| 1 | Physical, chemical and pasting features of maize Italian inbred lines |
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Abstract

In order to help predicting the performance of maize flour during food processing, a set of 23 Italian inbred lines derived from recent breeding programs has been analysed for chemical traits (protein, lipid, starch and amylose content) and pasting behavior and compared to US variety B73. Total antioxidant capacity (TAC) and soluble phenolic content (SPC) were also determined in maize flour. Two amylose extender lines (Lo1413*ae* and Lo1489*ae*, 31.60% and 48.41% amylose, respectively) were included in the set. A large variability was observed among the breeding lines for all the chemical parameters (protein: 9.66 - 14.79 % dm; lipid: 2.21 - 5.68 % dm; starch: 54.65 - 68.70 % dm; amylose content: 18.70 - 48.41 % dm). The range of variation of TAC (12.17 - 21.26 mmol TE/kg dm) and SPC (0.74 – 1,30 g_{GAE}/kg dm) was also quite large. As regards the pasting properties, the peculiar values shown by the *ae* lines during heating led to an absence of viscosity. Among the other lines, Lo1481, Lo1530, Lo1457, Lo1451 and Lo1473 might represent the best genotypes for pasta making, due to their strong tendency to retrogradation. On the other side, Lo1430, Lo1550, Lo1270 and Lo1404, showing a lower tendency to retrograde, seem to be suitable for bread production. The relevant variability of pasting properties in the Italian germplasm, therefore, suggests the possibility to choose the most appropriate genotypes according to the hydrothermal conditions used in food processes and/or to the characteristics of the final product.

Keywords: maize, inbred lines, pasting properties, amylose, gluten free products

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Introduction

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In the last few decades, the market of gluten free (GF) products greatly increased, due to the growing number of consumers suffering from coeliac disease or gluten intolerance who are seeking for GF foods with good sensory and nutritional properties. The difficulty in producing GF products is closely associated with the lack of visco-elastic proteins that play a technological role in cereal-based products. To overcome this issue, in GF formulations several ingredients, including non-gluten proteins, hydrocolloids, and emulsifiers, are included to mimic and partially replace gluten functionality [1 -4]. Maize (or corn) is widely considered a "key" raw material in GF formulations [3,5] where, in the form of isolated starch rather than flour, frequently, represents the first ingredient. Indeed, maize starch - together with rice starch, another recurring ingredient in GF recipes - is a "viscosity-builder" through the gelatinization and retrogradation phenomena that occur during the processing of GF foods [6]. Specifically, in GF pasta, a good cooking behavior is achieved when a regular starchy network is formed in the dried product during the pasta-making process [1]. This structure is guaranteed by using GF flours and/or starches exhibiting strong suitability to both gelatinization and retrogradation. Conversely, for bakery products, the optimal starch source has to present a scarce retrogradation tendency to assure a soft crumb for long time. Taking into consideration the role of amylose in defining the retrogradation extent and, therefore, the structure and sensory characteristics of GF products, the quality controls related to the end-uses of GF starch and/or flours mainly focus on the evaluation of either the amylose/amylopectin ratio [7] or the pasting properties [8-9]: the former influences the extent of gelatinization and retrogradation whereas the latter clearly describe the potential intensity of these phenomena during the technological processes. Devices able to measure the change in viscosity during heating and cooling treatments are of great interest for breeders since they need information on functional properties of lines in short time and using small amount of flour. Although starch is the main component (both for quantity and quality), maize is also an interesting source of bioactive compounds as polyphenols, carotenoids, vitamins and dietary fiber [10]. The high level of maize germplasm variability for starch quantity and quality and all the other macro- and micro-components might not only satisfy the technological requirements for producing GF food with a pleasant texture but also with balanced formulations from a nutritional point of view. Several works were carried out to screen maize genotypes according to their performance during GF bread/baked goods

and processing variables on the quality parameters of corn bread. Physicochemical and pasting properties of eight Brazilian maize landraces were analyzed by Uarrota et al. [12] for screening their suitability for industrial applications. A similar study was carried out on starches isolated from either six Indian [13] or 13 Argentinean [14] maize varieties. In the European context, variability in a collection of maize populations from Portugal [15] and Spain [16] have been explored for bread-making purposes. As far as the Italian context, traditional maize germplasm was analyzed with a focus on its richness in biocomponents [17-18] and safety [19-20]. In particular, since the 1970s, the Research Center for Cereal and Industrial Crops in Bergamo has been developing breeding programs with the aim to obtain maize hybrids with a strong adaptation to the Po Valley environment, improving the cold tolerance at the seedling stage and the resistance to Fusarium rot and European corn borer [21]. To the best of our knowledge, no information is available on the starch properties (i.e., pasting properties) of Italian maize genotypes. The increasing request of maize for food transformation stimulated the identification of Italian genotypes having specific qualitative parameters, including kernel hardness and peculiar starch properties, both strategic for food processing. In the present work, a set of 23 Italian inbred lines has been analyzed for chemical traits and pasting behavior and compared to US variety B73, normally used as reference line in several works. This information could not only help in predicting the performance of maize flour during food processing but also suggest the criteria useful for a shared end-use classification of this cereal for food processing.

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Materials and methods

76 Plant materials

- A set of 23 Italian inbred lines (Lo) and one US public line (purple seeded-B73), maintained at the CREA genebank
- 78 (Table 1), were multiplied by self-pollination during 2014 (planting date April 16) in Bergamo (249 msl, 45°68'N,
- 9°64'E). Fertilization (kg/ha: N=280, P2O5=115, K2O=120) and irrigation were applied during the growing season to
- 80 limit drought stress. Environmental conditions, such as temperature and rainfall, were recorded at the CREA Bergamo
- 81 Weather-Station. In 2014 the weather conditions were quite favorable for the crop, that were able to avoid the heat-
- 82 induced stress conditions observed in other agronomic seasons: the maximum temperature (28.2°C) was reached in June,
- and in the period between June and August the rainfalls were over 630 mm (Online Resource 1).

Sample preparation

- 85 Ears from each genotype were harvested separately and dried at 40°C for 20 days. Hardness and 1000 kernels weight (g)
- 86 were detected on bulked kernels. An aliquot of kernels (50 g) was milled into flour using a Retsch Zm200 Ultracentrifugal
- 87 Mill (Retsch GmbH & Co. KG, Haan, Germany), with a 0.5mm sieve, and stored at 7 °C until analysis.

88 Hardness

- 89 Flotation index (FI), was calculated as described in Lozano-Alejo et al. [22]. Kernels were classified as: very hard, hard,
- 90 intermediate, soft and very soft.
- 91 Color
- 92 The color of milled corn was measured using a CromaMeter CR 300 (Konica Minolta, Osaka, Japan), and expressed as
- 93 L*, a*, and b*. Five replicates were performed for each sample.
- 94 Chemical composition
- 95 Crude protein content was quantified by Dumas method (AOAC 990.03) using LECO FP 428. Crude lipid content was
- 96 determined gravimetrically by SOXHLET method (AOAC 920.39). Total starch was quantified using amylase-
- 97 amyloglucosidase method (AOAC 996.11, Kit Megazyme K-TSTA 07/11). The amylose content was evaluated using the
- 98 Megazyme commercial assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland). All analyses were performed
- 99 in duplicate.
- 100 Antioxidant capacity and soluble phenolic content
- Total antioxidant capacity (TAC) was determined by direct ABTS assay, as described in Alfieri et al. [17] and expressed
- as mmol of Trolox Equivalent per kilogram dried matter (mmol TE/kg dm) by means of a Trolox dose-response curve.
- Each sample was analyzed in three replicates.
- Soluble phenolic content (SPC) was measured as described by Tafuri et al. [18]. All samples were extracted in duplicate
- and expressed as grams of gallic acid equivalent for kilogram of dried matter (g_{GAE}/kg dm); the results are the average of
- three different measurements.
- **107** Pasting Properties
- 108 Starch gelatinization and retrogradation properties were determined using the Micro Visco Amylograph (MVAG;
- Brabender GmbH & Co.KG, Duisburg, Germany) and the method reported in Marengo et al. [23]. Data were elaborated
- with Viscograph program for Microsoft Windows (Brabender GmbH & Co.KG, Duisburg, Germany). Each sample was
- 111 analyzed in duplicate.
- 112 Statistical analysis
- 113 Minimum, maximum, average and median values were calculated using the software Excel (Microsoft® Office Excel
- 2016). Principal component analysis (PCA) and Pearson's simple correlation were carried out using the statistical program
- 115 PAST [24].
- 116 Results and discussion
- 117 Physical characteristics
- The results of kernel characterization of the maize genotypes used in this study, in addition to their origin, are shown in
- 119 Table 1; the ears of the maize lines are showed in Figure 1. These materials were characterized by a large variability of

1000 kernels weight, which ranged from 253 g (Lo 1489ae) to 403 g (Lo1530). For more than 50% of the samples, the seed range was 311 g (i.e. median value), higher than that of the US reference. The wide range of 1000 kernels weight cannot be attributable to soil or climate conditions, since all the samples were grown simultaneously in the same location. A large genetic variability was also observed for flour color, as indicated by the range of variation of parameters L* (75.26 - 88.12), a* (-2.65 - 1.51) and b* (12.95 - 47.94) (*data not shown*). Most maize lines presented hard of very hard grains; few lines had soft or very soft grains and one line (Lo1374) showed an intermediate grain hardness. The degree of hardness (from soft to very hard) is an important parameter to choose the end-uses of genotypes; in general, for food preparation the hard and semi-hard maize kernels are preferred due to their greater yield and higher quality meals and grits than soft maize [25].

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Chemical composition

The results of the chemical composition are shown in Table 2. The content of main components was extended to a very broad range. Starch content ranged from 54.65 % dm (Lo1489ae) to 68.70 % dm (Lo1451), with an average value (63.4%) which coincides with the median, suggesting a homogenous distribution of the data. As regards protein content, it ranged from 9.66 % dm (Lo1451) to 14.79 % dm (Lo1489ae), with an average and median values of 11.4% and 11.3%, respectively. Finally, lipid content extended from 2.21 % dm (Lo1526) to 5.68 % dm (Lo1489ae), with an average of 3.88% and a median of 4.07%. The amylose extender (ae) lines presented the lowest starch amount and the highest protein and lipid content: in particular, protein and lipid percentages of Lo1489 ae sample are noteworthy (14.79% and 5.68 %, respectively). Italian maize germplasm was previously analyzed by Alfieri and co-workers [17] who considered 14 lines. They reported high mean values for protein and lipid content (13.44 and 5.10 % dm, respectively). A set of open-pollinated populations, included in a core collection of European landraces [26], were also characterized, and presented mean values of 11.48 % dm and 5.00 % dm for protein and lipid content. The amylose content ranged from 18.70 (Lo1501) to 48.41 % dm (Lo1489ae), with an average of 23.21% and a median value of 21.00 % dm. The two ae lines considered in this study, Lo1413ae and Lo1489ae, showed a different percentage of amylose on total starch, 31.60 % and 48.41%, respectively. Both the average and median values were lower compared to the amylose content in B73. Nowadays, the amylose content of raw materials is recognized as an important trait because it affects the functional properties of the starch including the extent of the gelatinization and retrogradation phenomena, thus affecting the

technological performance of the ingredients and, therefore, the food texture. As an example, Tam et al. [27] investigated

amylose content of ≈28% has to be preferred for the production of this kind of product. Conversely, waxy (with 0.2–3.8% amylose content) and high-amylose (with 40.0–60.8% amylose content) maize starches failed to produce bihon-type noodles [27]. In spite of maize potential for GF bread-making, rarely bread-making assessments have been used for cultivars selection. It was found that waxy hybrids led to softer crumbs [28].

Besides the technological aspects, a cereal grain with high amylose content can assure the formation of resistant starch (RS) during the hydrothermal treatments present in GF technological processes. This fraction cannot be digested in the small intestine and passes to the colon, where is fermented by the microflora [29]. The positive physiological effects of RS include the decrease in the glycemic response, a lower calorie intake, a higher colon health, a modulation of fat metabolism and the prevention of cardiovascular diseases [30]. Although the unique functional properties of a high-amylose diet are gaining acceptance as a desirable outcome for consumers, the quantity of high amylose maize used so far in food is limited. Among the works as yet published on this subject, Granfeldt et al. [31], reported that "arepas", a typical corn bread from Colombia and Venezuela made from high amylose maize flour, was used in a clinical food trial giving good results concerning both glucose and insulin response.

Antioxidant capacity and soluble phenolic content

Concerning the antioxidant properties of Italian maize lines, TAC ranged from 12.17 (Lo1320A) to 21.26 (Lo1526) mmol

TE/kg dm, with a mean value of 15.80 mmol TE/kg dm (Table 2). In the paper published few years ago by Redaelli et

al. [32] and concerning a group of 107 Italian inbred lines, the values ranged between 9.88 and 32.35 mmol TE/kg dm,

with a mean value of 18.00 mmol TE/kg dm.

SPC varied in the range 0.74 (Lo1415B) -1.30 (B73) g_{GAE}/kg dm (mean value: 0.83 g_{GAE}/kg dm). In the present study, a

positive correlation was found between TAC and SPC (r=0.78, $p\le0.01$), as previously reported by other authors [18, 34-

37]. A negative and significant correlation, on the other hand, was found between SPC and L* (r= -0.60, p≤0.01).

Many molecules, pigmented or not, contribute to the antioxidant properties of this cereal: carotenoids, polyphenols,

flavonoids and anthocyanins [33]. The two white lines considered in this work (Lo1224w and Lo1446w), although the

lack of carotenoid in their kernel, showed an intermediate TAC value (13.27 and 15.30 mmol TE/kg dm, respectively),

probably due to the presence of other compounds such as polyphenols. Indeed, recent studies on white Italian landraces

[19,20] demonstrated that TAC in white genotypes has comparable values to those found in yellow genotypes, and in the

study by Tafuri and co-workers [18] no relation was found between kernel color and SPC. As expected, the TAC value

in the black line B73, was one of the highest (20.90 mmol TE/kg dm). As suggested by Redaelli et al. [32], although the

genotypes with black grains are surely characterized by high levels of TAC, the selection of genotypes for the antioxidant

activity taking into account only the grain color (white, yellow, orange or red) could be not efficient. Indeed, no correlation was found between TAC and color parameters L*, a* and b* (*data not shown*).

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Pasting properties

The indices of the pasting properties are reported in **Table 3**. Regarding the genotypes with an amylose content < 25-28%, the range of both the pasting temperature (i.e., temperature at the beginning of gelatinization) and the temperature at maximum viscosity extended to 5-7 °C of variation, from 71.2° to 75.8 °C, and from 87.9° to 95.5 °C, respectively. The highest value of maximum viscosity was registered for Lo1530 (298.5 BU), whereas the lowest was found in Lo1550 (76.5 BU). Both breakdown and setback values presented high variability (1.0 - 42.5 BU and 175.5 - 503.5 BU, respectively). The two ae lines showed peculiar values for all pasting parameters. To better appreciate the differences among the samples, the pasting profiles of selected Italian maize genotypes are summarized in Figure 2, where three types of gelatinization and retrogradation performances are presented. In the first group, the two amylomaize samples (i.e., Lo14189ae and Lo1413ae) were gathered. Both traces indicated a very low raising in viscosity during the whole temperature profile. The resistance to swell and gelatinize of this type of starch granules was responsible for the absence of viscosity during heating [30, 38]. Indeed, in Lo1413ae and Lo1489ae, the viscosity peak (the "marker" of starch gelatinization intensity) as well as the setback (the index describing the starch retrogradation phenomenon) were scarcely detectable. Samples belonging to the second group (e.g. Lo1430, Lo1550, Lo1270, and Lo1404) were characterized by a slow but continuous increase in viscosity during the heating step to 95°C and the maintenance at this temperature. Only the cooling step till 30° C induced a stronger increase in consistence. The samples of the third group (e.g. Lo1481, Lo1530, Lo1457, 1473, and Lo1551) exhibited a sharp increase in viscosity associated with heating at about 70°C. The mixing at 95°C promoted a modest breakdown followed by high setback during cooling. Since the breakdown index measures the paste stability during the holding phase at 95°C, it provides information on rigidity of the swollen starch granules. Specifically, the lower the breakdown, the higher the rigidity. Final viscosity indicates the ability of flour to form a viscous paste, and setback measures retrogradation tendency upon cooling of the cooked paste. Samples with higher viscosity values (i.e., Lo1530 and Lo1532) would be well suited for food applications that require stable thickening after heat treatment, such as soups, sauces, or puddings [39]. Conversely, samples more capable of forming a firm gel after cooling (i.e., showing high setback values) are undesirable for shelf-stable sauces and baked goods, as they could be more prone to precipitation, water separation, and staling [39]. Varieties that are more prone to both gelatinization and retrogradation are suitable for GF pasta production [1]. Among our samples, Lo1430 and Lo1550 would likely not be well suited for this application, as they had low setback values. Correlation analyses of the compositional attributes of the starches with their pasting properties potentially provide valuable insights into the

mechanisms contributing to the functional properties of the starches [14]. Negative and significant correlations were found between amylose content and peak viscosity (r = -0.50, $p \le 0.05$), temperature at peak viscosity (r = -0.74, $p \le 0.01$), and setback (r = -0.63, $p \le 0.01$), as previously reported by Acquistucci et al. [40]. Indeed, samples with high amylose content developed low viscosity after heating at 95 °C, and therefore they re-associated at less extent, providing a low final viscosity.

Principal component analysis

Explorative multivariate analysis via PCA was used to further explore the data and provide additional discriminatory power. The Principal Components Analysis (PCA) in **Figure 3** shows the distribution of the Italian maize genotypes according to the MVAG parameters and the amylose and starch content. The two *ae* genotypes were not included in this analysis, due to their inability to gelatinize during the heating process. The first three principal components (PC1, PC2 and PC3) provided a good summary of the data, accounting for about 76% of total variance. Moreover, the biplot visualisation easily distinguishes the variables affecting most sample distributions, which are the ones more distant from the origin of the biplot. PC1, which explained 36.2% of the variance, was mainly related to starch content, setback, breakdown and peak viscosity. PC2 accounted for 25.4% of total variance, which was attributed to the temperature at beginning of the gelatinization process and that at peak viscosity. An additional 14.2% was contributed by PC3, mainly related to amylose content. The regions I and IV of the graph are characterized by the presence of genotypes having high starch content, peak viscosity, breakdown and setback values. Based on these characteristics that indicate a strong tendency to retrogradation, forming a starch network, Lo1481, Lo1530, Lo1457, Lo1451 and Lo1473 might represent the best genotypes for pasta making. At the opposite side of graph, in the regions II and III, the genotypes having peak viscosity lower than 200 BU and low tendency to retrograde are grouped: consequently, Lo1430, Lo1550, Lo1270 and Lo1404 samples seem to be suitable for bread production.

Conclusions

In recent years, the screening of cereal genotypes according to their technological properties has been applied to breeding programs [41]. Indeed, describing the features of varieties anticipates the food application studies. Considering the more recent breeding results, lines potentially suitable for GF pasta and bread processes were identified in the present Italian maize germplasm. The relevant variability of pasting properties in the Italian germplasm, therefore, suggests the possibility to choose the most appropriate line according to the hydrothermal conditions used in food processes and, consequently, the related changes in viscosity in the food system. The lines with higher values of peak viscosity, breakdown and setback could be eligible for pasta formulation. On the contrary, the several lines with low set-back values

could be suitable for bread making. Finally, the amylomaize lines do not have suitable characteristics to be transformed, in purity, into food. This is due to the fact the high amylose corn required very high temperature for starch gelatinization. Nevertheless, they could be used - in percentage with other flours - to facilitate the formation and increase the amount of RS in the final food. The textural parameters and sensory evaluation of GF products made from these lines on a laboratory scale will enable to establish the role of amylose content and pasting properties of maize genotypes in defining a possible maize classification for GF foods. Specifically, a study on the use of maize varieties different in amylose content in the production of gluten-free snacks and pasta is underway.

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Table 1. Physical characteristics of the inbred lines (origin, 1000 kernels weight, seed color and hardness).

| Inbred line | Origin | 1000 kernels weight (g) | Seed color | Hardness |
|-------------|--------------------------|----------------------------|---------------|--------------|
| Lo1224w | P3463w | 259 | white | hard |
| Lo1270 | Lo1056 x Latina | 371 | orange | very hard |
| Lo1320A | Lo1142 x P3394 (Cecilia) | 311 | orange | hard |
| Lo1374 | BP42 515A x Lo863 | 309 | orange | intermediate |
| Lo1404 | Lo1230 x Lo1208 | 308 | orange | hard |
| Lo1413ae | Lo904ae x Lo1233ae | 286 | dark orange | very hard |
| Lo1415A | DK440 | 335 | yellow-orange | very soft |
| Lo1415B | DK440 | 323 | yellow-orange | very soft |
| Lo1430 | Lo1240 x Lo1208 | 298 | orange | hard |
| Lo1451 | Lo1279 x Lo1183 | 293 | yellow-orange | hard |
| Lo1457 | Lo1301 x Lo1106 | 361 | yellow | very hard |
| Lo1463 | Lo1289 x Lo1159 | 321 | orange | very hard |
| Lo1471 | PR38H67 | 268 | yellow | very soft |
| Lo1473 | Lo1313 x Lo1245 | 296 | yellow-orange | soft |
| Lo1481 | Lo1263 x Lo1301 | 298 | yellow-orange | very hard |
| Lo1489ae | Lo1339ae x Lo1309ae | 253 | yellow-orange | soft |
| Lo1501 | DSP5008C13 x Lo1279 | 373 | yellow-orange | hard |
| Lo1505 | Lo1301 x Lo1255 | 311 | yellow | very hard |
| Lo1526 | Plollen | 316 | orange | very soft |
| Lo1530 | PR31G98 | 403 | orange | very hard |
| Lo1532 | Lo1344 x DSP1771D | 377 | yellow-orange | soft |
| Lo1546w | Damiana | 278 | white | hard |
| Lo1550 | Lo1398 x Lo1270 | 376 | orange | hard |
| B73 | Iowa Stiff Stalk Syn. | 236 | black | hard |

Table 2. Chemical characterization of the inbred lines

| Inbred line | Starch (% dm) | Protein (% dm) | Lipid (% dm) | Amylose (% total starch) | SPC (ggae/kg dm) | TAC (mmol TE /kg dm) |
|-------------|------------------|------------------|-----------------|-----------------------------|---------------------|----------------------------|
| Lo1224w | 57.36 ± 0.02 | 11.18 ± 0.09 | 3.29 ± 0.11 | 24.61 ± 0.79 | 0.74 ± 0.02 | 13.27 ± 0.41 |
| Lo1270 | 61.70 ± 0.27 | 12.86 ± 0.10 | 4.07 ± 0.08 | 21.00 ± 0.84 | 0.71 ± 0.02 | 12.89 ± 0.35 |
| Lo1320A | 64.77 ± 0.07 | 12.87 ± 0.07 | 3.16 ± 0.09 | 19.33 ± 1.08 | 0.71 ± 0.00 | 12.17 ± 0.58 |
| Lo1374 | 66.02 ± 0.30 | 12.45 ± 0.14 | 3.78 ± 0.05 | 22.50 ± 0.39 | 0.79 ± 0.03 | 16.60 ± 0.78 |
| Lo1404 | 63.42 ± 0.30 | 10.98 ± 0.09 | 2.75 ± 0.02 | 21.92 ± 1.17 | 0.75 ± 0.03 | 15.16 ± 0.08 |
| Lo1413ae | 60.65 ± 0.36 | 12.72 ± 0.09 | 5.30 ± 0.03 | 31.60 ± 0.91 | 1.03 ± 0.04 | 19.93 ± 0.72 |
| Lo1415A | 66.04 ± 0.45 | 10.11 ± 0.11 | 3.34 ± 0.02 | 20.13 ± 0.04 | 0.53 ± 0.01 | 13.17 ± 0.35 |
| Lo1415B | 64.83 ± 0.27 | 10.51 ± 0.03 | 4.19 ± 0.01 | 22.14 ± 0.50 | 0.74 ± 0.01 | 16.20 ± 0.50 |
| Lo1430 | 60.30 ± 0.61 | 12.51 ± 0.06 | 4.65 ± 0.03 | 21.66 ± 0.88 | 0.77 ± 0.02 | 15.03 ± 0.40 |
| Lo1451 | 68.70 ± 0.06 | 9.66 ± 0.03 | 4.11 ± 0.05 | 20.25 ± 0.28 | 0.75 ± 0.03 | 14.38 ± 0.44 |
| Lo1457 | 65.68 ± 0.20 | 11.11 ± 0.07 | 4.50 ± 0.11 | 22.94 ± 0.70 | 0.75 ± 0.02 | 14.01 ± 0.45 |
| Lo1463 | 63.26 ± 0.08 | 11.63 ± 0.14 | 3.40 ± 0.07 | 20.50 ± 1.00 | 0.84 ± 0.01 | 13.59 ± 0.57 |
| Lo1471 | 65.01 ± 0.10 | 9.52 ± 0.03 | 3.22 ± 0.06 | 20.13 ± 0.06 | 1.04 ± 0.01 | 15.47 ± 0.46 |
| Lo1473 | 67.27 ± 0.18 | 11.67 ± 0.05 | 4.10 ± 0.05 | 19.48 ± 0.60 | 1.02 ± 0.02 | 20.30 ± 0.44 |
| Lo1481 | 65.89 ± 0.31 | 9.94 ± 0.19 | 4.15 ± 0.04 | 20.48 ± 0.03 | 0.65 ± 0.02 | 12.31 ± 0.14 |
| Lo1489ae | 54.65 ± 0.01 | 14.79 ± 0.14 | 5.68 ± 0.01 | 48.41 ± 0.75 | 0.85 ± 0.01 | 17.29 ± 0.27 |
| Lo1501 | 63.07 ± 0.01 | 12.53 ± 0.10 | 4.31 ± 0.01 | 18.70 ± 0.44 | 0.86 ± 0.03 | 15.41 ± 0.11 |
| Lo1505 | 63.33 ± 0.15 | 11.62 ± 0.01 | 4.47 ± 0.04 | 19.66 ± 1.11 | 0.85 ± 0.03 | 15.96 ± 0.21 |
| Lo1526 | 64.35 ± 0.38 | 10.78 ± 0.04 | 2.21 ± 0.03 | 20.50 ± 0.41 | 1.09 ± 0.04 | 21.26 ± 0.52 |
| Lo1530 | 63.22 ± 0.17 | 10.17 ± 0.11 | 4.17 ± 0.02 | 20.48 ± 0.67 | 0.82 ± 0.02 | 14.69 ± 0.20 |
| Lo1532 | 61.76 ± 0.18 | 11.34 ± 0.01 | 3.66 ± 0.02 | 25.96 ± 0.56 | 0.75 ± 0.01 | 18.95 ± 0.58 |
| Lo1546w | 64.94 ± 0.56 | 10.31 ± 0.08 | 3.54 ± 0.06 | 22.67 ± 1.33 | 0.66 ± 0.02 | 15.30 ± 0.39 |
| Lo1550 | 62.74 ± 0.46 | 11.58 ± 0.02 | 3.22 ± 0.02 | 28.80 ± 0.85 | 0.83 ± 0.02 | 16.39 ± 0.57 |
| Min | 54.65 | 9.52 | 2.21 | 18.70 | 0.53 | 12.17 |
| Max | 68.70 | 14.79 | 5.68 | 48.41 | 1.09 | 21.26 |
| Mean | 63.43 | 11.43 | 3.88 | 23.21 | 0.81 | 15.64 |
| Median | 63.42 | 11.34 | 4.07 | 21.00 | 0.77 | 15.30 |
| B73 | 65.97 ± 0.15 | 10.46 ± 0.09 | 3.45 ± 0.05 | 25.76 ± 1.32 | 1.30 ± 0.04 | 20.90 ± 0.76 |

SPC, Soluble Phenolic Content; TAC, Total Antioxidant Capacity

Table 3. Pasting properties of the inbred lines

| Inbred line | Pasting Temperature (°C) | Peak viscosity (BU) | Temperature at peak viscosity (°C) | Breakdown (BU) | Setback (BU) |
|-------------|--------------------------|------------------------|------------------------------------|-------------------|------------------|
| Lo1224w | 74.9 ± 0.4 | 249.0 ± 1.4 | 95.5 ± 0.0 | 61.5 ± 0.7 | 426.0 ± 4.2 |
| Lo1270 | 73.0 ± 1.1 | 109.5 ± 3.5 | 95.1 ± 0.2 | 4.5 ± 2.1 | 237.0 ± 7.1 |
| Lo1320A | 73.7 ± 0.2 | 157.0 ± 11.3 | 95.1 ± 0.1 | 12.5 ± 2.1 | 326.5 ± 7.1 |
| Lo1374 | 74.2 ± 0.1 | 173.0 ± 0.0 | 95.0 ± 0.0 | 7.5 ± 0.7 | 394.5 ± 0.7 |
| Lo1404 | 72.8 ± 0.1 | 102.5 ± 0.7 | 95.0 ± 0.0 | 1.0 ± 0.0 | 271.5 ± 3.5 |
| Lo1413ae | - | - | - | - | - |
| Lo1415A | 73.5 ± 1.1 | 203.5 ± 7.8 | 93.2 ± 3.5 | 37.5 ± 0.7 | 394.0 ± 4.2 |
| Lo1415B | 73.5 ± 0.1 | 130.0 ± 11.3 | 87.9 ± 0.8 | 17.5 ± 6.4 | 247.5 ± 4.9 |
| Lo1430 | 73.6 ± 0.6 | 81.5 ± 6.4 | 95.0 ± 0.0 | 1.5 ± 0.7 | 175.5 ± 2.1 |
| Lo1451 | 72.0 ± 2.1 | 233.0 ± 11.3 | 91.8 ± 4.7 | 43.0 ± 15.6 | 454.0 ± 11.3 |
| Lo1457 | 73.5 ± 0.6 | 253.0 ± 7.1 | 94.9 ± 0.6 | 49.0 ± 4.2 | 503.5 ± 3.5 |
| Lo1463 | 74.6 ± 0.6 | 151.5 ± 3.5 | 95.0 ± 0.0 | 12.5 ± 0.7 | 335.5 ± 13.4 |
| Lo1471 | 71.4 ± 0.5 | 229.5 ± 0.7 | 88.7 ± 0.8 | 79.5 ± 6.4 | 365.5 ± 7.8 |
| Lo1473 | 75.8 ± 0.3 | 243.5 ± 3.5 | 95.2 ± 0.1 | 44.0 ± 1.4 | 452.0 ± 4.2 |
| Lo1481 | 74.5 ± 2.1 | 247.5 ± 6.4 | 95.1 ± 0.4 | 42.5 ± 3.5 | 502.5 ± 9.2 |
| Lo1489ae | - | - | - | - | - |
| Lo1501 | 71.6 ± 0.1 | 137.0 ± 7.1 | 91.7 ± 4.0 | 17.5 ± 10.6 | 247.0 ± 2.8 |
| Lo1505 | 73.0 ± 2.3 | 169.5 ± 4.9 | 92.4 ± 4.2 | 27.0 ± 7.1 | 342.5 ± 4.9 |
| Lo1526 | 71.2 ± 1.0 | 201.0 ± 43.8 | 88.8 ± 1.1 | 50.0 ± 15.6 | 412.5 ± 48.8 |
| Lo1530 | 71.8 ± 0.1 | 298.5 ± 10.6 | 88.1 ± 0.4 | 87.5 ± 12.0 | 486.0 ± 5.7 |
| Lo1532 | 73.4 ± 0.6 | 276.5 ± 6.4 | 95.4 ± 0.2 | 65.5 ± 3.5 | 437.5 ± 2.1 |
| Lo1546w | 71.7 ± 0.1 | 170.5 ± 20.5 | 95.3 ± 0.5 | 23.0 ± 4.2 | 377.5 ± 33.2 |
| Lo1550 | 73.4 ± 0.1 | 76.5 ± 0.7 | 95.0 ± 0.0 | 2.5 ± 0.7 | 203.5 ± 3.5 |
| Min | 71.2 | 76.5 | 87.9 | 1.0 | 175.5 |
| Max | 75.8 | 298.5 | 95.5 | 87.5 | 503.5 |
| Mean | 73.2 | 185.4 | 73.2 | 32.7 | 361.5 |
| Median | 73.4 | 173.0 | 73.4 | 27.0 | 377.5 |
| B73 | 73.6 ± 0.4 | 243.0 ± 22.6 | 90.4 ± 0.1 | $70.0\pm\!17.0$ | 412.0 ± 17.0 |

- 378 Figure 1 Ears of the 24 maize inbred lines characterized in this study
- **Figure 2.** Pasting profiles of selected Lo lines.
- **Figure 3.** Principal Component Analysis considering starch and amylose content, and pasting profile indices.