Diversity among maize populations from Spain and the United States for dough rheology and gluten-free breadmaking performance

Running title: **Dough rheology and breadmaking in maize**

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Summary

Maize is used for bakery and for gluten free food for celiac patients. Our objective was assessing diversity for dough rheology and breadmaking in maize with different origins, grain types and growth cycles. Endosperm type affected bread crumb color having dent maize higher $L*$ and $a*$ and instant recovery speed. Population origin affected flotation index, onset pasting temperature, bread crumb color, hardness and instant recovery speed. Finally, growth cycle affected flotation index, crumb color $L*$ and $a*$, and cohesiveness. Water binding capacity, crumb color and hardness were the most discriminative parameters for maize. The maize population Andaluz/Daxa was the less distant from wheat parameters, and Tremesino was the most different.

Key words: Maize; flour; rheology; bread; gluten-free bread

Introduction

In the north of Spain and Portugal, maize (*Zea mays* L.) whole grain has been used for bakery for more than four centuries (Brites *et al*., 2010; Malvar *et al*., 2012; Revilla *et al*., 2003, 2008; Vaz Patto *et al*., 2007, 2009). In those countries, maize bread is usually made with mixtures of flour from maize, wheat and rye. Nevertheless, maize is also used for bakery in countries where wheat is not available at affordable prices (Nkhabutlane *et al*., 2014; Rai *et al*., 2012). Lately, a great potential value of maize is the manufacturing of bakery products for celiac patients (Brites *et al*., 2010; de la Hera *et al*., 2013). Indeed, maize lacks gluten, a mixture of storage proteins that gives wheat bread the viscoelastic network that enables the dough to hold the gas produced during the fermentation process, leading to an aerated crumb bread structure. However, gluten must be absent in the diet of celiac patients and bread made without gluten has physical characteristics that differ substantially from standard wheat bread (Matos & Rosell, 2011, 2015).

Quality criteria for maize have been defined for diverse culinary uses, e.g. maize is classified in the US into five different grades, based on grain density, proportion of whole grains, damaged grains, and grain color (FAO, 1992); nevertheless, less attention have been paid to their classification according to breadmaking potential. Genotypic variation and environmental growth conditions have a significant impact on physico-chemical quality of maize grain. This impact has been largely documented on the processing quality and sensory properties of masa and tortilla (Mexican products) (Vazquez-Carrillo *et al*., 2011; Harrigan *et al*., 2007), which required special processing. However, very limited information has been reported regarding fermented bread. Previous reports pertaining to the relationship between maize standard quality criteria and breadmaking potential have been focused on the production of breads containing gluten. Revilla *et al*. (2008) found genetic variability for yield and grain quality in a collection of open-pollinated maize populations used for making gluten containing bread in the north and northwest of Spain. Brites *et al.* (2010) found not significant differences among maize varieties for dough behavior during mixing and handling or specific volume, but sensory analysis revealed a preference for traditional maize populations over the hybrids. Accordingly, consumers prefer maize bread made with traditional flint populations than with modern commercial dent hybrids (Landa *et al*., 2006). Differences among maize samples were also significant for chemical traits such as protein, amylose and ash content (Brites *et al*. 2010). Vaz Patto *et al*. (2009) found variability for viscosity and chemical composition in a collection of maize populations from Portugal and concluded that Portuguese germplasm could be a valuable resource for quality improvement. To the best of our knowledge, diversity for bakery performance in the gluten free context has not been previously reported in a representative collection of maize germplasm.

Our objective was assessing diversity for dough rheology and breadmaking in representative collection of open-pollinated maize populations with different origins, grain types and growth cycles..

Material and Methods

Maize

Seven maize open-pollinated populations were selected as a representative sample of the maize germplasm available for breeding in the Spanish collection, including two populations from the USA (Table 1). The populations represent the grain types, the climatic adaptation and the growth cycles used in breeding programs. Each population was multiplied in Pontevedra (northwest of Spain) for three years in order to have seed from three environments for evaluations. Two commercial flours of wheat and rice were included in the tables of means for reference, but they were not included in the statistical analyses. Whole grain of maize populations and commercial rice were milled and used for making bread, along with wheat flour (Figure 1).

The assessment of the flotation index was used for characterizing the kernel hardness. One hundred kernels were placed in a beaker containing 300 mL of NaNO3 solution (1.25 g mL-1). They were stirred to separate the kernels and let stand for 1 min. The number of floating kernels indicates the flotation index (SAGARPA, 2002). The test was performed in duplicate.

Maize whole meal characteristics

Whole meal maize flour was obtained after milling the samples using a laboratory mill (IKA M20 Labortechnik, Staufen, Germany). Whole meal flour was sieved through 0.85 mm screen, and then larger particles were ground and sieved again to obtain less than 0.85 mm particles, this fraction was added back to the flour. Moisture flour content was calculated based on ISO 6540:1980 method (ISO, 1980). The pasting properties were determined by Rapid Visco Analyser (RVA 4500, Perten Instruments SA, Stockholm, Sweden) following the International Association of Cereal Chemists Approved Method 162 (ICC, 1997). The sample (3.5 g of flour based on 14 g of moisture per 100 g of flour) was dispersed in distilled water (25.0 mL). For RVA calculation, flour samples were hold for 60 s at 50 ºC, heated from 50 to 95 ºC in 282 s, hold at 95 ºC for 150 s and then cooled to 50 ºC. Each cycle was initiated by a 10 s, 960 rpm paddle speed for mixing followed by a 160 rpm paddle speed for the rest of the assay. Viscosity was recorded during a heating-cooling cycle using Thermocline software for Windows (Perten Instruments

SA, Stockholm, Sweden). Peak viscosity at 95ºC, final viscosity, setback (difference between final viscosity and peak viscosity at 95ºC) and onset temperature were evaluated.

The water hydration capacity, defined as the amount of water retained by the sample under centrifugation, was also evaluated. Samples (100 mg) were mixed with distilled water (1 ml) and vortexed for five minutes at 600 rpm, then they were centrifuged at 5,000 x *g* for 10 min. Water hydration capacity was expressed as grams of water retained per gram of solid. All the analyses were made by duplicate.

Breadmaking Process

The formula used for maize and rice was in flour basis: 2% sugar, 1% dry yeast, 0.5% xanthan gum and 100% water. For wheat flour breads were used the same formulation without the hydrocolloid (xanthan gum) and the amount of water was 60% (v/w flour basis). The doughs were optimally mixed at 100 rpm in stirrer with a turbine accessory (IKA Eurostar 40, Staufen, Germany). The breadmaking were carried out in a scale down method, 4 g of dough were put in well-greased glass pans, proofed for 30 min at 35 ºC and 50% relative humidity and then baked in an oven at 130 ºC for 7 min (Garzón & Rosell, 2014). Two batches were run for each sample.

Bread Analyses

In order to determine the bread quality, the crumb color and texture were quantified. Crumb bread color was determined by using a colorimeter (Chroma meter CR-400/410, Konica Minolta, Tokyo, Japan) after standardization with a white calibration plate (L^*=96.9, a^*=−0.04, b^*=1.84). The color was recorded using CIE-$L^*a^*b^*$ uniform color space, where L^* indicates lightness, a^* indicates hue on a green (−) to red (+) axis, and b^* indicates hue on a blue (−) to yellow (+) axis. Values are the mean of three replicates. Crumb hardness was determined in a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK) using a texture profile analysis (TPA). A bread slice of 10 mm thickness was compressed up to 50% of its original height at a test speed of 1 mm/s, with a cylindrical stainless steel probe having an adapted diameter for these slices (diameter 6 mm). Values were the mean of six replicates.

Statistical Analysis

The effect of maize population on grain hardness, flour characteristics and bread technological quality parameters were analyzed by analysis of variance being varieties fixed factors and all other sources of variation random factors. Means comparisons were performed by Fisher's protected LSD test. As the maize populations represent different origins, growth cycles and grain types (Table 1), subsequent analyses of variance were carried out for studying the effects of these factors on flour and bread quality parameters. For these analyses, we grouped the populations by origins (North of Spain, South of Spain and USA), growth cycles (early, medium and late), and grain type (dent and flint), respectively. In these analyses, means were calculated with the Least-square-means method because the number of data used for calculating each mean was different: consequently, mean comparisons were made by pairs of means using the Least-square-means method. Principal component analyses were performed with flour and bread parameters, respectively, in order to identify the most discriminant parameters for maize populations and to figure out the distribution of the maize populations in the multivariable space. All statistical analyses were conducted at a significant level of $P \leq 0.05$ with Statistical Analysis System (SAS Institute, 2010).

Results and discussion

Grain, whole meal and bread quality traits of seven open-pollinated maize populations with different grain type, origin and growth cycle (Table 1) were shown in Table 2. For comparison purposes, the values obtained for wheat and rice are included in Table 2, but not considered in statistical analysis. Grain quality was evaluated by recording flotation index, which is an estimator of grain hardness or grain density. For flotation index, there was enough variability from the most grain-dense population Tremesino (23.33) to the lightest-grain population ASG (76.67) (Table 2), but it was not possible to find significant relationship with the type of grain. Growth cycle affected flotation index, having early populations higher flotation index. Similarly, population origin affected flotation index, as the US populations were harder than the Spanish ones. The optimum value for flotation index depends on the intended use of the maize grain. Values lower than 40% are recommended for the masa and tortilla industry, although highlands landraces used for that purpose had an average flotation index of 61% (Vázquez-Carrillo *et al*, 2011). The preference for high density grains have been reported for bread (Thompson and Goodman, 2006; Revilla *et al*., 2008).

Maize flours had water binding capacity similar to rice and larger that wheat flour; onset pasting temperature was slightly larger in maize flours, compared to rice and wheat; maximum and final viscosity in maize was far below those of rice and wheat; and setback was similar in maize and wheat (Table 2). Moisture was higher for early than for late populations, and for American than for Spanish populations (Table 3). Water binding capacity varied with origin, growth cycle and grain type, being the lowest values obtained with Rastrojero and Sajambre, along with Tremesino and Tuy (Table 2). Pasting properties were determined since gelatinization and gelification has been reported as one of the most important predictors for gluten free bread development (Matos & Rosell, 2015). Viscosity recorded during the heating-cooling cycle (Figure 2) of whole meal maize suspensions showed different profiles compared to those of wheat or rice flours. Generally, viscosity patterns of maize traits lacked of a clear peak viscosity, showing a continuous increase in viscosity during heating and cooling, although two deep slopes were detected initially after the onset temperature and then at the end of the cooling gradient. No decay of viscosity, when holding temperature at 95 ºC, was detected when analyzing the different traits, indicating good stability of the starch granules during heating, in consequence, no breakdown was determined. The lowest maximum viscosity at 95 ºC was obtained for Tremesino, which together with Tuy showed the lowest final viscosity; Both populations had also the lowest flotation index and thus have the grains with the highest density. It would be expected lower breadmaking performance in those populations since Matos and Rosell (2015) found a significant positive correlation between the viscosity increase during cooling and both hardness and cohesiveness of bread crumbs. There was diversity for flour quality among these maize populations, as previously reported by Brites *et al*. (2010), who also found significant differences among maize populations for flour color and viscosity, since they reflected the variation of genotypes associated to starch structural properties (Ketthaisong *et al*., 2014). The population Tuy, which had high hedonic sensory qualification for gluten containing breads in a previous study (Revilla *et al*. 2008) was closer to wheat only for grain hardness and cohesiveness, while it was distant for onset pasting temperature, chewiness, and instant recovery speed.

Breads were made from each maize population to identify the effect of genetic variation on their breadmaking performance (Table 2, Figure 1). All maize populations produced bread clearly distinct from wheat and rice. Maize breads were far below wheat and rice values for crumb color L^*, retarded recovery elasticity, cohesiveness and chewiness (Table 2). Maize populations had variable values for crumb color a^* and b^*, being maize similar to wheat and rice for crumb color a^* and having higher values for crumb color b^*. The southern Spanish late dent population Andaluz/Daxa was the closest one to wheat for crumb color L^* and b^*, while the southern Spanish medium semident population Tremesino was the most distant to wheat for crumb color a^* and was quite distant for L^* and b^*. Endosperm type affected significantly bread traits (Table 3, Figure 1). Dent and flint populations were significantly different for some bread traits, particularly flint maize had lower crumb luminosity L^* and reddish color (a^*), but yielded higher yellowish tone (b^*). Southern Spanish maize led to brighter crumbs with reddish light yellow tones, and breads showed faster recovery of the crumb structure, similar to the US maize. Finally, growth cycle affected crumb color parameters L^* and a^*, and cohesiveness, having early populations higher crumb color b^* and lower L^* and a^* and instant recovery speed than late populations. Regarding crumb hardness, maize breads showed close hardness to rice breads, which, as expected, was higher than that of wheat bread. Population origin had significant effect on hardness, being southern Spanish populations the hardest ones. In addition, dent maize had higher instant recovery speed, which have being associated to lighter crumb structures with higher number of gas cells leading to faster recovery of the structure after compression (Matos *et al*., 2014). Rai *et al*. (2012) found that substitution of wheat flour by rice or maize altered the pasting properties and breadmaking qualities. As the proportion of wheat flour was reduced, the pasting temperature and the baking absorption increased, whereas bread specific volume decreased. Similar results were also reported by Nkhabutlane *et al* (2014), who found that steamed maize breads had lower volume, harder and denser crumbs, their texture was more chewy, dry, fibrous, brittle and needed higher compression force to deform than wheat bread. Therefore, bread quality parameters were quite distant from those of wheat or rice, and most of them depend on the maize variety chosen. There was diversity for bread quality among these maize populations but no clear pattern of geographic or growth cycle was observed for dough characteristics. Similarly, Revilla *et al.* (2008) concluded that there was no clear relationship between agronomic and breadmaking performance in gluten containing breads.

Altogether, our populations had lower pasting temperatures than those reported by Brites *et al.* (2010) for the two Portuguese populations analyzed and even lower than those of wheat and rice. These results agree with the results of Martínez and el-Dahs (1993) who found a reduction of maximum viscosity and an increase of final viscosity when maize flour was added to wheat flour. Several authors found clear differences between flint and dent maize for dough properties (Brites *et al.*, 2010; Almeida-Dominguez *et al.*, 1997; Sandhu *et al.*, 2007); although present study indicates that flour and bread characteristics are slightly related with flint or dent phenotype and dent grains were more distant than flint grains for this quality traits when a representative sample of maize populations with intermediate values for flint and dent grain are used.

Principal components analysis was performed to find possible clusters of maize populations according to flour and bread parameters. The first two principal components (CP) for flour traits had eigenvalue above one and explained 89% of the variability (Figure 3a). PC1 explained 67% of the variability and the quality traits with highest contribution were final viscosity and setback with positive sign, which were the most discriminative quality parameters for maize flour (Matos and Rosell 2015). PC2 explained 22% of the variability and the quality trait with highest positive contribution was maximum viscosity at 95ºC, while Water Binding Capacity had a negative score in PC2. Both US populations were located in the same sector of the plot, while the Spanish populations were dispersed. Furthermore, there was no clear distribution pattern for origin, cycle or grain type. However, traditional maize bread containing gluten in the humid Spain is made with populations like Tuy that is in the double negative sector.

For bread quality (Figure 3b), the first three PC had eigenvalue above one; PC1 explained 41% and had the highest positive contributions from hardness and crumb color $L*$, while crumb color $b*$ and cohesiveness had large negative contributions. PC2 explained 29% of the variability and the largest positive contributors were chewiness, retarded recovered elasticity, and crumb color *a**. Therefore, the bread quality parameters with the highest discriminant ability among maize populations were hardness, crumb color *L**, crumb color *b** and cohesiveness. The distribution of maize populations in the PCA plot, based on bread quality, was not related to that based on flour quality (Figure 3a). The distribution maize populations in the PCA plot based on bread parameters did not follow a clear pattern. Nevertheless, the two US populations were quite close and both populations from norther Spain were not far away along the PC2, suggesting that the origin of maize germplasm could have some effect on bread quality. Also both medium-cycle populations from the dry Spain (Rastrojero and Tremesino) were quite close, suggesting that growth cycle could affect bread quality.

Considering both PCA for flour and bread together, they resemble the PC1 obtained by Vaz Patto *et al* (2009) although the quality traits involved in each analysis were different except for the viscosity traits. Similarly, these authors obtained a PC1 that included color, as in our present work. We can speculate that the populations with positive scores in these PCs would produce flour with characteristics less distant from the dough of wheat, though they are not necessarily those with more potential value for the quality standards of traditional maize bread. Actually, the viscosity profile of standard wheat and rice samples and that of two typical maize flours (the European Flint Tuy, and the Corn Belt Dent AS3(HT)C3) was so different that we should not expect to imitate wheat bread replacing wheat by maize (Figure 2). The viscoelastic profile obtained for these populations was very different from those of wheat and rice, but it was also different than the profile found by Brites *et al.* (2010) by using maize flour produced by an electric mill.

Conclusion

There is large diversity among maize populations for flour and bread quality parameters, and maize was very different from wheat or rice for most parameters, particularly maximum and final viscosity, crumb color *b**, retarded recovery elasticity and chewiness. Considering all traits together, the southern Spanish medium semident population Tremesino was the most distant from wheat quality parameters, followed by the northern Spanish medium flint population Tuy; while the southern Spanish population Andaluz/Daxa and the US early semiflint population AS-G were the less distant to wheat. Maize origin affected most quality parameters, while growth cycle affected fewer parameters and grain type only a few bread parameters. These results open new possibilities for breeding maize for bread quality, but further research is needed in order to find out which types of maize are more appropriate for breadmaking.

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References

Almeida-Dominguez, H. D., Suhendro, E. L., & Rooney, L. W. (1997). Factors affecting rapid visco analyser curves for the determination of maize kernel hardness. *Journal of Cereal Science*, **25**, 93–102.

Brites, C., Trigo, M. J., Santos, C., Collar, C., & Rosell, C. M. (2010). Maize-Based Gluten-Free Bread: Influence of Processing Parameters on Sensory and Instrumental Quality. *Food Bioprocess Technology,* **3**, 707–715

de la Hera, E., Talegon, M., Caballero, P., & Gomez, M. (2013). Influence of maize flour particle size on gluten-free breadmaking. *Journal of the Science of Food and Agriculture*, **93**, 924–932.

FAO (1992). Maize in human nutrition. Food and Agriculture Organization of the United Nations, Rome.

Garzón, R., Rosell, C. M. (2014). A sistematic study of breadmaking settings for obtaining small scale bread. AACC International Annual Meeting.

Harrigan, G. G., Stork, L. G., Riordan, S. G., Ridley, W. P., MacIsaac, S., Halls, *et al*. (2007). Metabolite analyses of grain from maize hybrids grown in the United States under drought and watered conditions during the 2002 field season. *Journal of Agricultural and Food Chemistry,* **55**, 6169–617.

ICC (1997) Standard Methods of the International Association for Cereal Science and Technology, ICC Standards. The International Association for Cereal Science and Technology: Vienna.

IS0, 1980. International Standard 6540:1980. Maize: Determination of Moisture Content (on Milled Grains and on Whole Grains). Geneva: International Organization for Standardization.

Ketthaisong, D., Suriharn, B., Tangwongchai, R., & Lertrat, K. (2014). Changes in physicochemical properties of waxy corn starches after harvest, and in mechanical properties of fresh cooked kernels during storage. *Food Chemistry*, **151**, 561–567.

Landa, A., Revilla, P., Malvar, R. A., Butrón, A., & Ordás, A. (2006). Maíz para panificación. *Agricultura*, **886**, 506-509.

Malvar, R. A., Butrón, A., & Revilla, P. (2012). Maize bread: healthy and safe. En: *Bread consumption and health*, (MT Pedrosa Silva Clerici, ed.), pp.155-168, Nova Science Publishers, Nueva York, ISBN 978-1-62081-090-3.

Malvar, R. A., Revilla, P., Moreno-González, J., Butrón, A., Sotelo, J. *et al.* (2008). White maize: genetics of quality and agronomic performance. *Crop Science,* **48**, 1373-1381.

Martínez, F., & el-Dahs, A. A. (1993). Effect of addition of instant corn flour on rheological characteristics of wheat flour and breadmaking III. *Archivos Latinoamericanos de Nutrición*, **43**, 321–326.

Matos, M.E., & Rosell, C.M. (2011). Chemical composition and starch digestibility of different gluten free breads. *Plant Food for Human Nutrition*, **66**, 224-230.

Matos, M. E., Sanz, T., & Rosell, C. M. (2014). Establishing the function of proteins on the rheological and quality properties of rice based gluten free muffins. *Food Hydrocolloids*, **35**, 150-158.

Matos, M. E., Rosell, C. M. (2015). A review: understanding gluten free dough for reaching breads with physical quality and nutritional balance. *Journal of the Science of Food and Agriculture*, **95**, 653–661.

Nkhabutlane, P., du Randa, G. E., de Kock, H. L. (2014). Quality characterization of wheat, maize and sorghum steamed breads from Lesotho. *Journal of the Science of Food and Agriculture,* **94**, 2104–2117.

Rai, S., Kaur, A., Singh, B., & Minhas, K. S. (2012). Quality characteristics of bread produced from wheat,rice and maize flours. *Journal of Food Science and Technology,* **49**, 786–789.

Revilla, P., Landa, A., Rodríguez, V. M., Romay, M. C., Ordás, A., & Malvar, R. A. (2008). Maize for bread under organic agriculture. *Spanish Journal of Agricultural Research,* **6**, 241–247.

Revilla, P., Soengas, P., Cartea, M. E., Malvar, R. A., & Ordás, A. (2003). Isozyme variability among European maize populations and the introduction of maize in Europe. *Maydica*, **48**, 141–152.

SAGARPA (2002). Productos alimenticios no industrializados para consumo humano-Cereales-Parte 1: Maíz blanco para proceso alcalino para tortillas de maíz y productos de maíz nixtamalizado-Especificaciones y métodos de prueba. Dirección General de Normas México D.F NMX-FF-034/1- SCFI-2002.

Sandhu, K. S., Singh, N., Malhi, N.S. (2007). Some properties of corn grains and their flours I: Physicochemical, functional and chapati-making properties of flours. *Food Chemistry*, **101**, 938–946.

SAS Institute (2010). SAS Version 9.3. The SAS Institute, Cary, NC.

Thompson, D.L., & Goodman, M.M. (2006). Increasing kernel density for two inbred lines of maize. *Crop Science,* **46**, 2179–2182.

Vaz Patto, M. C., Alves, M. L., Almeida, N. F., Santos, C., Mendes Moreira, P., Satovic, Z. *et al*. (2009). Is the bread making technological ability of Portuguese traditional maize landraces associated with their genetic diversity? *Maydica*, **54**, 297–311.

Vaz Patto, M. C., Moreira, P. M., Carvalho, V., Pego, S. (2007). Collecting maize (*Zea mays* L. convar. mays) with potential technological ability for bread making in Portugal. *Genetic Resources and Crop Evolution,* **54**, 1555–1563.

Vázquez-Carrillo, G., García-Lara, S., Salinas-Moreno, Y., Bergvinson, D. J., Palacios-Rojas, N. (2011). Grain and Tortilla Quality in Landraces and Improved Maize Grown in the Highlands of Mexico. *Plant Foods for Human Nutrition,* **66**, 203–208.

Table 1. Maize populations evaluated during three years in Pontevedra (Northwestern Spain) for grain, flour and bread quality.

Maize population	Origin	Growth cycle	Grain type
Andaluz/Daxa	South of Spain	Late	Dent
AS3(HT)C3	Corn Belt Dent, USA	Medium	Dent
ASG	USA + Europe	Early	Semi flint
Rastrojero	East of Spain	Medium	Semi dent
Sajambre	Northern Spain	Early	Flint
Tremesino	South of Spain	Medium	Semi dent
Tuy	Northwest of Spain	Medium	Flint

Table 2. Means[a] of kernel, whole-meal and bread traits from seven maize open-pollinated populations cultivated in northwestern Spain during three years and standard references of rice and wheat.

	Grain traits	Flour traits					
Variety	Flotation index (%)	Moisture (%)	Water Binding Capacity (g H_2O/100g)	Onset pasting temperature (°C)	Maximum viscosity at 95ºC (cP)	Final viscosity (cP)	Setback (cP)
Andaluz/D	43 b	10.8 c	149 ab	77.3 a-c	684 a	1755 a	1188 a
AS3(HT)C3	73 a	11.9 a	151 a	76.7 bc	660 ab	1728 a	1164 ab
ASG	77 a	11.5 b	145 a-c	76.0 c	661 ab	1716 a	1185 ab
Rastrojero	75 a	11.1 c	134 d	77.5 a-c	679 a	1612 a	1058 bc
Sajambre	67 a	10.9 c	130 d	79.0 a	671 a	1624 a	1128 ab
Tremesino	23 c	10.9 c	138 b-d	78.3 ab	542 c	1300 b	885 d
Tuy	38 bc	11.0 c	137 cd	79.3 a	586 bc	1405 b	944 cd
LSD *P*=.05	19	0.4	11	2.2	83	179	129
Rice		11.3	128	69.0	4342	4164	1907
Wheat		14.0	88	64.5	2024	2209	1142

[a] Means followed by the same letter within the same column, are not significantly different at $P \leq 0.05$ according to the Fisher's protected LSD

Table 2 (continued). Means[a] of kernel, whole-meal and bread traits from seven maize open-pollinated populations cultivated in northwestern Spain during three years and standard references of rice and wheat.

	Bread traits							
Variety	Crumb color *L**	Crumb color *a**	Crumb color *b**	Hardness (g)	Retarded recovered elasticity	Cohesiveness	Chewiness (g)	Instant recovery speed
Andaluz/D	85 a	-0.9 b	13 d	189	0.7	0.2 c	25 bc	0.3 a
AS3(HT)C3	80 d	-1.3 cd	36 a	167	0.9	0.2 bc	23 c	0.3 a
ASG	81 c	-1.5 d	29 c	175	0.8	0.2 a-c	30 a-c	0.1 b
Rastrojero	83 b	-0.9 b	28 c	154	0.9	0.3 a	41 a	0.4 a
Sajambre	80 cd	-1.1 bc	30 c	141	0.8	0.3 a	31 a-c	0.1 b
Tremesino	81 c	-0.3 a	34 b	168	0.8	0.3 ab	35 ab	0.1 b
Tuy	81 c	-1.9 e	32 b	139	0.7	0.3 a	22 c	0.1 b
LSD P=.05	1	0.3	2			0.1	11	0.1
Rice	92	-1.7	6	146	1.2	0.7	114	0.3
Wheat	91	-1.8	11	57	5.3	0.8	149	0.3

[a] Means followed by the same letter within the same column, are not significantly different at $P \leq 0.05$ according to the Fisher's protected LSD

Table 3. Means[a] of kernel, whole-meal and bread traits from groups of open-pollinated maize populations: Dent *vs*. Flint, North Spain *vs*. South Spain *vs*. USA, and Early *vs*. Medium *vs*. Late cultivated in northwestern Spain during three years.

	Grain traits	Flour traits					
Maize population	Flotation index (%)	Moisture (%)	Water Binding Capacity (g H_2O/100g)	Onset pasting temperature (°C)	Maximum viscosity at 95ºC (cP)	Final viscosity (cP)	Setback (cP)
Grain type							
Dent maize	54	11.2	143	77.5	641	1599	1073
Flint maize	60	11.1	137	78.1	639	1582	1086
Origin							
North Spain	52 b	10.9 b	133	79.2 a	629	1515	1036
South-East Spain	47 b	11.0 b	140	77.7 ab	635	1555	1043
USA	75 a	11.7 a	148	76.3 b	660	1722	1174
Growth cycle							
Early	77 a	11.5 a	145	76.0	661	1716	1185
Medium	55 b	10.8 ab	149	77.3	684	1755	1188
Late	43 b	11.2 b	138	78.2	627	1534	1036

[a] Means followed by the same letter within the same set (grain type, origin, or growth cycle), are not significantly different at $P \leq 0.05$ according to the least-square-

means method

Table 3 (continued). Means[a] of gluten free breads from groups of open-pollinated maize populations: Dent *vs*. Flint, North Spain *vs*. South Spain *vs*. USA, and Early *vs*. Medium *vs*. Late cultivated in northwestern Spain during three years.

Maize population	Bread traits							
	Crumb color $L*$	Crumb color $a*$	Crumb color $b*$	Hardness (g)	Retarded recovered elasticity	Cohesiveness	Chewiness (g)	Instant recovery speed
Grain type								
Dent maize	82 a	-0.9 a	28 b	168	0.8	0.2	31	0.3 a
Flint maize	81 b	-1.5 b	31 a	154	0.7	0.3	28	0.1 b
Origin								
North Spain	81 b	-1.5 b	31 a	145 b	0.7	0.3	28	0.1 b
South Spain	83 a	-0.7 a	25 b	171 a	0.8	0.2	34	0.3 a
USA	81 b	-1.4 b	33 a	165 b	0.8	0.2	26	0.2 a
Growth cycle								
Early	81 b	-1.5 b	29	173	0.8	0.2 ab	30	0.1
Medium	81 c	-1.1 a	32	154	0.8	0.3 a	31	0.2
Late	85 a	-0.9 a	13	189	0.7	0.2 b	25	0.3

[a] Means followed by the same letter within the same set (grain type, origin, or growth cycle, are not significantly different at $P \leq 0.05$ according to the least-square-means method

Figure legends

Figure 1. Flour, bread and grain of standard wheat and rice samples and two typical maize populations (the Spanish flint Tuy and the Corn Belt Dent AS3(HT)C3).

Figure 2. Viscosity profile of two typical maize flours (produced in three locations indicated with color lines) obtained from laboratory mill determined by RVA (Rapid Visco Analyser). The three color lines represent years of seed origin: blue for the first year, green for the second and red for the third one. For comparison wheat and rice flour patterns are included.

Figure 3. Plot (PC1 x PC2) from the principal component analyses of whole-meal (a) and bread (b) quality traits for seven maize populations grown in three years in northwestern Spain. Populations with flint grains are represented as empty symbols while dent grains as full symbols. Red symbols are for populations from the USA, brown for those from south Spain and green for northern Spanish populations.

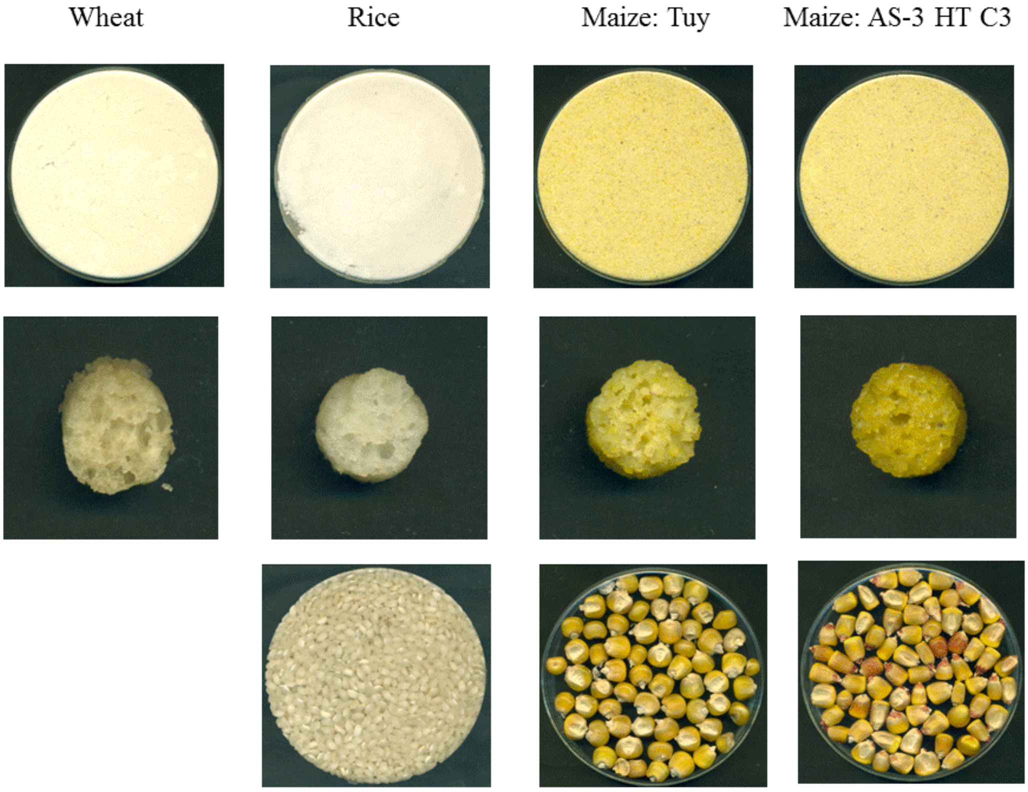

Figure 1

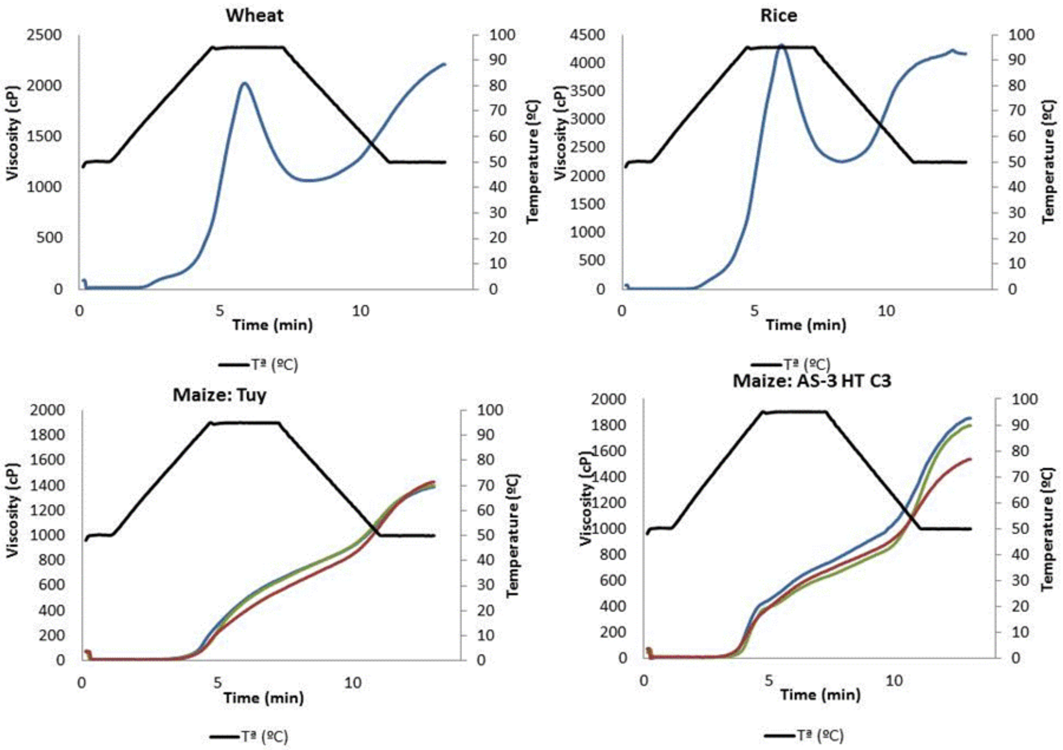
Wheat
Viscosity (cP)
2500
2000
1500
1000
500
0
Temperature (ºC)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
Time (min)
Tª (ºC)
Rice
Viscosity (cP)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Temperature (ºC)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
Time (min)
Tª (ºC)
Maize: Tuy
Viscosity (cP)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Temperature (ºC)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
Time (min)
Tª (ºC)
Maize: AS-3 HT C3
Viscosity (cP)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Temperature (ºC)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
Time (min)
Tª (ºC)

Figure 2

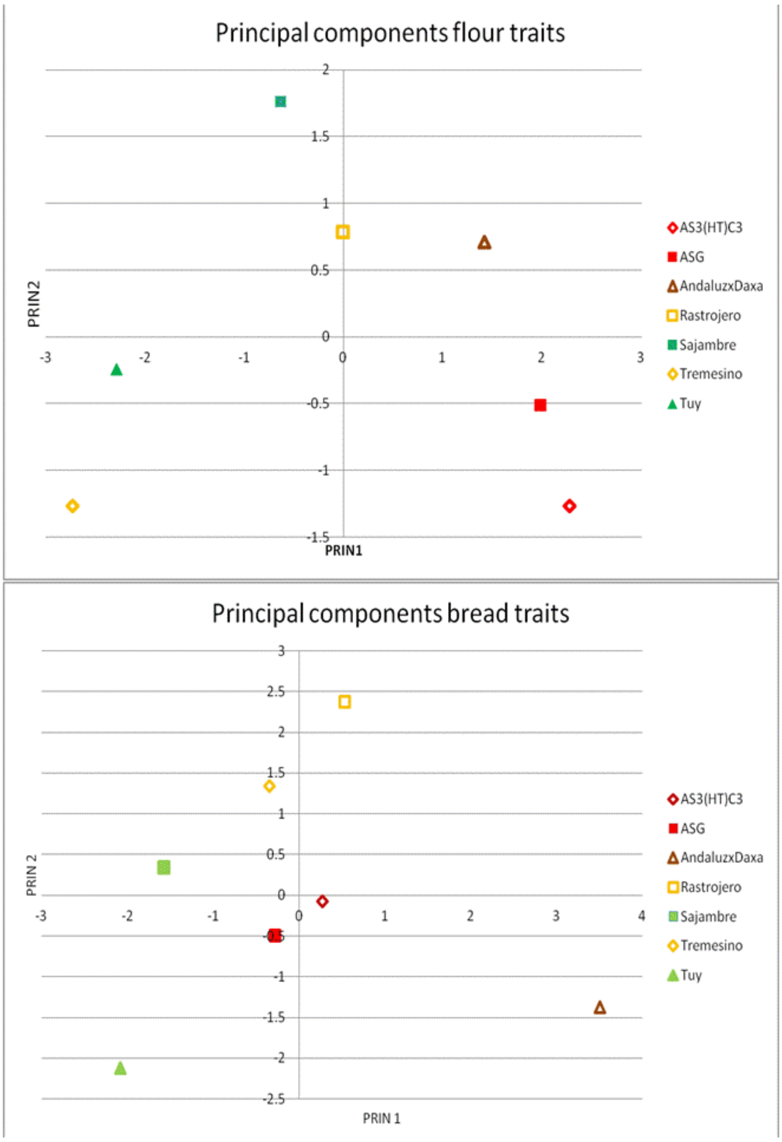
Principal components flour traits
PRIN2
PRIN1
2
1.5
1
0.5
0
-0.5
-1
-1.5
-3
-2
-1
0
1
2
3
AS3(HT)C3
ASG
AndaluzxDaxa
Rastrojero
Sajambre
Tremesino
Tuy
Principal components bread traits
PRIN 2
PRIN 1
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-2
-1
0
1
2
3
4
AS3(HT)C3
ASG
AndaluzxDaxa
Rastrojero
Sajambre
Tremesino
Tuy

Figure 3