



Genotype selection influences the quality of gluten-free bread from maize

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ARTICLE INFO

Keywords:

Maize flour
Gluten-free bread
Landraces
Hybrids
Africa

ABSTRACT

Making bread from maize is a technological challenge due to the poor viscoelastic properties of the dough. Maize germplasm as well as the thermoalkaline processing technique commonly used in Mexico can be harnessed for bread making purposes. We assessed the bread making performance of two maize hybrids, two landraces, and their thermoalkaline processed flour in addition to their blend with high zinc wheat. Significant differences ($P \leq 0.05$) were found in physical kernel characteristics such as flotation index, hardness, size and weight. Doughs had a higher consistency, springiness and gumminess than the untreated reference. Landrace L1 (Jala) had a larger specific volume (1.99 mL/g), softer texture (13.10 N) and faster springiness (0.90) but a relatively high staling (1.60), while landrace L2 (Cacahuacintle) and hybrid H1 (CSTH19001) had a lower staling (< 0.50). The specific volume and softness of bread reduced in all thermoalkaline processed flours. Genotypes demonstrated significantly different performances during bread making, indicating that the choice of maize genotype significantly affected the final product. Thermoalkaline processed flour did not seem to improve bread quality, hence its application in bread making requires further study.

1. Introduction

Bread is commonly made with wheat flour. The flours of other cereals, including rye, barley, maize, oats, sorghum, millet and rice, are used to a lesser extent. With the exception of rye, these cereals are usually combined with wheat to derive suitable rheological and textural properties. Maize is a staple crop in many countries in Africa and Latin America. Its uses are multiple in Latin America with more than 600 food products being made with maize, mainly derived from thermoalkaline processed flour (Ekpa, Palacios-Rojas, Kruseman, Fogliano, & Linnemann, 2019). However, maize proteins do not develop into a continuous network upon hydration and shear, resulting in doughs lacking extensibility and gas holding properties.

Maize germplasm has a vast genetic and functional variability, which could be exploited for bread making purposes. Identification of maize germplasm with proper bread-making properties could open opportunities for the growing gluten-free markets (Garzón, Rosell, Malvar, & Revilla, 2017). Garzón et al. (2017) observed differences in maize genotypes for dough rheology and gluten-free bread making performance. Mexico, the centre of diversity of maize, has around 60 landraces with most of them still cultivated throughout the country

(Arteaga et al., 2016). Cacahuacintle and Jala are two popular maize landraces characterized by large-sized grains with more than 80% floury endosperm, preferred for the preparation a soup called pozole, cookies and thermoalkaline processed tortillas (Figueroa Cárdenas et al., 2013).

Solutions to improve the bread-making performance of maize include the use of functional ingredients such as hydrocolloids, dough conditioners, protein sources, modified starches, and processing technologies such as high hydrostatic pressure (Zannini, Jones, Renzetti, & Arendt, 2012). However, these advanced technological solutions appear inappropriate for low and middle-income countries. Solutions such as using pre-gelatinized starch, thermoalkaline processing (so-called nixtamalization), sprouting and composite flours offer better possibilities for adoption (Brites, Trigo, Santos, Collar, & Rosell, 2010). Thermoalkaline processing improves dough viscoelasticity and network stability, which could ameliorate the bread-making performance of maize flour (Guadarrama-Lezama, Carrillo-Navas, Vernon-Carter, & Alvarez-Ramirez, 2016).

In this study, we compared the dough rheology and bread-making performance of two maize hybrids and two Mexican landraces, namely Jala and Cacahuacintle. Moreover, the physicochemical and functional

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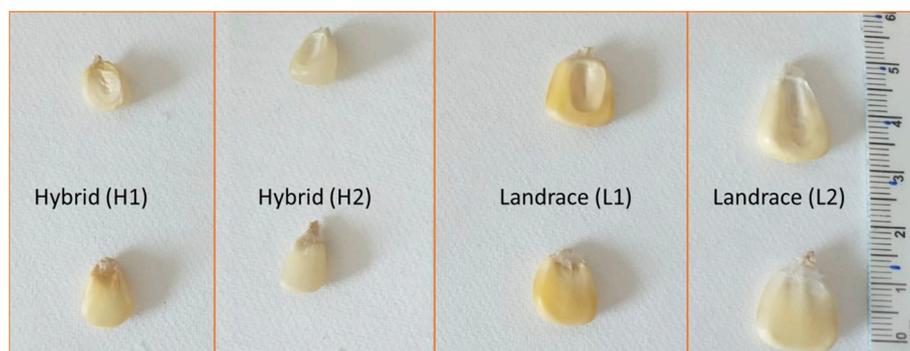


Fig. 1. Maize hybrids and landraces. H1 (CSTH19001), H2 (Commercial hybrid), L1 (Jala) and L2 (Cacahuacintle). Pictures showing the side views of maize kernels.

properties during bread making from thermoalkaline processed flour of these genotypes was evaluated. Finally, bread from a maize/high zinc wheat blend was analysed to test whether the observed differences in maize genotypes were sustained in a blend with wheat.

2. Materials and methods

2.1. Plant materials

Grains of the Cacahuacintle landrace were purchased from Ixtenco market and of Jala landrace from a farmer in Jala, Jalisco, Mexico (Fig. 1). Both landraces were produced in the spring-summer cycle of 2018 and harvested in December 2018. The hybrids, one commercial and one advanced hybrid from CIMMYT's tropical maize breeding program, were grown in the autumn-winter cycle 2019 in the experimental station of CIMMYT in Agua Fria, Mexico. Genotypes with contrasting quality parameters commonly used to prepare maize snacks were selected. High Zn wheat was produced at CIMMYT experimental station in Ciudad Obregon, Mexico, and was harvested in April 2019. Composite flour consisted of 75% high-Zn wheat and raw maize or thermoalkaline processed maize flour based on prior trials (Table S1-supplementary material).

2.2. Thermoalkaline processing, drying and milling

Thermoalkaline processing was performed as described by Roque-Maciel et al. (2016). Briefly, a pan with 3 L water containing 1 kg maize kernels and 10 g food-grade calcium hydroxide (Oxical®, Mexico) was placed on stove at a regularly monitored minimum steam level. Cooking time was based on grain hardness as described by Vázquez-Carrillo, García-Lara, Salinas-Moreno, Bergvinson, and Palacios-Rojas (2011). The cooked maize was steeped in the closed pan for 16 h. Next the cooking water was discarded, followed by washing the grains for three times in clean water. Then the grains were milled using a wet milling machine (Fumasa, M100, Mexico) to produce a paste, so-called masa, which was subsequently lyophilized with the Labconco equipment (model 7755041). The dried dough was re-milled using a hammer miller (Christy Turner 43220 Series 3000 mill - Suffolk, UK) equipped with a 0.5 mm mesh.

2.3. Physical and chemical properties of grains

The flotation index (FI) was used to determine kernel hardness by the method of Wichser (1961). One hundred grains were placed in a sodium nitrate solution with a specific density of 1.250 g/mL after which the number of floating grains after six strokes of mild stirring was recorded. Grain and flour colour were measured using the MiniEscan HunterLab colorimeter (Reston, VA, USA). The colour differences (ΔE^*_{ab}) after processing into thermoalkaline processed flour were calculated and interpreted according to Cserhalmi, Sass-Kiss, Tóth-Markus,

and Lechner (2006). Colour changes become more perceptible to human eyes with increasing values.

Grain size distribution was determined by passing 1 kg kernels through a set of meshes standard no. 24 (9.53 mm), 22 (8.73 mm), 20 (7.94 mm) and 18 (7.14 mm) with constant agitation for 1 min and determination of the percentage. Grain moisture, starch, protein and oil content were determined by Near-Infrared Spectroscopy (NIS) with the Infratec™ 1241 Grain Analyser (FOSS, Denmark) and calibrations provided by the manufacturer. Hundred kernel weight was determined by weighing 100 grains (Palacios Rojas, 2018). Mineral contents were analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Zarcinas, Cartwright, & Spouncer, 1987).

2.4. Bread making

Maize bread making was adapted from Falade (2014), namely on flour weight, as follows: 5% sugar, 3% dry yeast, 2% margarine, 2% salt and 130% hydration (based on dry matter). For wheat bread, the same formulation was used with adjusted hydration based on the solvent retention index and Mixolab®, i.e. a hydration level of 70% for whole wheat and 64% for refined wheat. Solvent retention capacity (SRC) and Mixolab® data were inadequate to determine the proper hydration level for baking maize bread. Hence empirical trials were conducted first to optimize the crumb structure. All samples were aligned based on the moisture content of the flour to establish equal treatment. For maize bread, the batter was placed in a loaf tin and kept for 1 h at room temperature for proofing, then baked for 25 min at 200 °C and relative humidity (RH) of 85%. Bread samples were prepared in duplicate.

2.5. Starch characterization

Total starch, amylose, amylopectin and resistant starch contents were determined in maize flour using the Megazyme assay kit (Megazyme, Bray, Ireland) based on AACC methods 76–13.01 and 32–40.01 (McCleary & Monaghan, 2002). In addition, the resistant starch content of thermoalkaline processed flour and all bread samples was determined. Bread samples were lyophilized prior to analysis.

2.6. Solvent retention capacity

SRC of all samples (including the flours of the maize/wheat blend) was determined by the AACC 56 - 11 method with slight modifications. Four solvents were individually used to determine the SRC values: 0.5 g/mL sucrose (SuRC); 0.05 g/mL sodium carbonate (SCRC), 0.05 g/mL lactic acid (LARC) and water (WRC). For each sample, 300 mg were weighed in duplicate in a 2 mL graduated round-bottom centrifuge tube after which 1.5 mL of solvent was added, followed by vigorous shaking for 10 s and immediate transfer to a thermomixer at 1400 rpm for 5 min at 25 °C. Next samples were centrifuged for 2 min at 4000 × g at 25 °C. The SRC was calculated as the weight of solvent retained by

Table 1
Mean values for physical and proximate characteristics of kernels from maize landraces and hybrids.

	Physical analysis of kernels				Proximate analysis of kernels			
	Flotation index (%)	Hardness *	HKW (g)	Lightness	Grain size** (Mesh 24 - %)	Protein (%)	Oil (%)	Moisture (%)
L1	79.0 ± 1.4 ^a	Soft	54.7 ± 0.2 ^b	79.4 ± 0.7 ^a	98.4	9.9 ± 0.1 ^b	5.3 ± 0.1 ^{ab}	11.4 ± 0.2 ^b
L2	83.0 ± 8.5 ^a	Soft	81.9 ± 1.9 ^a	78.9 ± 0.0 ^a	95.8	9.9 ± 0.2 ^b	5.6 ± 0.1 ^a	15.5 ± 0.5 ^a
H1	8.0 ± 3.5 ^b	Very hard	32.3 ± 0.6 ^c	78.7 ± 0.5 ^a	19.8	9.6 ± 0.2 ^b	5.1 ± 0.0 ^b	12.0 ± 0.0 ^b
H2	3.5 ± 0.7 ^b	Very hard	28.7 ± 0.3 ^c	77.3 ± 1.7 ^a	12.0	10.7 ± 0.2 ^a	5.7 ± 0.1 ^a	11.7 ± 0.1 ^b

Values in each column with different letters differ significantly (Tukey, 0.05).

*Based on flotation index scale (CIMMYT): 0–12 = very hard, 13–37 = hard, 38–62 = intermediate, 63–87 = soft, 88–100 = very soft; HKW - Hundred kernel weight, FI - Flotation index - to predict kernel hardness **Grain size based on % retention in mesh number 24 = 9.53 mm; for mesh size number 22 (8.73 mm), 1.4%, 2.5%, 43.0% and 36.0% L1, L2, H1 and H2, respectively, was retained. Values are mean ± standard deviation of triplicates.

samples after centrifugation and gel drainage for 10 min, and expressed as a percentage of sample weight on respective moisture content, as follows: (Haynes, Bettge, & Slade, 2009).

$$SRC(\%) = \left(\frac{\text{gel(g)}}{\text{flour(g)}} - 1 \right) * \left(\frac{86}{100 - \text{flour moisture}(\%)} \right) * 100$$

2.7. Thermal characterization of flour

Thermal properties were determined using a Differential Scanning Calorimeter (DSC) equipped with a thermal analysis data station (PerkinElmer Corp Norwalk, USA). For gelatinization analysis, a dry flour sample of approximately 5 mg was dispersed in distilled water at 1:3 w/v in stainless steel capsule, which was then hermetically sealed and incubated for 2 h to equilibrate the moisture. Sample and reference pans (balanced to within ± 0.5 mg) were loaded at ambient temperature, cooled to 10 °C, and held for 2 min before scanning to 120 °C. The temperatures of the characteristic transitions, onset (T_0), peak (T_p) and endpoint (T_e) were recorded and the enthalpy (ΔH) of the transition was expressed as J/g. The degree of gelatinization (DG) was calculated as $[1 - \Delta H_s / \Delta H_u] \times 100$, where ΔH_s is thermoalkaline processed flour and ΔH_u is untreated maize flour (González-Amaro, de Dios Figueroa-Cárdenas, Perales, & Santiago-Ramos, 2015).

2.8. Rheological behaviour (Mixolab®)

Rheological behaviour of flour was determined using the Chopin⁺ protocol with a dough weight of 100 g but without a target consistency (Dubat, 2013, pp. 85–88). The Mixolab® was originally developed to analyse wheat flour but can also be used for non-wheat flours after adjustment of the protocol, e.g. dough weight (Matos & Rosell, 2015; Xie et al., 2011). Rheological behaviour of maize dough was determined using a mixing speed of 80 rpm at a temperature regime of 6 min at 30 °C, heating of 4 K/min until 90 °C, 7 min at 90 °C, cooling for 4 K/min until 50 °C, and 5 min at 55 °C. Dough consistency and temperature during the entire process were measured to determine protein power, starch gelatinization, amylase activity and starch retrogradation. The tests were carried out for each sample at constant hydration of 110% (based on dry matter; corrected for moisture content of each sample).

2.9. Loaf characteristics

Loaf specific volume was measured upon cooling by the rapeseed displacement method according to AACC method 10–05.01, while bake loss was determined by the weight difference before and after baking. Texture profile analysis (TPA) was performed using a TA-XT texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell and a 20 mm aluminium cylindrical probe. Pre-test speed, test speed and post-test speed were 2 mm/s. The sliced samples (20 mm thickness) were tested using double compression (20%) with a trigger

force of 20 g at 5 s wait time between the first and second cycle. TPA measurements were performed 2 h after baking and on the fifth day. Bread staling was calculated using the following equation by Hager et al. (2012):

$$\text{Bread staling} = \frac{\text{crumb hardness on day 5} - \text{crumb hardness on day 0}}{\text{crumb hardness on day 0}}$$

3. Statistical analysis

Data analysis was by IBM SPSS® software (version 23) and XLSTAT® software (version 2018.5.52280, Addinsoft, New York). The significance level was fixed at $p < 0.05$. Data are presented as the mean of two or three determinations depending on the parameter.

4. Results and discussion

4.1. Physical and proximate characteristics of kernels from maize genotypes

Table 1 shows significant differences ($p < 0.05$) in all physical parameters measured, except colour (lightness). The highest FI (79–83%) was observed for the landraces, i.e. soft, while the hybrids showed the lowest FI (3.5–8%), indicating very hard kernels. This reflects the large proportion of floury endosperm of the popular landraces and the relatively high proportion of vitreous endosperm in the hybrids. Grain hardness is a crucial quality parameter in determining the type of food product to be made, and setting optimal processing conditions at industrial and household level. The average grain size (US mesh 24) varied from 95.8 to 98.4% in the landraces to 12–19.8% in the hybrids (Table 1 & Fig. 1). To date no relation between the physical properties of maize grains and their bread-making performance has been reported, but the association between tortilla (flatbread) quality and maize physical characteristics has been studied (Roque-Maciel et al., 2016; Santiago-Ramos et al., 2018). Industries producing thermoalkaline processed flour prefer grains with hard endosperm ($FI \leq 20\%$), kernel weight between 32 and 40 g, grain colour lightness of ≥ 50 and medium-sized grains (Vázquez-Carrillo et al., 2011). However, the masa-tortilla industry prefers softer kernels with a FI of about 40%. Garzón et al. (2017) found that soft maize improved gluten-free breadmaking performance compared to hard maize. The protein content of all samples did not differ significantly except for hybrid (H2), which had a higher protein content (Table 1). Hybrid (H1) with the highest starch content, had the lowest amount of other components.

4.2. Colour of maize flour

The colour values for untreated and thermoalkaline processed maize flour are shown in Table 2. The lightness (L^*) of the flour from both landraces decreased significantly ($P < 0.05$) after thermoalkaline cooking, signifying a darker colour. On the contrary, hybrid maize (H1) showed an increase in L^* . The ΔE^*ab values indicate that the flours

Table 2
Colour values for untreated and thermoalkaline processed maize flour.

	Maize flour			Nixtamalized maize flour			ΔE^{*ab}
	L ^a	a ^a	b ^a	L ^a	a ^a	b ^a	
L1	96.8 ^b	-0.4 ^a	15.1 ^b	93.8 ^b	-1.7 ^b	18.5 ^c	4.8
L2	100.5 ^a	-0.3 ^a	11.2 ^f	95.2 ^b	-1.6 ^a	18.3 ^f	8.9
H1	96.5 ^b	-0.5 ^a	11.7 ^e	97.7 ^c	-1.5 ^b	12.7 ^a	1.8
H2	97.7 ^b	-0.4 ^a	12.2 ^d	97.4 ^{bc}	-0.7 ^b	11.0 ^a	1.3

^a ΔE^{*ab} colour interpretation: 0–0.5 (not noticeable), 0.5–1.5 (slightly noticeable), 1.5–3.0 (noticeable), 3.0–6.0 (well visible) and 6.0–12.0 (greatly visible). Values are mean of triplicate.

from the landraces after thermoalkaline treatment differ visually in colour, while the colour changes in the hybrids are much smaller. [Vázquez-Carrillo et al. \(2011\)](#) reported that large differences (ΔE^{*ab}) in the colour of landrace flours could be due to the relatively high proportion of phenolic polymers, which easily oxidize under alkaline conditions.

4.3. Total starch, amylose, amylopectin and resistant starch

Maize samples were characterized for their chemical properties ([Table 3](#)). The hardest maize sample (H2) and the softest (L2) significantly differed ($P < 0.05$) in starch content with 74.5 ± 0.04 and 72.4 ± 0.22 , respectively. RS and its transformation during processing depend on variety ([García-Rosas et al., 2009](#)). After the thermoalkaline process, more than doubled the resistance starch content was quantified. L1, L2, H1 and H2 had RS contents of 0.83%, 0.81%, 1.19% and 1.18%, respectively. This agrees with data from [Villada, Sánchez-Sinencio, Zelaya-Ángel, Gutiérrez-Cortez, and Rodríguez-García \(2017\)](#) for masa (0.917%) and tortilla (1.477%) ([Table 3](#)). Three types of resistant starch have been reported in the processing of maize into masa: (1) physically inaccessible starch in the cellular matrix, (2) amylose-lipid complexes or amylose-calcium complexes, and (3) retrograded starch formed on cooling to low temperatures ([Santiago-Ramos et al., 2018; Villada et al., 2017](#)). RS has several health-supporting properties, such as lowering the risk of colorectal cancer, reduction of insulin sensitivity and lowering the glycaemic index ([Villada et al., 2017](#)). On the other hand, an increased RS content can negatively affect crumb hardness.

4.4. Solvent retention capacity

Both the untreated and thermoalkaline processed flours differed significantly in their ability to retain the tested solvents ($P < 0.05$), as shown in [Fig. 2](#). Soft maize flour (L1) had the lowest SRC profile for all solvents tested, possibly due to differences in chemical composition, especially the damaged starch content. Flour from hard or vitreous endosperm has been reported to contain significantly more ($p < 0.05$) damaged starch than flour from the floury (soft) endosperm because hard grains have a higher resistance to milling ([Xu et al., 2019](#)). The

Table 3
Total starch, amylose, amylopectin and resistant starch.

	Total starch	Amylose*	Amylopectin*	Resistant starch, dw %			
				Raw maize flour	Nixtamalized maize flour	Raw maize bread	Nixtamalized maize bread
L1	73.8 ± 0.5^{ab}	32.6 ± 1.7^a (a)	67.4^a (b)	0.43 ± 0.00^a	0.83 ± 0.01^a	1.9 ± 0.04^a	1.88 ± 0.04^a
L2	72.4 ± 0.2^b	32.9 ± 0.1^a (ab)	67.1^a (ab)	0.33 ± 0.03^a	0.81 ± 0.00^a	1.93 ± 0.03^b	1.48 ± 0.05^a
H1	72.7 ± 0.5^b	35.6 ± 1.1^a (b)	64.4^a (a)	0.38 ± 0.00^{ab}	1.19 ± 0.02^b	2.02 ± 0.05^{bc}	1.87 ± 0.03^a
H2	74.5 ± 0.0^a	35.0 ± 0.5^a (ab)	65.0^a (ab)	0.42 ± 0.01^b	1.18 ± 0.01^b	2.18 ± 0.01^c	1.85 ± 0.12^b

* Percentage in the starch of maize flour. Amylopectin determined by amylose difference. Values in each column with different letters are significantly different (Tukey, 0.05). Letters in bracket are based on (Fisher (LSD); 0.05). Values are mean \pm standard deviation of duplicates.

physicochemical and thermal properties of starch are significantly influenced by the damaged starch content.

Generally, the untreated maize flours and their corresponding thermoalkaline treated samples had different SRC values. Sucrose retention capacity, which corresponds to pentosan characteristics, increased in all thermoalkaline cooked samples. Hydrolysis and solubilization of maize pericarp during thermoalkaline processing generates pentosans, which in part act as hydrocolloids and confer good viscoelastic properties to dough ([Santiago-Ramos et al., 2018](#)). On the contrary, the lactic acid retention capacity (LARC) (associated with protein characteristics) significantly decreased in all samples up to 22% in L2 and H1. LARC was the most sensitive property, perhaps because of denaturation of the protein due to the thermoalkaline treatment.

High positive correlations were observed among the SRCs: SCRC and LARC; WRC and SCRC; WRC and LARC ($r = 0.98$, $P < 0.05$). However, a negative correlation was found between RS and all SRC profiles, e.g. RS and SuCR ($r = -0.85$, $P < 0.05$) ([Table S2](#)). SRC data show a significant difference ($P = 0.004$) between hybrid and landrace maize while no significant difference ($P = 0.937$) was found between raw and nixtamalized flour ([Table S3](#)). Principal Component Analysis (PCA) based on SRC values of untreated and thermoalkaline processed flour ([Fig. 3](#)) distinctively separated (PCA = 87.53%) landrace L1 (Jala) from the other maize genotypes. Furthermore, [Fig. S1](#) shows that solvent retention parameters of all maize flours are well distant from those of both whole and refined wheat, with L1 (Jala) being the least distant (PCA = 96.79%).

4.5. Thermo-mechanical behaviour of batter

The Mixolab® measures in real-time the torque generated by a dough between two paddles, thus allowing the study of baking quality and thermo-mechanical performance of hydrated flour. The profiles derived from the maize landraces, hybrids and their respective thermoalkaline cooked flour are summarized in [Fig. 4](#). The profiles show the parameters of maize batter behaviour during mixing (C1 & C2), cooking (C3 & C4) and cooling (C5) ([Matos & Rosell, 2015](#)). Substantial differences were found between the samples for all measured parameters. L1 (Jala) gave the lowest peak value for C1 and C2, namely 0.21 Nm and 0.04 Nm, respectively, which indicates a relatively poor protein quality during mixing at a fixed temperature (30 °C). However, this landrace also had the highest peak torque at C3 (starch gelatinization) as well as at C5 (starch retrogradation at cooling phase). Generally, the viscosity of untreated landrace dough at starch gelatinization was higher than for the hybrids. This confirms previous findings where flint maize showed a lower maximum viscosity and lower retrogradation than dent varieties ([Brites et al., 2010](#)). The high starch retrogradation value corresponds to a shorter shelf life, thus methods to lower starch recrystallization are necessary to improve stability of flour for bread making ([Matos & Rosell, 2015](#)). L2 (Cacahuacintle) had the slowest retrogradation value. [Matos and Rosell \(2015\)](#) found a significant correlation ($r = 0.7533$, $P < 0.001$) between C5 and hardness. The present study showed a positive correlation between C5 and cohesiveness ($r = 0.737$, $P < 0.05$) and resilience ($r = 0.818$, $P < 0.05$),

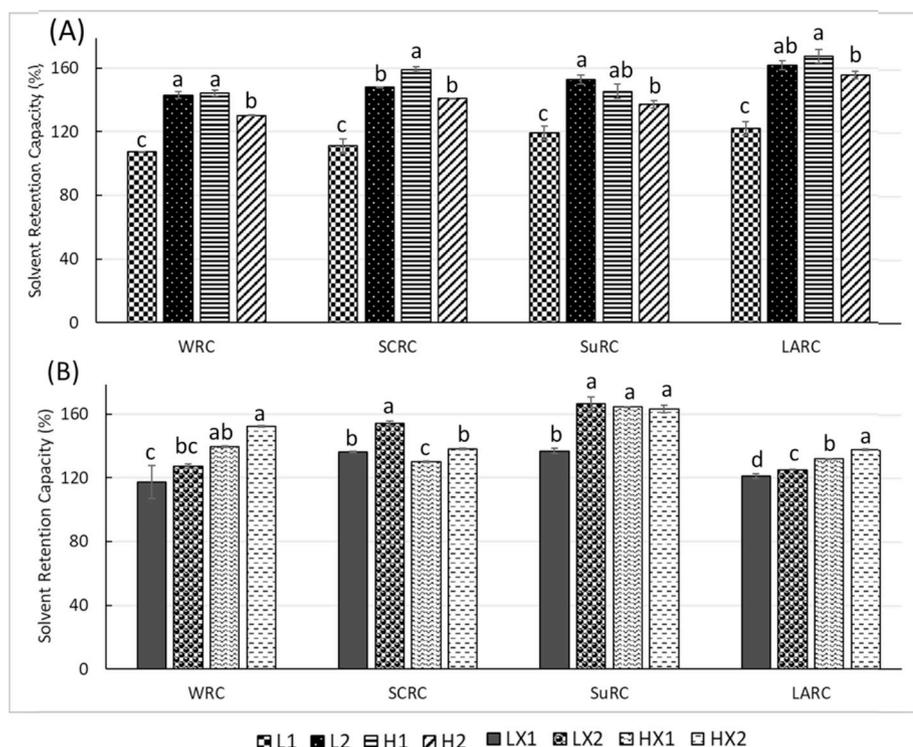


Fig. 2. Comparison of the solvent retention capacity of maize flour profiles for landraces and hybrids. Untreated maize (A) and thermoalkaline processed maize (B), within the same SRC parameters, bars with different letters are significantly different (P < 0.05). WRC – water; SCRC – sodium carbonate, SuRC – sucrose, LARC – lactic acid solution. L1 (Jala), L2 (Cacahuacintle), H1 (CSTH19001) and H2 (commercial hybrid) are untreated, while LX1, LX2, HX1 and HX2 are the corresponding thermoalkaline processed forms. Values are mean of duplicates.

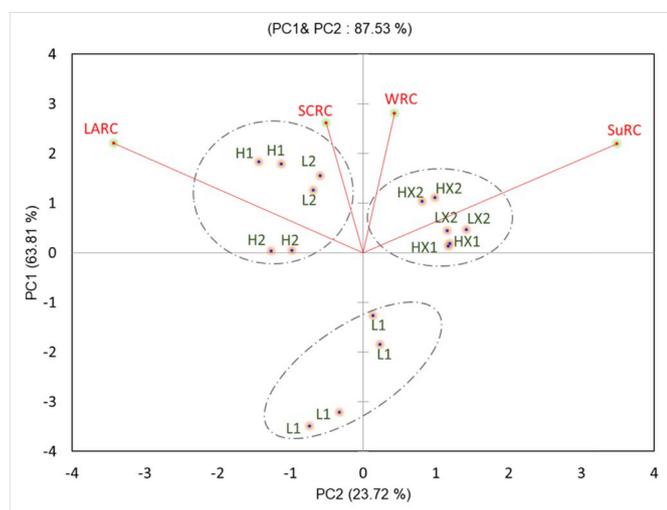


Fig. 3. Principal Component Analysis (PCA) for SRC values of untreated and thermoalkaline processed flour. L1 (Jala), L2 (Cacahuacintle), H1 (CSTH19001) and H2 (commercial hybrid) are untreated, while LX1, LX2, HX1 and HX2 are the corresponding thermoalkaline processed forms.

suggesting that genotypes with a low C5 like L2 (Cacahuacintle) could have better bread-making performance, particularly a slow staling.

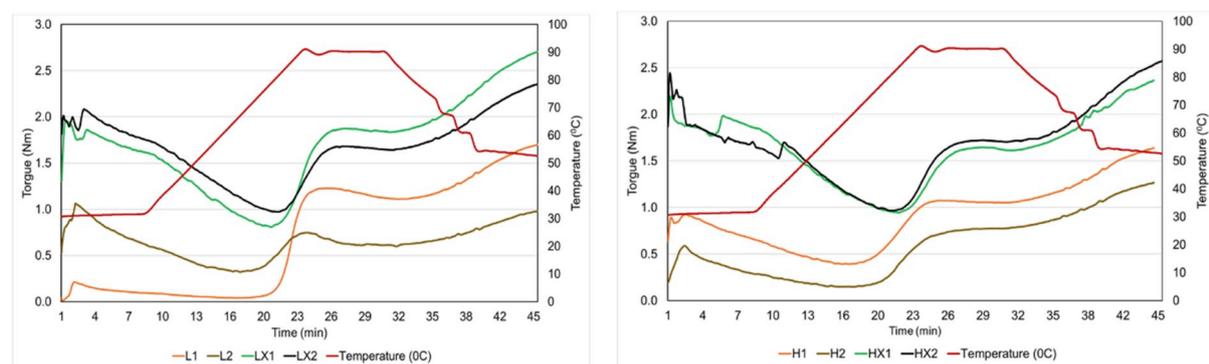
Thermoalkaline processed flour resulted in dough with at least a two-fold increase in the torque for all parameters, attributable to denaturation of proteins and partial gelatinization of starch (Fig. 4). Through calcium incorporation, thermoalkaline processing confers malleability and functionality to maize flour suggesting an improved viscoelasticity that can compensate for the lack of gluten in maize dough (Guadarrama-Lezama et al., 2016). From a techno-functional perspective, the enhanced dough consistency confers stability to maintain the shape of final product (Guadarrama-Lezama et al., 2016).

4.6. Thermal properties of untreated and thermoalkaline processed maize flour

The thermal characteristics of untreated and thermoalkaline processed maize flour measured by DSC are presented in Table 4. Untreated flour from L1 (Jala) had a significantly higher enthalpy (J/g) and gelatinization temperature than the other untreated flours. This indicates that the amylopectin content of the genotype is more stable during heating, making it harder for gelatinization to take place. However, hybrid maize H1 and H2 with hard endosperm had a significantly higher gelatinization temperature range. The decrease in the transition temperature range is influenced by a decrease in the onset temperatures (T_o) rather than a change in the end temperatures (T_c), possibly due to a lower expansion of the starch granule caused by a higher amylose content. The thermal behaviour of starch is influenced by granule size, damaged starch content, amylose/amylopectin ratio and granule crystallinity (González-Amaro et al., 2015; Miyazaki & Morita, 2005). Thermoalkaline processing raised the gelatinization temperature and decreased the enthalpy of all samples. Maize starch becomes difficult to gelatinize after heat treatment (Miyazaki & Morita, 2005). González-Amaro et al. (2015) reported similar thermal properties of maize landraces Cacahuacintle and Bolita, namely a T_p ($^{\circ}C$) of 66.4 and 67.0 respectively. Hybrid maize (H1 and H2) had a higher degree of gelatinization because hydration saturation is quicker in smaller grains.

4.7. Loaf characteristics

The produced breads differed in specific volume, springiness, resilience and staling. Notably, landrace L1 had a significant (P < 0.05) increase in specific volume. As expected, breads L1 and H1 with the highest specific volumes, 1.99 mL/g and 1.30 mL/g, respectively, also had the lowest hardness. However, this was not significantly different (P < 0.05) from other breads (Table 5). Lower hardness could theoretically be due to less amylose but this does not apply to the current research since the genotypes did not significantly differ in their amylose contents. Bread specific volume and firmness are strong indicators of



	Torque (Nm)					Amplitude (Nm)	Stability (min)
	C1	C2	C3	C4	C5		
L1	0.21	0.04	1.23	1.11	1.72	0.03	0.37
L2	1.06	0.32	0.75	0.60	0.98	0.04	1.87
LX1	1.95	0.81	1.87	1.83	2.71	2.88	0.42
LX2	2.08	0.97	1.68	1.64	2.36	3.28	0.40

	Torque (Nm)					Amplitude (Nm)	Stability (min)
	C1	C2	C3	C4	C5		
H1	0.90	0.41	1.07	0.05	1.64	0.09	4.27
H2	0.57	0.15	0.77	0.72	1.26	0.03	1.17
HX1	2.19	0.97	1.64	1.61	2.40	0.21	1.23
HX2	2.42	0.97	1.72	1.71	2.58	2.21	1.55

Fig. 4. Mixolab profiles of landrace, hybrid and their respective nixtamalized samples. L1 (Jala), L2 (Cacahuacintle), H1 (CSTH19001) and H2 (commercial hybrid) are untreated, while LX1, LX2, HX1 and HX2 are the corresponding thermoalkaline processed forms. C1 – dough development, C2 - protein reduction during heating, C3 - starch gelatinization, C4 - amylase activity/breakdown and C5 - starch gelling due to cooling/setback.

Table 4
Thermal parameters of untreated and thermoalkaline processed maize flour.^a

	T _o (°C) ^b	T _p (°C) ^b	T _f (°C) ^b	T _f – T _o (°C) ^b	ΔH (J/g) ^b	DG (%) ^b
Raw maize flour						
L1	64.5 ± 0.3 ^d	70.3 ± 0.6 ^d	76.4 ± 0.1 ^e	5.8 ± 0.3 ^b	10.4 ± 0.5 ^a	na
L2	59.0 ± 0.0 ^f	67.5 ± 0.0 ^g	73.0 ± 0.1 ^f	5.4 ± 0.1 ^c	7.4 ± 0.1 ^{bc}	na
H1	57.5 ± 0.1 ^f	69.5 ± 0.0 ^e	76.8 ± 0.0 ^e	7.3 ± 0.0 ^a	9.4 ± 0.1 ^{ab}	na
H2	61.0 ± 0.2 ^e	69.1 ± 0.0 ^f	76.6 ± 0.1 ^e	7.6 ± 0.1 ^a	9.6 ± 0.7 ^{ab}	na
Nixtamalized maize flour						
LX1	67.8 ± 0.1 ^c	73.3 ± 0.2 ^b	79.9 ± 0.2 ^c	6.6 ± 0.1 ^{bc}	7.8 ± 0.9 ^b	24.6 ± 5.0 ^a
LX2	66.2 ± 0.8 ^{cd}	71.4 ± 0.0 ^c	78.8 ± 0.0 ^d	7.4 ± 0.0 ^a	4.7 ± 0.6 ^d	36.7 ± 8.2 ^a
HX1	76.4 ± 0.4 ^a	76.7 ± 0.1 ^a	82.3 ± 0.3 ^a	5.5 ± 0.4 ^c	5.5 ± 0.7 ^c	41.8 ± 7.4 ^a
HX2	70.4 ± 0.7 ^b	76.7 ± 0.1 ^a	81.3 ± 0.1 ^b	4.6 ± 0.0 ^d	4.4 ± 0.5 ^d	54.3 ± 8.0 ^a

^a Data are means ± standard deviations. Values in the same column with different letters are significantly different (p < 0.05).

^b T_o = onset temperature; T_p = peak temperature; T_f = final temperature; ΔH = gelatinization enthalpy; and DG = degree of gelatinization. na = not applicable. Values are mean ± standard deviation of duplicates.

consumer preference; consumers from sub-Saharan Africa consider hard bread to be old and no longer fresh (Ekpa et al., 2019). High volume per weight is most preferred, thus giving landrace L1 an edge over other genotypes regarding consumer acceptance. Statistically significant differences existed for bread springiness; H2 hybrid (hard endosperm) showed the lowest value (0.82), while L1 landrace (soft endosperm) showed the highest value (0.90). Soft maize varieties have higher

springiness than hard maize, according to Garzón et al. (2017). Bread with a higher specific volume corresponds to higher springiness (Table 5). Bread with low springiness crumbles during cutting due to brittleness. Attributes such as hardness, springiness, cohesiveness, chewiness and resilience increased in all breads made from thermoalkaline processed maize flour of all genotypes. Guadarrama-Lezama et al. (2016) also observed increased hardness of bread from

Table 5
Loaf characteristics of bread from untreated and thermoalkaline processed flour.

Bread from untreated maize flour								
	Specific volume (ml/g)	Bake loss (%)	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience	Staling rate
L1	2.0 ^a	19.5 ^a	13.1 ^a	0.9 ^a	0.8 ^a	9.2 ^a	0.5 ^{ab}	1.6
L2	1.2 ^{bc}	14.8 ^{ab}	18.2 ^a	0.9 ^{ab}	0.8 ^a	11.8 ^a	0.5 ^{ab}	0.0
H1	1.1 ^{bc}	14.6 ^{ab}	17.6 ^a	0.9 ^{ab}	0.8 ^a	12.0 ^a	0.5 ^{ab}	0.5
H2	1.3 ^b	15.6 ^{ab}	12.0 ^a	0.9 ^{ab}	0.8 ^a	7.6 ^a	0.5 ^b	2.4
Bread from thermoalkaline processed flour								
LX1	1.0 ^c	12.5 ^b	18.9 ^a	0.90 ^a	0.9 ^a	14.0 ^a	0.6 ^{ab}	2.0
LX2	1.1 ^{bc}	13.2 ^b	14.8 ^a	0.8 ^b	0.8 ^a	8.9 ^{ab}	0.5 ^{ab}	0.8
HX1	1.1 ^{bc}	13.0 ^b	20.5 ^a	0.9 ^a	0.9 ^a	17.6 ^a	0.7 ^a	0.0
HX2	1.1 ^{bc}	12.9 ^b	20.1 ^a	0.9 ^a	0.8 ^a	15.2 ^a	0.6 ^{ab}	0.6

Values in the same column followed by different letter are significantly different (p < 0.05). Values are mean of duplicates.

thermoalkaline processed flour but other textural characteristics decreased. Pregelatinization generally increases dough consistency, gumminess and springiness (Brites et al., 2010). However, the rheological changes in the dough did not translate to improvement in specific volume and softness of the final product. Using morphological analysis (SEM), Guadarrama-Lezama et al. (2016) showed that dough from thermoalkaline processed flour can form a compact microstructure causing increasing hardness in bread.

Table S4 shows the loaf characteristics of composite bread from wheat and maize. As expected, refined wheat composite bread showed a higher specific volume than whole wheat composite bread. In both categories, composite bread made with L1 and H2 genotypes had the highest specific volume, 3.27–3.93 mL/g and 3.34–3.86 mL/g, respectively. In composite bread from thermoalkaline processed flour, volume was reduced by at least 30%. Wheat bran strongly binds to added water, making it inaccessible for gluten; reactive components in bran (such as glutathione and phytate) and bran-related enzymes (such as endopeptidases) can contribute to weakening of gluten (Hemdane et al., 2016). The volume of all maize bread was at least 40% lower volume than for their respective wheat composite bread. Concerning the overall texture, the L1_R (landrace 1 with refined wheat) composite was the most favourable with a high specific volume (3.93 mL/g), springiness (1.58), cohesiveness (0.87), chewiness (1.21 N) and low hardness (0.92 N).

Lastly, L2 with the lowest amylose content (32.91%) had the slowest staling. The retrogradation rate of amylose is faster than of amylopectin. Furthermore, the values of C1 showed a negative correlation with the specific volume of bread ($r = -0.721$) and positive correlation ($r = 0.714$) with hardness (Table S5 - supplementary material).

5. Conclusion

Bread making performance is not yet considered during maize breeding in spite of the potential for gluten-free bread applications and the available variability in maize germplasm. Furthermore, landrace germplasm traditionally used for bread making is losing its genetic diversity due to the very limited kernel characteristics and strong focus on productivity during the breeding process. In the current research, significant differences ($P < 0.05$) between maize landraces and hybrids were found in terms of physical kernel characteristics such as flotation index, hardness, size and weight. Maize landraces and hybrids had significantly different ($P < 0.05$) resistant starch content, solvent retention capacity and the produced bread had differences in specific volume, hardness, springiness and other quality parameters. Hybrid and landrace maize demonstrated significantly different performances during bread making, hence exploring different landraces with different kernel characteristics will be important to improve the quality of the final product.

CRedit authorship contribution statement

Onu Ekpa: Conceptualization, Formal analysis, Funding acquisition, Investigation, Visualization, Methodology, Writing - original draft, Writing - review & editing. **Natalia Palacios-Rojas:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing - review & editing. **Aldo Rosales:** Formal analysis, Methodology, Resources. **Stefano Renzetti:** Supervision, Writing - review & editing. **Vincenzo Fogliano:** Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing. **Anita R. Linnemann:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing - review & editing.

Acknowledgements

We thank Hongyun Choi for support in laboratory work and data

analysis, Velu Govidan and Felix San Vicente, wheat and maize breeder, respectively, for providing the grain used for analysis. We also thank Cristian Zapata of the CIMMYT GenBank for providing Jala landrace maize. We appreciate the staff of the CIMMYT maize and wheat quality laboratories for technical assistance, and the European Cereal Atlas Fund (ECAAF) for financial support of the internship of OE at CIMMYT. Financial support from MAIZE-CRP is also appreciated.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2020.109214>.

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