - T_{oG}. After analysis, all pans were stored for 14 days at 4°C to allow starch retrogradation and re-analyzed (25–120°C at a rate of 5°C/min). Onset temperature (T_{oR}), peak temperature (T_{pR}), endset temperature (T_{eR}), retrogradation enthalpy (ΔH_R) and retrogradation temperature range (R_R) (T_{eR} - T_{oR}) were obtained. Retrogradation percentage (%R) was calculated as $\Delta H_R/\Delta H_G$ x100. All measurements on DSC were evaluated at least in duplicate and expressed in J/g of starch.

Statistical Analysis

Statistical analyses were performed using Infostat/Professional statistical software (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba). Data were examined by ANOVA and Di Rienzo, Guzmán and Casanoves' multiple comparison test (DGC) (Di Rienzo et al. 2002) was used. The differences among genotypes (average of each genotype from the two harvest, 2012 and 2013) and between crop years (average of all genotypes from each year, 2012 or 2013) were analyzed. In addition, the individual progenies were compared with the original population used as starting material and two non-opaque genotypes (white corn and popcorn) used as controls. The relationships between the parameters measured were determined by Pearson correlation test (significant level at $P < 0.05$).

RESULTS AND DISCUSSION

Chemical Composition

Table I shows the flour chemical composition of genotypes analyzed. Significant differences were obtained in protein content in *opaque-2* genotypes. These values are comparable to those reported by Gibbon and Larkins (2005) in *o-2* mutant lines and by Mendoza-Elos et al. (2006) in QPM varieties. No significant differences were observed when average protein content of all *opaque-2* genotypes was compared to that of white corn and popcorn used as controls. Robutti et al. (2000) reported similar protein content in two landrace varieties of white dent corn and popcorn from Argentina. The *o2* recessive mutation in homozygous state confers higher lysine and tryptophan amounts, but does not change total amount of protein present in the grain (Gibbon and Larkins 2005).

No significant differences were found in the average starch content of all *opaque-2* genotypes in relation to that of white corn and popcorn (Table I), but Joshi et al. (1980) reported that *opaque-2* corn endosperms contain less protein and starch content than normal maize endosperms. However, average amylose content obtained in *opaque-2* genotypes was significantly lower than that in non-opaque controls (Table I). In hard endosperm corn, high amylose content results in increased compressibility of starch granules in the protein matrix, unlike soft grains with more amylopectin (Dombrink-Kurtzman and Knutson 1997). Robutti et al. (2000) and Agama-Acevedo et al. (2013) reported lower amylose content in soft than in hard corn endosperm. Amylose content correlated positively with total starch ($r = 0.4$; $P < 0.05$) in *opaque-2* genotypes. The original population (POr) showed the lowest starch and amylose contents when compared with individual progenies obtained from it. Resistant starch content (RS) was lower than 1% in all opaque and non-opaque genotypes. As it can be observed from the total starch content, no significant differences were found in digestible starch content (NRS) between *opaque-2*

genotypes when compared with that in white corn and popcorn. These results are similar to those reported by Méndez-Montealvo et al. (2005).

Variations in phenotypic traits can result from three components: the effect of genotype, environment, and genotype-by-environment interaction (Poehlman and Sleper 1995). The weather conditions did not show large differences between years during the crop cycle (Supplementary Table I) and temperatures for grain filling were below 30°C in both years under non-limiting water conditions. This suggests that the genotypes studied were not affected by high temperature stress or drought conditions at this stage. However, a significant annual average decrease was found in amylose and total starch content. Consistently, an increase in protein content was observed from one crop year to another (Table I) and a negative correlation between protein and starch was seen (r = -0.34; $P < 0.05$) in *opaque-2* genotypes. In maize endosperm, a lower amount of starch results in an increase in protein content, related to the type of endosperm. Floury endosperm contains higher starch content than vitreous endosperm, with higher protein content (Serna-Saldivar 2010). On the other hand, the genetic variability within progenies could have influenced in changes of the amount of grain constituents and could have translated into higher protein content in 2013.

The average lipid and ash content obtained in *opaque-2* genotypes was higher than that found in white corn and popcorn (Table I). Lower lipid and ash content was reported in QPM genotypes by Corcuera et al. (2013) and Mendoza-Elos et al. (2006).

A significant annual increase was observed in lipid content between crop years. In maize grains, lipids are found mainly in the germ, so that when the germ is large, a higher lipid content is found (Lambert et al. 1998). The correlated responses for germ and endosperm size and the change of the ratio of germ to endosperm can result in variations in the kernel components (Lambert 2001). The changes observed from one harvest to another suggest differences in grain composition,

which could be mainly attributed to differences within each family and their progenies. The genetic variability within half-sib families is greater than that in inbred lines or hybrids (Borojevic 1990).

Pasting Properties

Table II shows the pasting parameters of the whole grain flour obtained from all studied genotypes. A great variability was observed in progenies. The values of peak viscosity (PV), trough viscosity (TV) and breakdown (BD) shown by *opaque-2* genotypes were significantly higher than those of white corn and popcorn. Genotypes with soft grains develop high viscosity since starch granules are larger and less compacted, allowing water diffusion within the grain (Narváez-González et al. 2006; Almeida-Dominguez et al. 1997). A similar behavior was reported in soft wheat flour samples compared to that of hard wheat flour (Ragaee and Abdel-Aal 2006).

Breakdown (BD) is associated with the stability of starch granules (Ragaee and Abdel-Aal 2006). Higher swelling capacity of granules results in higher peak viscosity. The higher BD shown by *opaque-2* genotypes indicates lower stability of starch granules in relation to that of non-opaque corns used as controls. Consistently, a strong correlation between PV and BD was obtained (r = 0.82; $P < 0.05$) in *opaque-2* genotypes. In addition, an annual average decrease was found in PV and BD (Table II), along with a decrease in starch content from 2012 to 2013 (Table I).

Swelling of starch granules is inhibited with higher amylose content, resulting in lower viscosity (Debet and Gidley 2006). The P10 and P3 samples showed the lowest PV (Table II) and the highest amylose content (Table I). Conversely, P13, P18, P22 and P28 samples showed the highest PV and low amylose content. Jane et al. (1999) found particularly low PV in two high-amylose maize starches. The P18 sample showed the highest PV and BD, indicating its ability to

paste formation. Besides, RVA parameters, especially PV and BD, showed a negative correlation with protein content (r = -0.36 and r = -0.45, respectively; $P < 0.05$), as reported by Sandhu and Singh (2007), and a positive correlation with total starch content (r = 0.36 and r = 0.37, respectively; $P < 0.05$). However, no significant correlation with lipid content was observed. Proteins and lipids can reduce the starch water absorption with the ensuing decrease in viscosity, in agreement with the results obtained by Baxter et al. (2014) in rice starch. Proteins develop lower viscosity than starch, so the inverse correlation between them (r= -0.34) explains the lower PV and FV observed when corn protein increases in the grain.

Pasting temperature (PT) was significantly lower in *opaque-2* genotypes than in white corn and popcorn (Table II). In hard endosperms, the protein matrix is thicker than soft endosperms (Wolf et al. 1969) and the starch granules are tightly packed (Christianson et al. 1969). Therefore, higher temperature is required in the hard endosperm of white corn and popcorn to allow hydration and swelling of starch granules as compared to opaque grain endosperms.

During cooling, re-association between starch molecules, especially amylose, produces an increase in viscosity called setback (SB) and is related to starch retrogradation (Biliaderis 2009). In *opaque-2* genotypes, FV correlated with SB (r = 0.93; $P < 0.05$), but amylose content correlated negatively with both (FV r = -0.47; $P < 0.05$ and SB r = -0.37; $P < 0.05$). This might be associated with the re-association of starch with other components after gelatinization, rather than with amylose content. The starting material (POr) showed the lowest amylose content (Table I) and the highest FV and SB of all individual progenies obtained from it (Table II). White corn did not show significant differences in FV and SB with opaque samples; yet, PV was significantly lower. The higher amylose content of white corn with respect to *opaque-2* genotypes (Table I) might explain these results. However, popcorn showed the lowest FV, SB and PV (Table II). In popcorn endosperm, polygonal starch granules are tightly packed in the

protein matrix, which inhibits their swelling in water (Narváez-González et al. 2006; Sandhu et al. 2004).

Thermal Properties

Gelatinization

Table III shows the gelatinization parameters of the whole grain flour obtained from the studied genotypes. The gelatinization properties of starches are affected by many factors including size of starch granules, amylose/amylopectin ratio (Inouchi et al. 1983), fine structure of amylopectin molecules (chain length and branching ratio) (Jane et al. 1999), protein and lipid content, and architecture of granules (amorphous to crystalline ratio) (Tester 1997). A great variability was found in the gelatinization properties in genotypes. The ΔH_G average of *opaque-2* was significantly higher than that of white corn and popcorn. Higher ΔH_G has been reported by White et al. (1990) in open-pollinated varieties of dent and floury corn and by Ng et al. (1997a) in inbred lines (S3) of yellow semi-dent corn. On the other hand, Méndez-Montealvo et al. (2005) obtained lower ΔH_G between QPM simple hybrids. High gelatinization enthalpy reflects a high percentage of crystallinity of the amylopectin, thus a larger amount of energy is required to fuse its crystallites (Sandhu and Singh 2007; Uarrota et al. 2013). Lower amylose content implies higher amylopectin proportion, which might explain the higher ΔH_G shown by *opaque-2* genotypes in relation to non-opaque genotypes used as controls in this study, containing higher amylose. In *opaque-2* genotypes a negative correlation was found between amylose content and ΔH_G (r = -0.36; $P < 0.05$). Krueger et al. (1987) observed lower ΔH_G in high-amylose maize starch as compared to that in other mutant corns.

Significant differences were observed in *opaque-2* genotypes in T_{oG} and T_{pG}, showing slight differences in relation to white corn and popcorn (Table III). A similar variability was reported by Li and Glover (1994) in 35 tropical and subtropical maize populations. Seetharaman et al. (2001) found a similar T_{oG} range in Argentinean landraces. *Opaque-2* genotypes showed significantly lower R_G average than in white and popcorn. This suggests a higher homogeneity of crystalline region in starch granules in *opaque-2* grains due to narrow gelatinization ranges. Narrow-range starch allows a more rapid gelatinization (Hegenbart 1996) and may be useful for industrial applications. Seetharaman et al. (2001) showed higher R_G in six floury corn races. In *opaque-2* genotypes, ΔH_G correlated positively with T_{oG} and T_{pG} (r = 0.4 and r = 0.42, respectively; $P < 0.05$). In addition, samples with greater T_{oG} and T_{pG} were more likely to have smaller R_G (r = -0.86 and r = -0.67; $P < 0.05$). A similar correlation pattern was reported by Eyhérabide et al. (2007a) in 12 inbred lines from Argentina.

Original population (POr) showed the highest ΔH_G and lowest R_G with respect to progenies obtained from it (Table III). These results show differences in composition and crystalline structure of starch granules in genetic material used as original population. In addition, the genetic variability among progenies could have manifested in the differences obtained in the starch gelatinization parameters.

The annual average variation in *opaque-2* genotypes did not show important differences in ΔH_G, while slight differences in T_{oG} and T_{pG} were observed. In this study, mean temperatures (Supplementary Table I) during the starch synthesis period were similar in both crop years and environmental conditions did not have a major impact on gelatinization properties, which remained similar in both crop years.

Retrogradation

Table IV summarizes data of retrogradation parameters of the whole grain flour obtained from the studied genotypes. Two endotherms were observed in DSC analysis (Fig. 1). The first was considered crystallization of mainly amylopectin chains (ΔH_{R1}) (Sandoval-Aldana et al. 2005). The second endotherm corresponds to the melting of amylose-lipid complex (ΔH_{R2}), and both enthalpies ($\Delta H_{R1} + \Delta H_{R2}$) were considered total starch retrogradation (ΔH_{RT}). A wide range of variation was seen in ΔH_{RT} in *opaque-2* genotypes. Higher retrogradation enthalpies were reported by Ng et al. (1997a) in 62 corn inbred lines. The average ΔH_{RT} shown by *opaque-2* genotypes was not significantly different to that of white corn and popcorn. Seetharaman et al. (2001) showed no significant differences in ΔH_R averages between Argentinean flint and floury corns.

Significant differences were obtained in T_{oR1}, T_{pR1} and R_{R1} in *opaque-2* progenies (Table IV). The T_{oR1} average of *opaque-2* samples was lower than in white corn and higher than in popcorn. Lower average values of T_{oR} were reported in landraces of floury corn and flint corn (Seetharaman et al. 2001) and inbred lines (Ng et al. 1997a). The relationship found between ΔH_{R1} with R_{R1} ($r = 0.72$; $P < 0.05$) in *opaque-2* genotypes indicates that a higher temperature range was required to melt the crystalline structure resulting from molecular reordering during storage. The breadth of endothermic peak is related to the heterogeneity of the molecular associations in the retrograded starch (Yuan et al. 1993). Amylopectin and intermediate materials also play an important role in starch retrogradation during refrigerated storage (Yamin et al. 1999). Hence, an increase of intermediate materials might result in broader retrogradation ranges (Ng et al. 1997b; Yuan et al. 1993).

The averages of T_{oR2} and T_{pR2} observed in *opaque-2* genotypes were slightly different from those in white corn and popcorn (Table IV). Similar endotherm temperatures for the melting of

amylose-lipid complexes were reported by Chung and Liu (2009) in corn, amylomaize and rice, and by Ng et al. (1997b) in normal and mutant corns. A wide variation range was seen in ΔH_{R2} in *opaque-2* genotypes, with an average value significantly higher than that found in popcorn and lower than that found in white corn. Chung and Liu (2009) reported similar enthalpies for the melting of the amylose-lipid complex during cooling in normal maize, amylomaize and rice. The P3 sample showed the highest amylose and lipid content (Table I) and the highest ΔH_{R2} (Table IV). Conversely, P28 presented low amylose and lipid content and consistently low ΔH_{R2}. Lipid content influences the amylose chain association (Boltz and Thompson 1999). Amylose chains form complexes with lipids, and they are less able to form junction zones, influencing the formation of extended intermolecular networks in starch gels (Blazek and Copeland 2009). An annual average increase was found in ΔH_{R2} (Table IV) and lipid content (Table I) and, concomitantly, positive correlations between lipid with ΔH_{R2} ($r = 0.45$; $P < 0.05$) and R_{R2} ($r = 0.45$; $P < 0.05$) were obtained in the *opaque-2* genotype group. These results suggest that an increase in lipid content within grain contributes to the formation of stable amylose-lipid complexes, showing higher peak breadth (R_{R2}) for their melting and higher enthalpy. Chung and Liu (2009) found a positive relationship between enthalpy of melting of the amylose-lipid complex with free and bound lipid content.

The %*R* is a measure of the tendency of the gelatinized starch to retrograde after low-temperature storage (Ng et al. 1997a). A great variability was shown by *opaque-2* genotypes in %*R*, with an average value significantly lower than that in white corn and popcorn (Table IV). Similar %*R* ranges have been reported in maize inbred lines by Ng et al. (1997a) and Eyhérabide et al. (2007b). The higher amylose content shown in white and popcorn used as controls (Table I) might explain the higher %*R* with respect to that found in *opaque-2* genotypes. Jane et al. (1999) found similar %*R* in high-amylose corn. In addition, the %*R* correlated positively with starch

content (r = 0.35; $P < 0.05$) and negatively with protein content (r = -0.38; $P < 0.05$). The primary effect of proteins on retrogradation is a decrease in the starch rate and not in an interaction with proteins (White 2001). Yuan et al. (1993) reported that starch content was determinant in the shapes and enthalpies of the retrogradation thermograms in mutant corn genotypes.

CONCLUSIONS

A great variability was found in starch properties of *opaque-2* genotypes, revealing differences in composition and structure. Differences among starting genetic material (POr) and half-sib families suggest the existence of genetic variability within the original population. However, a larger sample size is required to make inferences about genetic variability of a population.

The prevailing environmental variables analyzed (temperatures and rainfall) were similar during both crop years, suggesting that variations observed in annual average values among families and their progenies could result mainly from the effect of genotype in the flour composition and, specially, in starch structure. Indeed, pollination in half-sib families occurs with several individuals of the same family, and therefore, the genetic variability in each family is greater than that seen in inbred lines or hybrids, where differences in phenotypic traits are mainly attributed to the environmental effect. However, many other environmental variables not analyzed in this study may have had an influence on the measurements.

The lower pasting temperature and the higher peak viscosity developed by *opaque-2* maize indicate that these genotypes would be more suitable for food processing requiring greater viscosity development during heating. The variability found among genotypes indicates different starch behavior, which would have important implications for the end-product quality.

The results from DSC assays suggest that differences seen in the gelatinization and retrogradation properties between opaque and non-opaque genotypes may be mainly influenced by amylose content. The higher ΔH_G values provided by *opaque-2* genotypes suggest greater molecular order within starch granules. The average values of %*R* in both years, which indicate a similar tendency to retrograde in both generations of *opaque-2* genotypes, were significantly lower in non-opaque genotypes used as controls. However, differences observed in retrogradation peaks (ΔH_{R1} and ΔH_{R2}) indicate that interaction with other grain components could have played an important role due to unusual values shown by some samples. High lipid content shown by *opaque-2* genotypes could have played a fundamental role in starch retrogradation, mainly ascribed to differences found in the melting enthalpy of amylose-lipid complex. Additionally, higher %*R* found in white corn and popcorn indicates their greater tendency to retrograde for which the higher amylose content may have been a determining factor.

Taking into account that *o2* mutation increases essential amino acids content even when it produces a soft floury endosperm, the differences found in starch behavior would make it possible to identify individual families with particular starch properties, thus varieties with specific traits may be developed and used in the production of processed starchy food items with improved nutritional properties.

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TABLE I Flour Chemical Composition of Maize Genotypes

[a]Genotype	Protein (%)	Starch (%)	Amylose (%)	Lipid (%)	Ash (%)
P3	11.63d	61.94a	23.88b	10.86d	1.9b
P5	11.25c	65.49b	21.68a	7.65b	1.73a
P10	11.03c	63.64b	22.30a	8.54c	1.8a
P13	10.06a	66.32b	21.10a	7.91b	1.9b
P15	10.11a	63.28b	20.69a	8.72c	1.96c
P18	9.76a	64.47b	21.37a	8.77c	1.88b
P19	11.11c	60.85a	20.04a	8.06b	1.87b
P20	10.09a	65.21b	21.54a	8.64c	1.76a
P22	10.44b	65.72b	21.05a	9.07c	2.14d
P25	11.90d	63.5b	19.61a	6.68a	1.97c
P26	10.67b	61.77a	19.96a	7.68b	1.92b
P28	12.28e	66.03b	19.52a	8.04b	2.09d
POr	10.57b	61.24a	18.55a	8.64c	2.06d
[b]**2012**	10.26a	64.97b	22.16b	8.24a	1.87a
2013	11.42b	62.64a	19.58a	8.57b	1.97b
[c]**Opaque**	10.84a	63.8a	20.87a	8.41a	1.92b
BL	11.17a	66.34a	24.58b	6.73a	1.68a
PS	10.33a	66.22a	25.35b	7.83a	1.54a

Values followed by different letters in the same column are significantly different ($P < 0.05$).

[a] Values of each genotype (P_n) and Original Population (POr) correspond to the average between both crop years (2012 and 2013).

[b] Comparison of annual averages of all *opaque-2* half-sib families harvested in 2012 and their individual progenies grown in 2013. The data showed in 2012 correspond to the average of the twelve *opaque-2* half-sib families harvested in that year. Data showed in 2013 correspond to the average of their twelve individual progenies, in order to compare annual average variation on analyzed genotypes.

[c] Comparison of the average between all *opaque-2* genotypes (Opaque) in both crop years, with white corn (BL) and popcorn (PS) used as controls.

TABLE II Pasting Properties Parameters[a] of Whole Grain Flour Measured by Rapid Visco Analyzer (RVA)

[b]Genotype	PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)	PT (°C)
P3	1574.00a	1195.50c	378.50a	3089.75d	1872.25d	80.09a
P5	1781.00b	1330.00d	451.00a	3509.50e	2060.25f	78.50a
P10	1500.25a	911.25a	589.00b	2227.50a	1269.25a	73.74a
P13	2418.75f	1482.00e	936.75d	3545.25e	1868.00d	77.10a
P15	2239.75e	1247.75d	992.00e	3206.00d	1850.25d	77.70a
P18	2790.25g	1588.00f	1202.25f	3859.50f	2148.00f	76.88a
P19	2171.50e	1137.00c	1034.50e	2801.75c	1491.50c	78.38a
P20	1595.50a	1140.25c	455.25a	3086.00d	1805.50d	79.84a
P22	2369.50f	1462.75e	906.75d	3546.50e	1934.50e	78.13a
P25	1909.50c	1076.00b	833.50c	2647.75b	1407.25b	75.88a
P26	2003.25d	1430.00e	573.25b	3256.25d	1572.00c	78.26a
P28	2343.00f	1322.50d	1020.50e	3405.75e	1996.75e	77.48a
POr	1861.00c	1274.50d	586.50b	4048.00g	2722.50g	78.30a
[c]**2012**	2114.04b	1163.73a	950.31b	2807.81a	1640.15a	76.56a
2013	1971.69a	1389.73b	581.96a	3689.04b	2051.85b	78.86b
[d]**Opaque**	2042.87c	1276.73c	766.13b	3248.42b	1846b	77.71a
BL	1007.5b	940b	67.5a	3660b	2196.5b	89.6b
PS	397a	319.5a	77.5a	1462.5a	1143a	93.63c

Values followed by different letters in the same column are significantly different ($P < 0.05$).

[a] PV = peak viscosity; TV = trough viscosity; BD = breakdown; FV = final viscosity; SB = setback; PT = pasting temperature.

[b] Values of each genotype (P_n) and Original Population (POr) correspond to the average between both crop years (2012 and 2013).

[c] Comparison of annual averages of all *opaque-2* half-sib families harvested in 2012 and their individual progenies grown in 2013. The data showed in 2012 correspond to the average of the twelve *opaque-2* half-sib families harvested in that year. Data showed in 2013 correspond to the average of their twelve individual progenies, in order to compare annual average variation on analyzed genotypes.

13 [d] Comparison of the average between all *opaque-2* genotypes (Opaque) in both crop years, with
14 white corn (BL) and popcorn (PS) used as controls.

15

TABLE III Gelatinization Parameters[a] of Whole Grain Flour Measured by Differential Scanning Calorimetry (DSC)

[b]Genotype	ΔH_G (J/g)	T_{oG} (°C)	T_{pG} (°C)	R_G (°C)
P3	6.47a	68.23c	73.41d	9.95d
P5	6.55a	67.47b	72.33b	9.46c
P10	5.84a	68.48c	72.88c	8.73b
P13	6.18a	68.81c	73.22d	8.53b
P15	8.17c	68.83c	73.55d	8.92b
P18	5.87a	67.64b	72.73c	9.61c
P19	6.52a	68.1c	72.95c	9.29c
P20	5.49a	65.21a	71.4a	11.32e
P22	7.37b	67.75b	73.31d	10.44d
P25	9.32d	67.74b	72.97c	10.02d
P26	8.32c	67.64b	72.72c	10.33d
P28	6.8a	67.83b	72.84c	9.23c
POr	10.09d	70.93d	74.94e	8.1a
[c]**2012**	7.14a	68.5b	73.52b	9.41a
2013	7.17a	67.59a	72.51a	9.65b
[d]**Opaque**	7.15b	68.05b	73.02b	9.53a
BL	5.58b	65.25a	72.09a	15.09c
PS	3.01a	68.79b	73.91b	11.42b

Values followed by different letters in the same column are significantly different ($P < 0.05$).

[a] ΔH_G = enthalpy of gelatinization; T_{oG} = onset temperature; T_{pG} = peak temperature; R_G = range of temperatures (T_{eG} - T_{oG})

[b] Values of each genotype (P_n) and Original Population (POr) correspond to the average between both crop years (2012 and 2013).

[c] Comparison of annual averages of all *opaque-2* half-sib families harvested in 2012 and their individual progenies grown in 2013. The data showed in 2012 correspond to the average of the twelve *opaque-2* half-sib families harvested in that year. Data showed in 2013 correspond to the average of their twelve individual progenies, in order to compare annual average variation on analyzed genotypes.

[d] Comparison of the average between all *opaque-2* genotypes (Opaque) in both crop years, with white corn (BL) and popcorn (PS) used as controls.

TABLE IV Retrogradation Parameters[a] of Whole Grain Flour Measured by Differential Scanning Calorimetry (DSC)

	First Endotherm				Second Endotherm					
[b]Genotype	ΔH_{R1} (J/g)	T_{oR1} (°C)	T_{pR1} (°C)	R_{R1} (°C)	ΔH_{R2} (J/g)	T_{oR2} (°C)	T_{pR2} (°C)	R_{R2} (°C)	ΔH_{RT} (J/g)	*%R*
P3	0.45a	51.43b	56.46b	9.74a	2.38d	82.24a	90.9a	21.12c	2.91d	28.25b
P5	1.95d	46.43a	54.29a	14.19b	0.97a	88.85b	97.35b	13.79b	2.92d	43.28d
P10	0.48a	48.85a	53.06a	8.91a	0.64a	92.06c	99.18b	11.68b	1.1a	13.96a
P13	1.94d	46.83a	54.97a	15.16b	1.34b	91.79c	96.65b	8.26a	3.28d	48.9d
P15	3.04f	47.42a	56.01b	15.63b	1.95c	87.04b	97.44b	16.87b	4.99f	62.27e
P18	2.9f	47.36a	55.88b	16.09b	1.21b	90.6c	95.77b	13.43b	4.1e	69.92f
P19	0.77b	51.63b	57.43b	10.81a	1.49b	91.9c	98.52b	11.81b	2.26c	35.02c
P20	1.56c	49.32a	54.39a	12.75b	2.26d	83.99a	91.17a	16.91b	3.82e	71.94f
P22	1.39c	49.14a	57.24b	12.4b	1.7c	87.91b	97.9b	14.24b	3.09d	41.87d
P25	2.37e	48.29a	55.9b	15.72b	1.84c	88.12b	97.05b	15.97b	4.2e	47.92d
P26	1.84d	49.02a	57.02b	12.69b	2.22d	87.14b	95.31b	15.4b	4.06e	49d
P28	0.85b	48.91a	56.66b	13.14b	0.87a	86.82b	95.14b	13.95b	1.72b	26.45b
POr	2.05d	47a	53.97a	15.17b	1.46b	84.13a	94.81b	18.72c	3.52d	34.81c
[c]**2012**	1.86b	49.04b	55.68a	12.67a	1.48a	89.37b	96.83b	13.19a	3.6b	44.15a
2013	1.46a	48.13a	55.59a	13.85b	1.64b	86.75a	95.32a	15.88b	3.1a	44.1a
[d]**Opaque**	1.66a	48.59a	55.64a	13.26b	1.56a	88.06b	96.07a	14.64a	3.11a	44.12a
BL	1.38a	52.28b	56.67a	9.32a	2.86b	82.49a	92.55a	20.62b	4.23a	75.91b
PS	1.95a	46.97a	54.59a	14.88b	0.89a	86.68b	93.43a	13.55a	2.84a	94.72b

Values followed by different letters in the same column are significantly different ($P < 0.05$).

[a] ΔH_R = retrogradation enthalpy; T_{oR} = onset temperature; T_{pR} = peak temperature; R_R = range of temperatures (T_{eR} - T_{oR}); ΔH_{RT} = total retrogradation enthalpy (ΔH_{R1} + ΔH_{R2}); %R = percentage of retrogradation calculated as $\Delta H_{RT}/\Delta H_G$ x 100.

[b] Values of each genotype (P_n) and Original Population (POr) correspond to the average between both crop years (2012-2013).

[c] Comparison of annual averages of all *opaque-2* half-sib families harvested in 2012 and their individual progenies grown in 2013. The data showed in 2012 correspond to the average of the twelve *opaque-2* half-sib families harvested in that year. Data showed in 2013 correspond to the average of their twelve individual progenies, in order to compare annual average variation on analyzed genotypes.

[d] Comparison of the average between all *opaque-2* genotypes (Opaque) in both crop years, with white corn (BL) and popcorn (PS) used as controls.

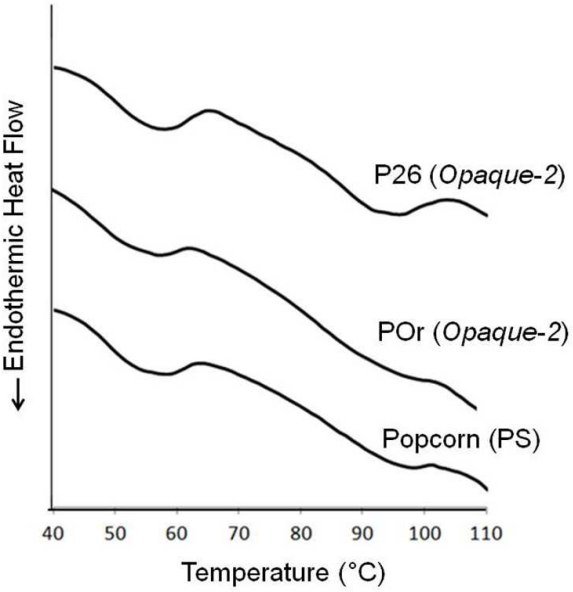

Fig. 1. DSC endotherms of retrogradation of maize genotypes: Starting material (POr) and P26 progeny were considered as representative of *opaque-2* samples. Popcorn (PS) was used as non-opaque control.

SUPPLEMENTARY TABLE I Average Monthly Temperatures and Total Precipitation of Crop Years

Crop Year Month	Temperature Minimum (°C)	Temperature Maximum (°C)	Temperature Average (°C)	Total Precipitation (mm)
2012[a]				
January	16.2	32.7	24.4	142.2
February	17.9	29.6	23.8	166
March	13.6	28.4	21.0	54
April	11.6	24.4	18.0	22.2
May	9.4	23.1	16.2	10
Jun	3.8	19.6	11.7	0.8
Average	12.1	26.3	19.2	
2013				
January	16.9	31.7	24.3	95.6
February	15.5	28.2	21.8	90.6
March	12.6	25.8	19.2	44.4
April	10.2	25.9	18.1	47.4
May	7.2	21.9	14.5	33
Jun	4.5	20.2	12.4	2
Average	11.2	25.6	18.4	

[a] Grain-filling occurred during April and May in both crop years.