**Effects of high amylopectin (*waxy1*) and high-quality protein (*opaque2*) maize mutants in
agronomic performance and bakery quality**

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Abstract

Maize is used in bakery and gluten free food for celiac patients. The objective of this study was to assess the effects of maize mutants with high- amylopectin (*waxy1*) or quality protein (*opaque2*) in dough bread by using near-isogenic maize hybrids for *waxy1* (*wx1*) and *opaque2* (*o2*). Differences between *wx1* and its wild type allele (*Wx1*) were significant more often than for *o2*. Agronomic performance was low for *o2* and fair for *wx1* hybrids. Among the meal traits, *wx1* decreased flotation, onset pasting temperature, maximum and final viscosity, trough, breakdown, total set back, and area under the apparent viscosity curve, and increased water binding capacity. Concerning bread traits, *o2* had no significant mean effects, while *wx1* increased reddish crumb color, retarded recovery elasticity and cohesiveness, and reduced hardness, chewiness and instant recovery speed. Therefore, we can use *waxy* maize for improving maize bread quality, and the choice of the maize genotype could significantly affect the final results

Key words: Maize flour; *opaque2*; *waxy1*; gluten-free bread

1. Introduction

Maize is one of the main cereal crops worldwide and at the same time, it has been one of the primary genetic model plants. Important genetic progress has been achieved on maize leading to varieties with better adaptation to climatic conditions and also higher yields (Andorf *et al.*, 2019). Maize germplasm has a huge variability, which could be exploited in different applications. Maize is appropriate for manufacturing gluten-free bakery products for celiac patients (Brites *et al.*, 2010; de la Hera *et al.*, 2013). Maize lacks gluten, which is the mixture of proteins that gives viscoelasticity but to which some persons are intolerant, like celiac patients. The only way that celiac disease can be treated is the avoidance of gluten consumption throughout the patient's lifetime. Consequently, bakery products must be developed without gluten to primarily meet the demand of consumers that require absence of gluten for medical reasons.

Previous findings confirm the potential of using maize for making gluten free breads. 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 commercial hybrids. Actually, Landa *et al.*, (2006) found that consumers prefer maize bread made with traditional flint populations than with modern commercial dent hybrids. Vaz Patto *et al.* (2007) reported the diversity of maize populations collected in Portugal from farmers that had selected maize for a special type of bread called 'broa' that usually contains maize, wheat and rye. Further, 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. Garzón *et al.* (2017) evaluated dough rheology and breadmaking in a diverse maize collection with different origins, grain types and growth cycles and detected significant effects of endosperm type on bread crumb color; maize origin affected flotation index, onset pasting temperature, bread crumb color, hardness and instant recovery speed; and growth cycle affected flotation index, crumb color, and cohesiveness. Maize varieties differed mainly for water binding capacity, crumb color and hardness. Although some attempts to obtain gluten-free maize bread have been made, there are some studies that present negative attributes partly due to the distinctive yellow color, flavor or hard crumbs (Sanni *et al.*, 2007, Hager *et al.*, 2012). The potential of maize cultivars could be important as main gluten-free commodity. In fact, results reported encourage the search of further variability in maize with diverse endosperm composition for improving bakery quality.

61 However, previous studies have been rather limited regarding the variability of the maize
62 varieties, despite the huge possibilities that traits variability offers for extending applications. Mostly
63 those studies have been focused on *waxy* wheat to improve the bread quality (Mouliney et al., 2011) or to
64 retard bread staling (Hug-Iten et al., 2003), but also *waxy* traits from other grains like barley have been
65 tested for breadmaking (Purhagen et al., 2011). Similarly to *waxy* traits, protein digestibility has been the
66 focus for extending cereals' variability, which is the case of sorghum novel lines for improving flour and
67 breadmaking quality (Elhassan et al., 2015; Goodall et al., 2012).

68 Regarding maize, *waxy1* (*wx1*) is a single recessive mutant of a gene located on chromosome 9,
69 which encodes the granule-bound starch synthase GBSS-I that catalyzes amylose synthesis from ADP-
70 glucose in the endosperm (Mason-Gamer *et al.*, 1998). The germplasm base of *waxy* maize is narrow
71 compared to normal maize, as few countries have active *waxy* maize breeding programs. Breeding of
72 *waxy* maize is carried out by introducing the recessive mutant *wx1* in elite dent lines through backcrossing
73 (Boyer and Hannah, 2001). *Waxy* maize is a popular component of the diet in South Asia (Xiaoyang *et*
74 *al.*, 2017). The *wx1* mutant produces highly digestible starch due to the lack of amylose. The high
75 amylopectin content increases viscosity and digestibility (Lu and Lu, 2012). Because of these
76 characteristics, *waxy* maize is widely used in frozen food processing and livestock feeding industries, as
77 well as in textile, adhesive and paper industries (Bao *et al.* 2012).

78 The most important mutant affecting protein in maize is *opaque2* (*o2*) (Vasal, 1994; Mertz *et al.*,
79 1964) as a mutant that reduces yield and agronomic performance (Vasal, 1994) and increases lysine and
80 tryptophan values between 60 and 100% compared to those of the wild type (Lawton and Wilson 2003).

81 Therefore, the objective of this study was to evaluate effects *waxy* maize (*wx1*) with high
82 amylopectin and opaque maize with high-quality protein (*o2*) in production performance and quality of
83 bread in terms of the dough rheology and bread making quality of gluten free matrix.

2. Material and Methods

2.1. Maize varieties and field trials

Near-isogenic maize hybrids for high amylopectin (*waxy1*) or high-quality protein (*opaque2*) were used for assessing the effects of modified maize starch or protein on bakery quality. The endosperm types included three *waxy1* maize hybrids and their respective wild type (*Wx1*) hybrids, two *opaque2* hybrids and their respective wild type (*O2*) hybrids, and three unrelated flint hybrids (Table 1). The *waxy1* inbred mutants were provided by the University of Purdue in 1977, while the *opaque2* inbred mutants were provided by the Spanish National Institute for Agronomic Research in 1974. Following a well-established backcross method, each inbred mutant (for example A632wx) was produced more than 50 years ago by crossing the original wild-type inbred (in this case A632 homozygous for the wild-type allele *Wx1Wx1*) to an anonymous donor of the mutant (*wx1wx1*) and then backcrossing to the wild-type inbred (A632 *Wx1Wx1*) at least 7 times, while selecting the mutant phenotype. The donors of *wx1* and *o2* were not published by the breeder that released the inbred mutants. The pairs of isogenic hybrids were made by manually crossing each pair of mutant inbred lines (for example A632wx × C103wx) and, separately, each pair of wild-type inbreds (in this case A632 × C103).

All available mutants and wild-type inbreds are Corn Belt Dent inbred lines from the USA, while maize bread in the Atlantic coast of Spain and Portugal is usually made with European flint varieties. Therefore, we included three European flint hybrids made by crossing five inbred lines that represent the germplasm of this area, i.e. EA2087 and EP1 from the north of Spain, EP42 from the northwest of Spain, PB97 from the north of Portugal and EP39 from the south of Spain.

The 13 hybrids (three waxy maize hybrids and the respective three wild-type hybrids, two opaque hybrids and the two corresponding wild-type hybrids, and three flint hybrids) were evaluated in 2016 and 2017 in Pontevedra (42° 24' N, 8° 38' W and 20 m above sea level) and Barrantes (42° 30' N, 8° 46' W and 50 m above sea level). Both fields are located in northwestern Spain with cool and wet springs and a short summer. Field trials followed a randomized complete block design with two repetitions. Plots consisted on four rows spaced 80 cm with 13 hills per row spaced 21 cm, given an approximate density of 60000 plants ha⁻¹. Sowing dates were April 16th of 2016 and April 25th of 2017 in Pontevedra, and May 15th of 2016 and 22th of 2017 in Barrantes. Data and samples were taken only in the two central rows of each plot, in order to minimize the xenia effects.

Agronomic traits recorded were proportion of emergence (proportion of emerged plants over sown grains), early vigor (by using a scale from 1 = weak to 9 = vigorous), number of ears per plant (number of ears / number of plants at harvest), days to anthesis and silking (days to 50% of plants sowing anthers or silks, respectively), grain moisture (proportion of moisture in the harvested grains determined by drying the grains at 80 °C during five days), grain yield (weight of kernels, in Mg ha⁻¹, adjusted at a grain moisture of 140 g kg⁻¹), and plant and ear height (distance from the soil to the top of the plant or to the node of insertion of the uppermost ear, respectively). Moreover, ten-ear samples from the two central rows of each plot were used for further quality analyses.

2.2. Kernel and whole meal characteristics

Flotation index was used as an index of kernel hardness by placing 100 kernels in a beaker containing 300 mL of NaNO₃ solution (1.25 g ml⁻¹) that was stirred to separate the kernels and let stand for one minute. The proportion of floating kernels indicates the flotation index (SAGARPA, 2002). The test was performed in duplicate.

Maize kernels were milled using a laboratory mill (IKA M20 Labortechnik, Staufen, Germany) in order to produce whole meal that was sieved through 0.85 mm screen and the procedure was repeated with the larger particles. Flour moisture content was calculated based on ISO 6540:1980 method (ISO, 1980). The pasting properties were determined by using a 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). Apparent viscosity of the flours was recorded along a heating-cooling cycle. Settings were 60 s at 50°C, then heating from 50 to 95°C in 282 s, hold at 95°C for 150 s and then cooling to 50°C. 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. Apparent viscosity was recorded using Thermocline software for Windows (Perten Instruments SA, Stockholm, Sweden). Parameters identified were onset temperature, maximum viscosity at 95°C, trough, breakdown (difference between maximum viscosity and trough), final viscosity, total setback (difference between final viscosity and trough) and the area under the apparent viscosity curve were evaluated. The water hydration capacity (grams of water retained per gram of solid) was calculated with 100 mg-samples that were mixed with distilled water (1

ml) and vortexed for five minutes at 600 rpm, followed by centrifugation at 5,000 x g for 10 min. All the analyses were made by duplicate.

2.3. Breadmaking procedure

Maize flour was mixed with 2% sugar, 1% dry yeast, 0.5% xanthan gum and 100% water (v/w flour basis), and bread formulation was based on previous research (Garzón *et al.*, 2017). Doughs were mixed at 100 rpm in a stirrer with a turbine accessory (IKA Eurostar 40, Staufen, Germany). The breadmaking was carried out in a scale down method, 4 g of dough were put in well-greased glass pans, proofed for 30 min at 35°C and 50% relative humidity and then baked in an oven at 130°C for 7 min (Garzón & Rosell, 2014). Two batches were run for each sample and the breadmaking analyses were done only for both environments of 2016.

2.4. Evaluation of bread quality

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. Three replicates were made. The force required to compress bread crumb was determined in a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK) using a double compression test of 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 30 s delay between first and second compression. The test was made with a cylindrical stainless steel probe having an adapted diameter for these slices (diameter 6 mm). The parameters recorded were hardness, retarded recovered elasticity, cohesiveness, chewiness and instant recovery speed. Values were the mean of six replicates.

2.5. Statistical Analyses

Analyses of variance were made for each environment, as well as combined analyses of variance across environments with hybrids as fixed factors and all other sources of variation random factors. Separate analyses of variance were made for the three *waxy1* and wild type hybrids, and for the *opaque2* and its wild type hybrids for comparisons between each mutant and the corresponding wild type allele.

175 Means comparisons were performed by Fisher's protected LSD test. All statistical analyses were
176 conducted at a significant level of $P=0.05$ with Statistical Analysis System (SAS Institute, 2010).
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3. Results and discussion

3.1. Agronomic performance

The combined analyses of variance detected significant differences among hybrids for all agronomic traits, and the genotype $\times$ environment interaction was significant for vigor, days to anthesis, grain moisture and plant and ear height. Interactions were mainly of magnitude, rather than rank; and rank interactions were considered only when they affect the discussion of results. Considering all traits together, the flint hybrids were among those with lowest yield and agronomic performance, but they were also the earliest ones (Table S1 and Figure 1). The hybrids with the most favorable agronomic performance were B37 $\times$ Oh43, A632 $\times$ C103, and A632wx $\times$ 103wx; while those with the worst performance were B37o2 $\times$ Mo17o2, A619o2 $\times$ A632o2, and EP42 $\times$ EP39. The *opaque2* hybrids were among those with lowest yield and agronomic performance; furthermore, A619o2 $\times$ A632o2 had less vigor and grain yield than its wild type A619 $\times$ A632; and B37o2 $\times$ Mo17o2 had worse emergence, ears per plant, and yield than its wild type (Table S1 Figure 1). Therefore, *opaque2* decreased significantly vigor and grain yield, though the effects were not significant for both hybrids; furthermore, this mutant had detrimental effects on emergence and ears per plant in B37o2 $\times$ Mo17o2 respect to its wild type hybrid. The findings of this study are in agreement with previous reports by Vasal (1994) showing that *opaque2* hybrids had less yield compared to wild type versions. The effects were more often significant for the *wx1* mutant that had lower emergence and vigor as well as higher days to flower and grain moisture. These effects were not consistent across hybrids, as differences between the mutant and the wild type hybrids were significant for vigor and days to flower in A632wx $\times$ C103wx; emergence, vigor and moisture in B37wx $\times$ C103wx; and vigor, days to flower and yield in B37wx $\times$ Oh43wx. Those findings agree with previous reports showing that *waxy1* maize yield is similar to that of wild type hybrids (Ferguson, 1994). Altogether, *waxy1* reduced vigor and earliness consistently, while there was significant gene $\times$ genotype interaction for emergence and yield (Table 2).

3.2. Kernel and whole meal characteristics

Differences among hybrids were significant for all whole meal traits and the genotype $\times$ environment interaction was significant for all traits except flour moisture and water binding capacity; however, most interactions were of magnitude rather than rank. Flotation index was the lowest for flint hybrids and A632wx $\times$ C103wx, and the highest for dent hybrids; but neither *o2*, nor *wx1* hybrids differed

from their respective wild type isogenic hybrids; however, the mean flotation index of *wx1* hybrids was lower than that of *Wx1* (Table S2, Figure 2). Flotation index is an estimator of grain hardness that was not affected by type of grain in maize, according to Garzón *et al.* (2017), who found that early populations had high flotation index, contrarily to what happens in the current study for early flint hybrids and the type of grain *wx1* that affected flotation index. The reduction of flotation index of *wx1* may be due to the compaction of the endosperm caused by the highly branched amylopectin. The optimum value of flotation index depends on the final use (Vázquez-Carrillo *et al.*, 2011; 2006; Revilla *et al.*, 2008). The flour moisture showed similar trend as flotation index.

Water binding capacity was low for flint hybrids and high for *waxy* hybrids (Table S2, Figure 2). Actually, *wx1* increased water binding capacity, while *o2* had not significant effects on this trait. Garzón *et al.* (2017) reported similar values for the local populations than for our flint hybrids, which were closer to the values of commercial rice than to those of wheat.

The viscosity profiles of whole meal maize suspensions from the diverse types of maize endosperm were recorded during the heating-cooling cycle (Figure 3). All five types of endosperms, except *opaque2*, followed common patterns that were similar to those previously reported for maize (Garzón *et al.*, 2017). They were characterized by an increase of apparent viscosity during heating associated to starch gelatinization and then during cooling that corresponded to amylose retrogradation. Accordingly, Martínez and el-Dahs (1993) found that maize reduced maximum viscosity and increased final viscosity of wheat flour. However, the profile of the *opaque2* endosperm was not so different from that of wheat as the other maize endosperms (Matos and Rosell, 2015; Garzón *et al.*, 2017). The diversity detected among these endosperm types was higher than among the maize populations previously reported by Brites *et al.* (2010) and Garzón *et al.* (2017). Main differences were observed in B37o2 × Mo17o2 that showed a slow increase of apparent viscosity during heating, without a defined peak viscosity, probably because *o2* increases endosperm hardness in the B37 × Mo17 genetic background.

Considering that reported studies stated the preference for breads made with flint maize instead of dent maize, viscosity parameters more related to flint maize were considered of election. Onset pasting temperature was larger in flint maize than in dent maize, and neither *o2* nor *wx1* hybrids differed from their respective wild type isogenic hybrids; however, the mean onset pasting temperature of *wx1* hybrids was lower than that of *Wx1* (Table S2). Therefore, *waxy* maize had values for onset pasting temperature more appropriate than wild type hybrids for making breads, considering reported consumer preferences

(Landa et al., 2006). For trough, breakdown and area under the curve, the pattern of variability for hybrids and mutants was similar to that of onset pasting temperature.

Maximum viscosity was highest for *o2* hybrids; furthermore, B37o2 × Mo17o2 was superior to the corresponding wild type. Conversely, the *wx1* mutant had a detrimental effect that was significant also for A632wx × C103wx. However, none of the hybrids had values close to those of wheat. Therefore, opaque maize was more appropriate than other endosperm types for bread than the wild type hybrids, based on maximum viscosity (Table 2). For final viscosity, the *o2* hybrids were also among those with the highest values, but none of them were superior to the corresponding wild type hybrids; contrariwise, the *wx1* mutant had a detrimental effect that was significant also for A632wx × C103wx. The performance of the flint hybrids was opposite for maximum viscosity than for final viscosity, as they had the lowest values for the first trait and were not significantly different from the highest value for the last one. It was noticeable that the hybrids with the highest final viscosity had similar magnitudes than commercial wheat (Garzón *et al.*, 2017). Therefore, *o2* maize had values of apparent viscosity more appropriate than wild type maize for bakery (Table 2).

3.3. Bread quality

In spite of maize potential for gluten free breadmaking, rarely breadmaking assessments have been used for cultivars selection. In the present study, breads were made from maize traits and results discussed with previous findings (Figure 4). Differences among hybrids were significant for all bread traits except retarded recovered elasticity (data not shown). Crumb color L^* was highest for the flint hybrid EP1 × EP42, but several hybrids, including the opaque hybrids and A632wx × C103wx, were not significantly different, while the other two *waxy* hybrids were among those with lowest values for crumb color L^* (Table S3, Figure 5). Neither differences between mutant and wild type isogenic hybrids nor between means for each mutant and wild type allele were significantly different for crumb color L^* . The a^* parameter was significantly lower for B37o2 × Mo17o2 than for its wild type isogenic hybrid, and higher for B37wx × Oh43wx than for its wild type isogenic hybrid, while the flint hybrids had the lowest crumb color a^* ; moreover, *wx1* increased crumb color a^* respect to the wild type allele, having a reddish tone. Neither isogenic couples nor the means for mutants and the respective wild type allele were significantly different for b^* parameter, showing similar yellowness. Therefore, effects of both mutants on crumb color depended on the gene × genotype interaction for a^* . Accordingly, Garzón *et al.* (2017)

found diversity for a^* and b^* color parameters among maize populations, having flint maize lower crumb luminosity L^* and reddish color (a^*), but yielded higher yellowish tone (b^*); however, they found that early genotypes had higher crumb color b^* and lower L^* and a^* than late populations, while our current results indicate that early hybrids had high color L^* .

The opaque gene had no significant effects on crumb hardness, while all *waxy* hybrids led to softer crumbs than their respective wild type isogenic hybrids; furthermore, the mean hardness was lower for *wx1* than for *Wx1* (Table 2). If *waxy* hybrids are not considered, differences among hybrids for hardness were not relevant, in agreement with Garzón *et al.* (2017), who concluded that there was not variability among local populations for hardness. The *o2* mutant had no significant effects on cohesiveness, which was significantly increased by the *wx1* mutant, except for the hybrid A632wx × C103wx; and the flint hybrids had the lowest cohesiveness, although some dent hybrids were not significantly different. The effects on chewiness were not significant for the *o2* mutant, while the *wx1* mutant reduced chewiness though that reduction was only significant for A632wx × C103wx respect to its wild isogenic hybrid. Finally, instant recovery speed was lower for *wx1* than for *Wx1*, but differences were not significant for any of the isogenic couples of hybrids. Garzón *et al.* (2017) found that early genotypes had higher instant recovery speed than the late ones, which would be similar here if *waxy* hybrids were not considered. According to Matos *et al.* (2014), instant recovery speed is associated to lighter crumb structures with higher number of gas cells leading to faster recovery of the structure after compression.

4. Conclusions

Agronomic performance was reduced in maize with high quality protein and not affected in *waxy* maize. The mutant *wx1* decreased several quality traits, particularly flotation, onset pasting temperature, maximum and final viscosity, trough, breakdown, total set back, and area under the apparent viscosity curve, and increased water binding capacity. Bread traits were not significantly affected by opaque2 maize, but *waxy* maize increased reddish crumb color, retarded recovery elasticity and cohesiveness and reduced hardness, chewiness and instant recovery speed. Given the extensive variability of the maize traits, particularly for flotation index and apparent viscosity, *waxy* maize offers very good alternatives for food applications, which could replace further chemical modifications of starches. In

addition, breeding for *wx1* or *o2* hybrids should consider the appropriate genotype for introducing these recessive mutants.

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Table 1. Near-isogenic maize hybrids for high amylopectin (*waxy1*) or protein (*opaque2*) evaluated during two years in two locations of Pontevedra (Northwestern Spain) for grain, flour and bread quality.

Maize hybrid	Endosperm mutant	Grain type	Origin
A632wx × C103wx	<i>waxy1</i>	Dent	USA
A632 × C103	Wild type (<i>Waxy1</i>)	Dent	USA
B37wx × C103wx	<i>waxy1</i>	Dent	USA
B37 × C103	Wild type (<i>Waxy1</i>)	Dent	USA
B37wx × Oh43wx	<i>waxy1</i>	Dent	USA
B37 × Oh43	Wild type (<i>Waxy1</i>)	Dent	USA
A619o2 × A632o2	<i>opaque2</i>	Dent	USA
A619 × A632	Wild type (<i>Opaque2</i>)	Dent	USA
B37o2 × Mo17o2	<i>opaque2</i>	Dent	USA
B37 × Mo17	Wild type (<i>Opaque2</i>)	Dent	USA
EA2087 × PB97	Wild type	Flint	Spain
EP1 × EP42	Wild type	Flint	Spain
EP42 × EP39	Wild type	Flint	Spain

Table 2. Means ^a for near-isogenic maize hybrids for *waxy1* or *opaque2* evaluated during two years in two locations of Pontevedra (Northwestern Spain).

Hybrid	Agronomic traits									
	Emergenc e (%)	Vigor (1- 9)	Ears per plant	Days to anthesis	Days to silking	Grain moisture (%)	Grain yield (Mg ha ⁻¹)	Plant height (cm)	Ear height (cm)	
<i>o2</i>	50.8	4.1 b	1.05	76.8	77.7	21.9	5.2 b	257	105	
<i>O2</i>	69.4	5.1 a	0.94	77.5	78.8	22.3	8.9 a	265	109	
LSD (0.05)		0.7					1.8			
<i>wx1</i>	71.5 b	4.4 b	1.00	79.4 a	81.1 a	24.8 a	8.2	269	109	
<i>Wx1</i>	83.0 a	5.8 a	1.01	76.7 b	78.5 b	22.4 b	9.0	272	113	
LSD (0.05)	5.2	0.6		1.2	1.1	1.2				
Hybrid	Whole-meal traits									
	Flotation index	Flour Moisture (%)	Water Binding Capacity (g H ₂ O/100 g)	Onset pasting temperature (°C)	Maximum viscosity at 95°C (cP)	Trough	Breakdown (cP)	Final viscosity (cP)	Total setback (cP)	Area under the Curve
<i>o2</i>	83.8	14.1	141	75.4	1497	1072	425	2615	1543	13451
<i>O2</i>	87.1	14.4	144	75.2	1155	1002	250	2365	1460	12130
LSD (0.05)					244					
<i>wx1</i>	48.8 b	14.3	151 a	74.7 b	778 b	686 b	92 b	1478 b	793 b	8252 b
<i>Wx1</i>	56.9 a	14	139 b	75.3 a	994 a	828 a	166 a	2177 a	1349 a	10452 a
LSD (0.05)	8		3	0.6	97	70	62.7	122	75	650

Hybrid	Bread traits							
	Crumb color L^*	Crumb color a^*	Crumb color b^*	Hardness (g)	Retarded recovered elasticity	Cohesiveness	Chewiness (g)	Instant recovery speed
<i>o2</i>	66.4	0.50	41.4	97.6	0.60	0.27	26.7	0.085
<i>O2</i>	65.4	1.03	47.8	100.0	0.65	0.27	27.5	0.077
LSD (0.05)								
<i>wxI</i>	63.1	2.45 a	44.9	47.3 b	0.66 a	0.31 a	14.8 b	0.053 b
<i>WxI</i>	64.5	1.87 b	47.6	80.3 a	0.53 b	0.24 b	19.6 a	0.065 a
LSD (0.05)		0.5		14.4	0.12	0.05	3.5	0.006

^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

Figure 1. Vigor and Yield of near-isogenic maize hybrids for *waxy1* or *opaque2* evaluated during two years in two locations of Pontevedra (Northwestern Spain).

Figure 2. Water bindign capacity, final viscosity and flotation index of near-isogenic maize hybrids for *waxy1* or *opaque2* evaluated during two years in two locations of Pontevedra (Northwestern Spain).

Figure 3. Apparent viscosity profile obtained by RVA (Rapid Visco Analyser) for flour of (from left to right and top to bottom) a *opaque2* hybrid, its wild relative, a *waxy1* hybrid, its wild relative, and a flint hybrid evaluated in 2017.

Figure 4. Experimental maize breads made with six maize hybrids representing the genotypes evaluated.

Figure 5. Hardness and cohesiveness of near-isogenic maize hybrids for *waxy1* or *opaque2* evaluated during two years in two locations of Pontevedra (Northwestern Spain).