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# Assessment of the attitude to ensilability of different FAO classes and maturities at harvest of maize hybrids grown in areas with different yield potential

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# 1 Assessment of the attitude to ensilability of different FAO classes and maturities at harvest of

# 2 maize hybrids grown in areas with different yield potential

- 3 Running title: Effect of different factors on maize ensilability
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- 14
- 15 Abstract

The aim of this study was to verify the influence of the FAO class of maize hybrids harvested at 16 different levels of maturity and grown with different yield potential on their attitude to ensilibility, 17 described by their fermentation products and summarized by a fermentation quality index (FQI). 18 Maize hybrids belonging to early (n = 14) and late (n = 15) FAO classes, were grown in low, medium 19 and high potential yield areas and harvested at 1/3 (EH), 2/3 (MH) and 5 d after the 2/3 milk line 20 phase (LH), according to a factorial design with two replications. Upon harvest, each sample (n =21 522) was chopped and analysed for dry matter (DM) and water-soluble carbohydrates (WSC) before 22 being ensiled in vacuum-packed bags (n = 1044). After 60 days of conservation samples were 23 analysed for DM and fermentation products. In the pre-ensiling phase DM was higher in early 24 hybrids, low yield area and at LH maturity, whereas the WSC content was higher in early hybrids, 25 medium yield area and at EH maturity. As regards maize silage, early hybrids led to a higher FQI 26 than late hybrids and in the early ones the FQI was optimised in areas with high production compared 27 with the others and at an EH maturity compared with MH and LH maturity. Late hybrids seemed to 28 be better suited for low yield areas compared with early hybrids and had a higher FQI at an early or 29 medium maturity than at LH maturity. Further research is warranted. 30

31 Keyword: maize silage, attitude to ensilability, hybrid, maturity at harvest, yield potential.

#### 32 1. INTRODUCTION

Maize (*Zea mays*, L.) silage represents one of the most important forages used in dairy cows and beef cattle rations in temperate regions (Grant and Adesogan, 2018; Marchesini et al., 2017), owing to its high productivity, ease of ensiling and nutritional profile, including both starch and physically effective fiber (Grant and Ferraretto, 2018; Khan et al., 2015). For these reasons it is often included in the rations at over 30 - 40 % of dry matter (DM) and over 50 % of ration's fodder (Castillo-Lopez et al., 2014., Grant and Adesogan, 2018; Silva et al., 2018).

It is therefore evident the importance of maize silage quality in terms of DM and nutrients
composition (Krämer-Schmid et al., 2016), absence of harmful compounds (Cavallarin et al., 2011;
Driehuis, 2013), fermentation profile (Gallo et al., 2016; Kung et al., 2018) and aerobic stability
(Borreani et al., 2014; Elferink et al., 2000) to obtain high performance by healthy animals.

This is even more true when highly productive animals are fed, because they have very high metabolic needs and are easily affected by any alterations of the ration that can lead to reduced DM intake (DMI), performance (Gerlach et al., 2013; Grant and Ferraretto, 2018) and poor health (Borreani et al., 2008).

The main purpose of ensiling is to prevent the loss of DM and energy from a substrate and the 47 production of inedible and toxic compounds by aerobic and anaerobic microbial activity (Grant and 48 Adesogan, 2018). Immediately after harvsting, in the presence of air, an oxidative degradation of 49 organic compounds and proteolysis occur as a result of the activity of enzymes present in the plant 50 tissue. Furthermore, when oxygen is in contact with plant tissue, either in the early phases of ensiling 51 or during feed-out processes, aerobic microbial activity occurs, especially by means of moulds and 52 yeasts, leading to a decay of the substrate (McDonald et al., 2011). On the other hand, when anaerobic 53 54 conditions are achieved, it is necessary to prevent the activity of undesirable microorganisms, such as clostridia and enterobacteria which increase DM, aminoacid and energy losses (Pahlow et al., 55 2003), by lowering the pH of the substrate (Kung et al., 2018; McDonald et al., 2011). 56

Ideally, during the anaerobic fermentation, all water-soluble carbohydrates (WSC) should be metabolised by homolactic acid bacteria to lactic acid, which contributes the most to the decline in pH and minimize energy and DM loss (Pahlow et al., 2003), but a great number of factors, including the DM content, the amount of WSC and the buffering capacity of the substrate, lead to a more complex array of compounds such as volatile acids, alcohols and esters which can alter many nutritive charcteristics of a forage (Kung et al., 2018). Their analysis, along with the measure of pH, still

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represents the main way of assessing maize silage fermentation quality (Andrighetto et al., 2018;

Ethanol, for example, is produced by yeasts, enterobacteria and heterofermentative bacteria (Mc 65 Donald et al., 1991; Nishino et al., 2004, Weiss et al., 2016), whereas others derive from clostridia 66 methabolism (propionate, butyrate and ammonia) and protein or aminoacid degradation (ammonia), 67 68 as reported by Muck (1988), Pahlow et al. (2003) and Khun et al. (2018). These compounds alltoghether are associated with a loss of silage DM and a drop in animal DMI and performance 69 (Borreani et al., 2018). Acetic acid, that after lactic is the most abundant acid found in silage, when 70 is present in moderate concentration seems to be beneficial, as it improves silage aerobic stability, 71 owing to its antifungal activity (Nishino et al., 2004). However, in order to guarantee an optimal 72 silage quality, the lactic/acetic acid ratio should not be lower than 2.5 (Kung et al., 2018), with the 73 exception of silages treated with L. buchneri which show a lower lactic/acetic acid ratio without 74 affecting the overall silage quality. This result is due to the metabolism of some lactic acid to acetic 75 76 acid, as reported by Kleinschmit and Kung (2006).

77 There are many factors that can affect the fermentation processes of a silage, such as the DM content 78 and chemical characteristics of the green chopped whole plant, stage of maturity at harvest, mechanical processing, speed of packing, pack density, addition of inocula, sealing material used on 79 80 silos, etc. (Borreani et al, 2018; Ferraretto et al., 2018; Wilkinson and Rinne, 2018). However, to optimize the ensiling processes, it would also be necessary to better characterize the different maize 81 hybrids or maize hybrid classes about their attitude to ensiling, through verifying their fermentative 82 response to different growing conditions and management factors, such as their maturity at harvest. 83 84 The knowledge of the response of each hybrid to these conditions would allow both the farmer to 85 choose the most suitable varieties in order to attein the best yields together with the best silage quality, 86 and the seed companies to better plan their strategies for improvement.

In this regard, there are numerous studies on the use of inocula during ensiling (Holzer et al., 2003; Kleinschmit and Kung, 2006; Wilkinson and Davies, 2013) and on different hybrids and maturity at harvest (Ferraretto et al., 2015; Gerlach et al., 2018; Hunt et al., 1989; Johnson et al., 2002), but to our knowledge few researches have focused on the effect of the interaction between the FAO class of hybrids, maturity at harvest and the yield potential of an area on the fermentative characteristics of maize silage, nor have included such a number of hybrids in order to assess the variability of their response to these conditions.

In this regard, a possible help, at least as screening phase, can derive from studies involving the use 94 of different hybrids, ensiled in standard and controlled conditions in lab-scale silos and without the 95 use of inocula. The different response of a hybrid or hybrid class in terms of silage fermentation 96 quality at given growing conditions could also give information about the most suitable inocula to 97 use. This would help on one hand to optimize the fermentation process and the stability of the 98 substrate, and on the other, to advise the farmer when it is necessary using inocula and on what 99 inoculum fits better. Extending the knowledge in this field could help farmers to select a hybrid or a 100 hybrid class not only on the basis of its productivity, but even because it shows the best fermentation 101 102 profile in certain environmental conditions and could give them more information about the proper time of harvest. 103

The aim of this study was to verify the influence of the FAO class of maize hybrids harvested at different levels of maturity and grown in areas with different yield potential on their attitude to insilability, described by their fermentations products and summarized by a fermentation quality index (FQI).

## 108 2. MATERIALS AND METHODS

#### 109 2.1. Experimental design

The trial was performed in the summer of 2016 in the Po Valley (Northern Italy), using 29 maize 110 hybrids of early (FAO class 200, n = 14) and late (FAO class 600-700, n = 15) maturity classes, which 111 reflect the whole plant DM concentration (Gerlach et al., 2018). All the hybrids were grown in 3 112 localities (Baura: Ferrara province; Granze and Montagnana: Padova Province) with different 113 pedoclimatic characteristics, that on the basis of historical maize silage crop production data (the 114 areas were planted with monocrops of maize for the last three years), could be referred as areas of 115 low (466 g/ha), medium (563 g/ha) and high (686 g/ha) potential yield, respectively. Baura is a high 116 stressed area with a medium-heavy soil, characterised by a very poor water availability for irrigation, 117 a medium crop drydown and a medium to poor yield potential. Granze is a medium-high stressed area 118 with a medium soil, characterised by a poor water availability for irrigation, a medium crop drydown 119 and a medium to poor yield potential. Montagnana is a high stressed area with a light clay soil, 120 characterised by a high water availability for irrigation, a fast crop drydown and a high yield potential. 121 In each locality the different hybrids were grown in adjacent plots under the same tillage and 122 fertilization practices, applying 300 kg/ha of N, 150 kg/ha of P and 150 kg/ha of K. 123

The plants were harvested at three maturity phases which were determined through the observation of the kernel milk line (Ferraretto et al., 2015): early (EH, 1/3 milk line phase), medium (MH, at 2/3 milk line phase) and late (LH, 5 d after the 2/3 milk line phase). Early and late hybrid populations

were sown at the standard densities recommended by the Italian Ministry of Agriculture for the different FAO classes, corresponding to 95000 and 70000 plants/ha, respectively. Each hybrid was subjected to each growing and harvesting condition in a factorial design. At the time of cropping, whole plants belonging to each hybrid were randomly sampled from the central area of each plot and were chopped at a theoretical length of cut of 2 cm on a self-propelled forage harvester. Two replicates were made for each thesis, up to a total of 522 samples.

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# 134 *2.2. Sample collection, preparation and analysis*

In order to verify the attitude to ensiling of a large number of different hybrids grown and harvested under different conditions, it was necessary to reduce the differences related to the ensiling system and the management factors as much as possible (Gallo et al., 2016). For this reason it was decided to standardize the ensiling phase using the vacuum-packed bags technique and avoiding the use of inocula, which could mask the initial attitude to ensiling of whole maize plants.

Each whole maize sample was immediately chopped and subjected to the determination of DM and water-soluble carbohydrate (WSC) content by means of a portable near infrared (NIR) system (poliSPEC<sup>NIR</sup>, ITPhotonics, Breganze, Italy). After the analysis, for each of the 522 whole maize samples, two replicates (n = 1044) were immediately ensiled in vacuum-packed bags (Orved 2633040, Orved SpA, Musile di Piave, VE, Italy) as described by Andrighetto et al. (2018).

All the replicates were stored at 23°C and opened for the analysis of maize silage after 60 days of 145 conservator. The silage content of each vacuum-bag was scanned in duplicate (n = 2088) using a 146 FOSS NIRSysistem 5000 scanning monocromator (FOSS NIRSystem, Silver Spring, MD, USA) 147 using the calibration curve, reported by Andrighetto et al. (2018), for the analysis of dry matter (DM), 148 lactic, acetic, propionic and butyric acids, ethanol, ammonia and pH. All these fermentation products 149 were used to assess the quality of fermentation, as reported also by Kung et al. (2018) and besides, 150 the concentrations of lactate, ammonia, ethanol, acetate and butyrate were used to calculate an index 151 of maize silage fermentation quality (FQI), described by Andrighetto et al. (2018) as index 15. 152

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#### 154 2.3. Statistical analysis

Statistical analyses were performed using SAS release 9.4 (SAS Institute Inc., Cary, NC, 2012). All data of whole maize plant and maize silage composition and FQI were first tested for normality with the Shapiro–Wilk test (> 0.9 = normally distributed) and then submitted to an ANOVA model with hybrid class (two levels: early and late), yield potential (three levels: low, medium and high), maturity at harvest (three levels: EH, MH and LH) and their interactions, as fixed effects. Within each maturity

160 class whole maize plant, maize silage composition and FQI were submitted to a second ANOVA 161 model with hybrid (14 levels for FAO class 200 and 15 levels for FAO class 600-700), yield potential 162 (three levels: low, medium and high), maturity at harvest (three levels: EH, MH and LH) and their 163 interactions, as fixed effects. In both models post-hoc pairwise comparisons were run between factor 164 levels using Bonferroni correction. Assumptions of the linear model on the residuals were graphically 165 tested.

As this paper focuused more on the effect of hybrid maturity class than on the effect of single hybrids, for practical reasons, in the tables reporting the hybrid effect on whole plant and silage characteristics, only the range of values was shown (Tables 2, 4 and 5).

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## 170 **3. RESULTS**

At the time of harvest, as reported in Table 1, the early varieties had a higher DM content than the late ones (p = .001). Despite their higher level of DM, the hybrids of the class 200 showed a higher content of WSC than hybrids of the class 600-700 (p < .001).

- 174 The DM content of maize in low and high yield areas, was the the highest and the lowest, respectively
- 175 (p < .001). Unlike DM, WSC showed the highest concentration in the medium yield area and the
- 176 lowest content in the low yield area (Table 1).
- As the maturity stage progressed, an increase in the percentage of dry matter was observed (p < .001), whereas an opposite trend was observed for the content of WSC (p < .001).
- Within each FAO class the effect of the hybrid was significant both for DM (p < .001) and WSC (p < .01), as reported in Table 2. A significant interaction between hybrid and maturity at harvest was observed only for DM in the class 600-700. Significant interactions were found also for DM and WSC between maturity at harvest and yield potential and between hybrid and yield potential, with the exception of DM in hybrids of Class 200.
- As reported in Figures1 and 2 the relationship between DM and WSC content is poor when the class 600-700 ( $R^2 = 0.19$ , p < .001) is taken into account (Fig. 1), whereas it is better ( $R^2 = 0.55$ , p < .001) when the hybrids of the class 200 are considered (Fig. 2).
- The fermentative characteristics of maize silage were affected by the FAO class, maturity at harvest,yield potential and their interactions (Table 3).

Overall, as reported in Table 3 the FQI was higher in the maize silage ripening class 200 compared with the class 600-700 (p < .001), indicating a better quality of fermentation, characterized by a slightly reduced production of ammonia (p = .002), acetic acid (p < .001) and propionic acid (p = .002). The early hybrids led also to higher DM content (p < .001) and slightly higher pH (p < .001).

The area characterized by high yield, compared with the others, led to higher FQI (p < .001), lower ethanol (p < .001), propionic acid concentration (p = .02) and to an intermediate level of pH (Table 3). High and medium yield areas were instead similar and led to lower DM and to higher ammonia, lactic and butyric acid concentrations compared with low yield areas.

The progressive maturity at harvest, led to a progressive decrease of the fermentaion quality index (Table 3), with the early harvest leading to the lowest DM and pH and the highest lactic acid, ammonia, ethanol, propionic and butyric acid concentrations. Acetic acid concentration was similar between EH and MH and the lowest in LH (Table 3).

The plant maturity at harvest and the yield potential had different effects on the fermentation profile depending on the FAO class, as there were found significant interactions between these two factors and the FAO class (Table 3).

As regards the interactions between factors it should be noted that in the class 200, passing from EH to LH there was a progressive and significant reduction of FQI (Fig. 3), while in the class 600-700, the FQI was not different between EH and MH, while it was significantly lower (p < .001) in LH (Figure 3). Besides, the difference in FQI between the two FAO classes seems negligible at the 2/3 milk line phase.

With regards to yield potential, it can be noticed that there was a significant increase in the fermentation qualiy index in the class 200 passing from low to high yield (Fig. 4), whereas in the class 600-700 there was not such a trend and the lowest FQI was found in the medium yield area (Figure 4).

Furthermore, as reported in Figure 5, the FQI in class 200 was generally lower in the low yield area and in such conditions there was no difference between the different maturity phases at harvest.

In the hybrids of class 600-700 the FQI declined when the harvest was done at the LH phase, no
matter the yield potential of the area and reached its minimum value in the medium yield area (Figure
6).

Table 4 shows the maximum and minimum values of FQI and the various parameters of the fermentation profile measured in the different hybrids belonging to the class 200. For all the parameters the effect of hybrid was significant. As regards the interactions between hybrid and maturity and between hybrid and yield potential, they were not significant either for FQI or for any parameter, with the exception of lactic and butyric acids. The interaction between maturity and yield potential was always significant.

The effect of the hybrid within the class 600-700 on the fermentative profile is reported in Table 5, in which it could be noticed that the interaction between hybrid and maturity at harvest is significant for pH and lactic acid, while the interaction between hybrid and yield potential is significant for FQI and for all the parameters, excluding acetic acid.

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## 229 4. DISCUSSION

Given the outstanding spread of maize silage as main ingredient both in dairy cows and beef cattle rations (Borreani et al., 2018; Grant and Adesogan, 2018; Wilkinson and Rinne, 2018), it was decided to investigate some of the many factors that could affect its quality, and especially its fermentative profile, that is known to be related with DMI, feed refusal and DM losses during conservation (Elferink et al., 2000; Grant and Ferraretto, 2018; Mc Donald et al., 2011; Muck, 1988).

In this regard, we focused on the effect of hybrids and their FAO classes (early vs. late maturity) grown in areas characterized by different yield potential (low vs. medium vs. high) and harvested at different maturity phases (1/3, 2/3 and 5d after 2/3 of milk line phase).

The whole maize plant content of DM and WSC at harvest was in line with data reported in literature (Ferraretto et al., 2015; Hatew et al., 2016; Lynch et al., 2013) for whole maize crops intended for the production of maize silage.

The highest DM and WSC content of hybrids belonging to class 200 was likely due to their higher radiation use efficiency that led to a higher speed in the synthesis of carbohydrates before flowering (Andrieu et al., 1993) and in the deposition of starch during the period of grain filling (Martins et al., 2017), as also reported by Millner et al. (2005) and Lynch et al. (2013) who found a higher percentage of grain in the early maturing hybrids, compared with late hybrids.

The higher DM and the lowest WSC content of plants grown in the low yield area was expected, because non-optimal conditions of soil texture, hydration and presence of nutrients, lead to a slower

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maturation of the plant, affecting the content of cell walls, carbohydrate synthesis and graindevelopment (Andrieu et al., 1993).

As the plant grows, it increases its DM (Hatew et al., 2016; Martins et al., 2017), along with its crude protein and lignin content (Ferraretto et al., 2015) and the deposition of starch in the grain, which derives from both the photosynthesis and the conversion of WSC (Lynch et al., 2013). This also justifies the highest DM and the lowest content in WSC observed in plants harvested 5 days after the 2/3 milk line phase, compared with the ones harvested erlier.

Within each FAO class the differences between one hybrid and another are significant both for the 255 DM and the WSC content. Furthermore, looking at the interactions between factors, it can be noticed 256 how the interactions between hybrid and maturity at harvest and between hybrid and yield potential 257 for DM are not significant in the hybrids of class 200, while they are significant for that of class 600-258 700. The same difference there is also for the content in WSC, but limited to the interaction between 259 hybrid and maturity at harvest. This means that with regard to class 600-700, there are hybrids which 260 are specifically suited to areas with different yield potential and different harvest times, whereas for 261 262 class 200 this differentiation and specialization among hybrids is lacking. This is partly confirmed by 263 the lower coefficient of determination between DM and WSC found in late hybrids compared with early hybrids. As reported in the literature (Andrieu at al., 1993; Linch et al., 2013) in fact, the increase 264 265 of the DM corresponds to a decrease in the WSC of the plant, as the WSC synthesized in the flowering phase and stored at the level of the stem and leaves are used to synthesize the starch contained in the 266 267 grains, which contributes for the most part to the increase of the DM (Andrieu et al., 1993). However, relationship is better in early ( $R^2 = 0.55$ ) than in late hybrids ( $R^2 = 0.19$ ), because some of the late 268 hybrids showed a low WSC content even when the DM content was low. This could be likely due to 269 270 different proportions of the morphological constituents of the plant (grain, cob, leaf, stem), as reported 271 by Millner et al. (2005).

The FAO class, the level of maturity at harvest and the different yield potential of the areas have alsosignificantly affected the fermentative profile of maize after the ensiling process.

In this study, the fermentation quality was measured using fermentation products such as lactic acid, volatile acids, ethanol, ammonia and pH, as reported in literature (Kung et al., 2018) and was summarized by an index tested and validated by Andrighetto et al. (2018), that resulted to be particularly suitable for discriminating the differences in the quality of fermentations obtained under controlled ensiling conditions, such as those obtained using vacuum-packed bags (Andrighetto et al., 2018; Johnson et al., 2005). This index, ranging from 1 to 100, weighs the presence of compounds such as lactate, ethanol, acetate, butyrate and ammonia, that are produced during homolactic,
heterolactic, clostridia and yeast fermentations and characterize the fermentation quality of maize
silage (Nishino et al., 2004; Romero et al., 2017).

This type of ensiling usually leads to high quality silages (Romero et al., 2017), with rather low values of ethanol, acetic, butyric and ammonia, as can be seen by comparing the atteined values with those suggested by Kung et al. (2018). For this reason it was decided to use the above reported FQI to summarize the quality characteristics of fermentation, because it proved to be particularly sensitive in diversifying the quality of fermentation between silages with low, medium and high levels of DM, obtained in lab conditions (Andrighetto et al., 2018).

In this study, all the hybrids showed a FQI value higher than 48.2, which according to Andrighetto et al (2018) represents the cut-off between excellent and not excellent maize silage, from a fermentative point of view, confirming the goodness of the ensiling method used. In this regard, the early hybrids have generally shown a slightly lower production of ammonia, acetic and propionic acid, as also reported by Gerlach et al. (2018), although both compounds have a concentration well below the suggested critical limit (Kung et al., 2018). Better fermentations were also found in plants grown in the area characterised by the highest fertility, and in those harvested earlier.

In the most suitable growing areas, where water, nutrients and temperature are not limiting factors, 296 plants quickly develope grain and increase the ratio between cytoplasmic carbohydrates and cell wall 297 constituents, affecting the composition of the whole plant and facilitating the fermentation process 298 accordingly (Andrieu et al., 1993). On the other hand, when the growing conditions are difficult the 299 synthesis of WSC is less intense and the hemicelluloses of the cell wall, which together with WSC 300 are used by micro-organisms as substrate for fermentations (Gerlach et al., 2018) are less available. 301 Moreover, as the plant ages the WSC content decreases (Andrieu et al, 1993) and DM content 302 increases, leading to a reduction in bacterial growth caused by low availability of water (De Bedrosian 303 et al., 2012; Ferraretto et al., 2015) and consequentely to a lower content in lactic acid which can 304 305 explain the better FQI of the plants harvested earlyer compared with the ones harvested last.

Through a more accurate investigation, it can be seen how the effects of yield potential and maturity at harvest vary depending on whether early or late hybrids are taken into account. Delaying the harvest from the 1/3 milk line ripening phase till 5 days after the 2/3 milk line phase resulted in a progressive reduction of the fermentation quality in the early hybrids, while in the late ones this reduction was observed only in the last phase.

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On the basis of these results, it can therefore be stated that, after taking into account productivity, 311 from a fermentation perspective, when using early maturity hybrids it is advisable to harvest at an 312 early stage of maturity, whereas with the late hybrids the harvest can be delayed until the 2/3 milk 313 line phase without reducing the fermentaion quality. This would give to the farmer a wider time 314 interval to wait for the most suitable wheather conditions. This rule, however, does not seem to be 315 valid either for early hybrids grown in low yield areas, where the FQI is low no matter the maturity 316 at harvest, or for late hybrids grown in high yield areas, where it is still convenient to harvest at 1/3 317 318 milk line phase.

As regards the yield potential, growing early maize hybrids in more and more suitable conditions led to a progressive increase in fermentation quality, whereas this trend was not seen in late hybrids. Besides, in good and excellent areas the early hybrids led to a better FQI than late hybrids, whereas in the worst conditions the FQI was higer in hybrids of class 600-700. This result implies that in the presence of good or excellent areas, it is better to use early hybrids, while in the poor ones, stressful for plants, from a point of view of the fermentation profile, it is better to use late FAO hybrids.

Within each FAO class, the difference between the various hybrids was always significant both for the FQI and for the various fermentation parameters, indicating that different hybrids within a class lead to a diversified fermentation quality. However, based on the interactions between factors, as seen for DM and WSC in the whole plants, it seems that early hybrids did not behave differently in different yieald potential areas and harvesting conditions and that, on the other hand, late hybrids displayed fermentations of different quality depending on the yield potential of an area and therefore appeared to be more suitable for specific growing conditions.

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## 333 5. CONCLUSIONS

This study showed that the attitude to ensiling measured in lab-scale ensiling conditions of maize 334 335 silage can be significantly affected by the FAO class of the hybrid, the single hybrid and its interaction with the yield potential of an area and the maturity at harvest. In particular, it seems that early maize 336 hybrids led to a better fermentation quality of maize silage, but mainly in very favorable areas and 337 when harvested at an early maturity phase. On the other hand, the hybrids of class 600-700 appeared 338 to guarantee a good ensilability even in medium or low yield potential areas and when they are 339 harvested within a wider period that goes from 1/3 up to 2/3 of the of the milk line phase. These 340 results show also that lab-scale ensiling and FQI are effective tools in comparing the different attitude 341 to ensiling of a large number of hybrids and the effect of the different factors involved. Further 342 research is warranted in order to confirm these results at field level. 343

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# 349 CONFLICT OF INTEREST

- 350 The authors declare there is no conflict of interest.
- 351

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492 Content of dry matter (DM) and water-soluble carbohydrates (WSC) in pre-ensiled maize belonging

to different FAO classes (class 200 vs. class 600-700), grown in different yield potential areas (low

- 494 vs. medium vs. high) and harvested at different maturity phase (early, LH vs. medium, MH vs. late,
- 495 LH). (Is means and standard error of means [SEM])
- 496

FAO class	DM (g/kg)	WSC (g/kg DM)
- Early (200)	366 <sup>a</sup>	100 <sup>a</sup>
- Late (600-700)	343 <sup>b</sup>	89.0 <sup>b</sup>
SEM	4.40	1.50
p	.001	<.001
Yield potential		
Low	373 <sup>a</sup>	88.7°
Medium	353 <sup>b</sup>	102ª
High	333°	93.0 <sup>b</sup>
SEM	1.50	1.00
p	< .001	< .001
Maturity at harvest		
EH	310°	106 <sup>a</sup>
MH	351 <sup>b</sup>	94.4 <sup>b</sup>
LH	403 <sup>a</sup>	83.0 <sup>c</sup>
SEM	1.50	0.90
p	< .001	<.001
FAO class × Maturity at harvest		
p	< .001	.042
FAO class × Yield potential		
p	< .001	< .001
Maturity at harvest × Yield potential		
p	<.001	< .001

497 a-c Means within columns not sharing a common superscript are significantly different (p < 0.01).

- 499 Effect of maize hybrids belonging to classes 200 and 600-700 on dry matter (DM) and water-
- soluble carbohydrates (WSC) content range at harvest, before the ensiling process. (Is means and
- standard error of means [SEM])

		class		
		200	6	00-700
	DM	WSC (g/kg	DM	WSC (g/kg
	(g/kg)	DM)	(g/kg)	DM)
Minimum	339	88.0	326	83.9
Maximum	416	111	362	100
SEM	4.60	2.82	4.10	2.71
p	< .001	< .001	< .001	.002
Hybrid × Maturity at harvest				
P-value	.1054	.174	.014	.203
Hybrid × Yield potential				
P-value	.217	.029	<.001	< .001
Maturity at harvest × Yield				
potential				
p	< .001	<.001	< .001	< .001

504 Fermentation profile and DM recovery (DMR %) of maize silage. belonging to different FAO

- classes (class 200 vs. class 600-700), grown in different yield potential areas (low vs. medium vs.
- 506 high) and harvested at different maturity (early, LH vs. medium, MH vs. late, LH). (Is means and
- 507 standard error of means [SEM])

	DM (g/kg)	рН	Ammonia (%Nitrogen)	Ethanol (g/kg DM)	Lactic acid (g/kg DM)	Acetic acid (g/kg DM)	Propionic acid (g/kg DM)	Butyric acid (g/kg DM)	FQI
FAO class									
Early (200)	360 <sup>a</sup>	3.87ª	5.70 <sup>b</sup>	11.2	52.2	8.51 <sup>b</sup>	0.02 <sup>b</sup>	0.563	57.0ª
Late (600-700)	333 <sup>b</sup>	3.83 <sup>b</sup>	5.97 <sup>a</sup>	11.6	50.1	9.80 <sup>a</sup>	0.04ª	0.643	53.5 <sup>b</sup>
SEM	1.11	0.002	0.023	0.191	0.294	0.130	0.001	< 0.001	0.29
р	< .001	< .001	.002	.510	.134	<.001	.002	.002	<.001
Yield potential									
Low	362 <sup>a</sup>	3.89 <sup>a</sup>	5.68 <sup>b</sup>	10.3 <sup>b</sup>	48.3 <sup>b</sup>	8.92	0.04ª	0.562 <sup>b</sup>	54.1 <sup>b</sup>
Medium	336 <sup>b</sup>	3.82°	5.90 <sup>a</sup>	14.5ª	52.2ª	9.50	0.04ª	0.604 <sup>a</sup>	53.5 <sup>b</sup>
High	341 <sup>b</sup>	3.85 <sup>b</sup>	5.91 <sup>a</sup>	9.30°	53.0 <sup>a</sup>	9.13	0.01 <sup>b</sup>	0.602 <sup>a</sup>	58.1ª
SEM	1.40	0.003	0.029	0.240	0.360	0.162	< 0.001	< 0.001	0.360
р	< .001	< .001	<.001	< .001	< .001	<.001	.002	<.001	<.001
Maturity at harv	vest								
EH	301°	3.82 <sup>b</sup>	6.13 <sup>a</sup>	12.9ª	57.9ª	10.1ª	0.05ª	0.640 <sup>a</sup>	58.8ª
MH	341 <sup>b</sup>	3.87 <sup>a</sup>	5.79 <sup>b</sup>	9.82°	50.8 <sup>b</sup>	9.90ª	0.02 <sup>c</sup>	0.591 <sup>b</sup>	55.8 <sup>b</sup>
LH	398ª	3.86 <sup>a</sup>	5.57°	11.4 <sup>b</sup>	44.9°	7.54 <sup>b</sup>	0.03 <sup>b</sup>	0.530 <sup>c</sup>	51.1°
SEM	1.40	0.003	0.029	0.240	0.360	0.162	< 0.001	< 0.001	0.360
р	< .001	<.001	<.001	< .001	< .001	<.001	.002	<.001	<.001
FAO class × Ma	aturity at l	narvest							
р	< .001	< .001	< .001	.206	<.001	.004	<.001	<.001	<.001
FAO class × Yi	eld potent	ial							
р	< .001	< .001	.312	< .001	<.001	<.001	<.001	<.001	<.001
Maturity at harv	vest × Yie	ld potentia	.1						
p	< .001	< .001	< .001	< .001	<.001	<.001	.011	<.001	<.001
<sup>a-c</sup> Means wi	thin col	umns n	ot sharing a	common s	uperscrip	t are signif	icantly differe	nt ( $p < \overline{0}$ .	01).

- 511 Effect of hybrid within class 200 on fermentation profile and DM recovery of maize grown in
- 512 different yield potential areas and harvested at different maturity phases. (Is means and standard
- 513 error of means [SEM])

	рН	Ammonia (%Nitrogen)	Ethanol (g/kg DM)	Lactic acid (g/kg DM)	Acetic acid (g/kg DM)	Propionic acid (g/kg DM)	Butyric acid (g/kg DM)	FQI
Minimum	3.81	5.58	8.10	45.8	8.10	0.01 <sup>b</sup>	0.570	52.4 <sup>b</sup>
Maximum	3.86	6.59	14.3	57.1	11.3	0.07ª	0.650	62.2 <sup>a</sup>
SEM	0.009	0.091	0.730	1.11	0.510	0.007	< 0.001	1.10
р	<.001	<.001	<.001	< .001	<.001	<.001	<.001	<.001
Hybrid × Mat	urity at harv	est						
р	.733	.164	.194	.007	.646	<.001	.014	.130
Hybrid × Yiel	d potential							
р	.137	.200	.103	<.001	.887	.187	<.001	.400
Maturity at ha	rvest × Yiel	d potential						
р	<.001	< .001	<.001	<.001	<.001	.323	<.001	.013

514 <sup>a-b</sup> Means within columns not sharing a common superscript are significantly different (p < 0.01).

- 517 Effect of hybrid within class 600-700 on fermentation profile and DM recovery of maize grown in
- 518 different yield potential areas and harvested at different maturity phases. (Is means and standard
- 519 error of means [SEM])

	рН	Ammonia (%Nitrogen)	Ethanol (g/kg DM)	Lactic acid (g/kg DM)	Acetic acid (g/kg DM)	Propionic acid (g/kg DM)	Butyric acid (g/kg DM)	FQI
Minimum	3.85	5.47	7.20	54.0	7.40	0.02 <sup>b</sup>	0.500	51.2 <sup>b</sup>
Maximum	3.94	5.94	13.8	58.7	9.60	0.07ª	0.600	58.7ª
SEM	0.009	0.091	0.730	1.11	0.510	0.007	0.010	1.13
р	<.001	.001	< .001	<.001	.059	<.001	<.001	<.001
Hybrid × Mat	turity at har	vest						
р	.026	.194	.116	.001	.371	.408	.079	.487
Hybrid × Yie	ld potential							
р	.002	<.001	<.001	.005	.241	. 165	.006	.052
Maturity at ha	arvest × Yie	ld potential						
р	<.001	.287	<.001	<.001	.049	<.001	<.001	<.001

520 <sup>a-b</sup> Means within columns not sharing a common superscript are significantly different (p < 0.01).

FIGURE 1 Relationship between DM (g/kg) and WCS (g/kg DM) taking into account the maize
hybrids belonging to the late FAO class (class 600-700).

- FIGURE 2 Relationship between DM (g/kg) and WCS (g/kg DM) taking into account the maize
  hybrids belonging to the early FAO class (class 200).
- **FIGURE 3** Effect of the interaction between FAO class (class 200 vs. class 600-700) and maturity at harvest (Early, EH vs. medium, MH vs. late, LH) on the fermentation quality index (FQI). <sup>a-d</sup> Means not sharing a common letter are significantly different (p < 0.01).
- **FIGURE 4** Effect of the interaction between FAO class (class 200 vs. class 600-700) and yield potential (low vs. medium vs. high) on the fermentation quality index (FQI). <sup>a-d</sup> Means not sharing a common letter are significantly different (p < 0.01).
- **FIGURE 5** Effect of the interaction between yield potential (low vs. medium vs. high) and maturity at harvest (Early, EH vs. medium, MH vs. late, LH) in class 200 hybrids on the fermentation quality index (FQI). <sup>a-c</sup> Means not sharing a common letter are significantly different (p < 0.01).
- **FIGURE 6** Effect of the interaction between yield potential (low vs. medium vs. high) and maturity at harvest (Early, EH vs. medium, MH vs. late, LH) in class 600-700 hybrids on the fermentation
- 537 quality index (FQI). <sup>a-c</sup> Means not sharing a common letter are significantly different (p < 0.01).





**FIGURE 1** Relationship between DM (g/kg) and WCS (g/kg DM) taking into account the maize hybrids belonging to the late FAO class (class 600-700).

O class (class out- , ...,



**FIGURE 2** Relationship between DM (g/kg) and WCS (g/kg DM) taking into account the maize hybrids belonging to the early FAO class (class 200).

n DM (g/ĸ<sub>b</sub>, AO class (class 200).



**FIGURE 3** Effect of the interaction between FAO class (class 200 vs. class 600-700) and maturity at harvest (Early, EH vs. medium, MH vs. late, LH) on the fermentation quality index (FQI). <sup>a-d</sup> Means not sharing a common letter are significantly different (p < .01).



**FIGURE 4** Effect of the interaction between FAO class (class 200 vs. class 600-700) and yield potential (low vs. medium vs. high) on the fermentation quality index (FQI). <sup>a-d</sup> Means not sharing a common letter are significantly different (p < .01).



**FIGURE 5** Effect of the interaction between yield potential (low vs. medium vs. high) and maturity at harvest (Early, EH vs. medium, MH vs. late, LH) in class 200 hybrids on the fermentation quality index (FQI). <sup>a-c</sup> Means not sharing a common letter are significantly different (p < .01).

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**FIGURE 6** Effect of the interaction between yield potential (low vs. medium vs. high) and maturity at harvest (Early, EH vs. medium, MH vs. late, LH) in class 600-700 hybrids on the fermentation quality index (FQI). <sup>a-c</sup> Means not sharing a common letter are significantly different (p < .01).

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