Use of near-infrared spectroscopy and multivariate approach for estimating silage fermentation quality from freshly harvested maize

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ABSTRACT
The study aimed to evaluate the most predictive traits of fresh maize and the most appropriate multivariate approach for estimating silage fermentation quality. The use of near-infrared (NIRs) instruments allowed rapid, accurate and cheap analysis. Samples of fresh maize plant (n = 822) from hybrids (Class Cultivar) of early and late classes, were harvested at three maturity stages: early, medium and late, in three areas (level input field) of ‘low’, ‘medium’ and ‘high’ soil fertility, along three consecutive years. Several algorithms of feature selection, regression, classification and machine learning, were tested. Maize silage fermentative quality was summarised through a Fermentative Quality Index (FQI). We found the most predictive traits as dry matter (DM), starch, and acid detergent lignin (ADL), with negative coefficients, or water-soluble carbohydrates (WSC) with a positive coefficient. FQI was significantly (p < 0.0001) affected by year (negatively for 2018), level input field (positively for high level) and maturity stage (negatively for the late harvest). The most satisfying results were attained using a stepwise regression algorithm (R² = 0.48), improved by the introduction of fixed effects (R² = 0.55) and partial least square discriminant analysis (PLS-DA), which was assessed through the Matthew Correlation Coefficient (MCC) in validation (MCC = 0.57). Concluding, among the tested approaches, the use of linear regression after stepwise algorithm or the use of PLS could be of practical help for the farmers to the effective management of the ensiling process of maize plants, even though environmental conditions should be considered to improve the predictions.

HIGHLIGHTS
• The prediction of FQ at harvest would allow the farmer to tune up the ensiling process
• The prediction of FQ through the use of portable NIRs instruments was successful
• DM, starch and ADL were negatively related to FQ index

Introduction
In the context of dairy farming, efforts are being made to increasingly combine the production and economic needs of farmers with those of reducing the environmental impact, for greater sustainability (Britt et al. 2018). A possible unifying element between these needs is represented by the improvement of production efficiency (Connor 2015), which is however difficult to achieve due to the extreme variability that characterises the composition and quality of the ration, animals, environment and farm management. Feed quality, which includes nutrient composition (Krämer-Schmid et al. 2016), digestibility, absence of contamination (Driehuis 2013), fermentation quality (Kung et al. 2018) and even the homogeneity of the ration (Marchesini et al. 2020), can vary over time and between different batches of feed, silo-bunkers and even between different areas within the same silo-bunker (Gallo, Bertuzzi, et al. 2016).

To limit this variability and always guarantee the animals a high quality ration, it is necessary to frequently measure feed characteristics as recommended within the view of precision feeding (Wathes et al. 2008; Mottram 2016). In this regard, repeated measures are made feasible by the use of Near Infra-red spectroscopy (NIRs) technique, a type
of analysis that is more and more extensively adopted in agriculture (Fernández-Ahumada et al. 2008) for its low costs, high speed and ease of use and for the availability of portable devices which allow performing the analyses in real-time at field level (Marchesini et al. 2017; Harris et al. 2018). Among the ingredients used in the ration of dairy cows, maize silage is one of the most important (Grant and Adesogan 2018) and is characterised by a great variability both in composition and fermentation quality (Grant and Ferraretto 2018; Marchesini et al. 2019), which if not adequate, could reduce DM intake (DMI) and production (Gerlach et al. 2016), this study aimed to evaluate the best fitting multivariate approach, compared with the multiple linear regression, to predict the potential of maize silage fermentation quality based on the chemical composition of the fresh plant. This possibility would allow the farmer to comprehend the ensiling attitude of fresh maize plants at the time of harvest and decide how to tune up the ensiling process adopting suitable choices, such as by consciously delaying the harvesting period to reach maturity and the ideal composition (Filya 2004; Xie et al. 2012) or for example, by the proper choice of inoculants (Wilkinson and Davies 2013). Since the use of multiple parameters to assess the silage quality requires a multivariate approach, which has already been proved useful in characterising silage conservation methods, post-ensiling composition, digestibility, and fermentation traits as well (Gallo, Bertuzzi et al. 2016; Gallo, Giuberti et al. 2016), this study aimed to evaluate the best fitting multivariate approach, compared with the multiple linear regression, to predict the potential of maize silage fermentation quality based on the chemical composition of harvested fresh whole maize plants.

Materials and methods

Experimental design

Data were collected in the harvest season among three consecutive years (2016, 2017 and 2018) from maize cultivated in the Veneto Region (Northeast Italy), using a total of 37 maize hybrids of early (FAO class 200, n = 19) and late (FAO class 600–700, n = 18) ripening classes, as reported: in 2016, 14 early and 15 late; in 2017, seven early and eight late; in 2018, five early and five late. The average yield per hectare was 23.5, 22.3 and 24.0 tons for the years 2016, 2017 and 2018, respectively. In 2016 each hybrid was harvested in three plots, corresponding to three areas (level input field) defined by different pedoclimatic characteristics. Each area was characterised by an average yield that could be referred to as soils of ‘low’ (medium-heavy soil with an average production of 496 q/ha), ‘medium’ (medium-light soil with an average production of 543 q/ha) and ‘high’ fertility (clay soil with an average production of 686 q/ha). In 2017 and 2018, the trial was performed only in ‘medium’ and ‘high’ productivity plots. Per each plot, every hybrid was harvested twice in two different subplots. Each hybrid was sown in both subplots in a land area of 4 rows × 10 plants. In total, 40 plants were grown per hybrid and subplot. In the main plot field, some external rows of generic seeds have been sown and excluded from the trial. In the core of the field, early and late hybrid were sown at precise densities to maximise the production, corresponding to 95,000 and 70,000 plants/ha, respectively. Corn silage was manually harvested at a stubble height of approximately 20 cm, and chopped to a theoretical length-of-cut of 20.0 mm by the means of a self-propelled forage harvester. Per each plot and subplot, plants were harvested at three maturity phases: 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). Per each plot, subplot and maturity phase, about five plants were harvested, chopped and mixed to obtain one sample. Each sample was split into two subsamples. Any processing and NIRs analysis were executed with two scans on subsamples, but averages of scans and subsamples were performed before statistical analysis was performed.

The number of samples collected per each year was 522 (29 hybrids × 3 plots × 2 subplots × 3 maturity phases), 180 (15 hybrids × 2 plots × 2 subplot × 3 maturity phases) and 120 (10 hybrids × 2 plots × 2 subplots × 3 maturity phases), for 2016, 2017 and 2018, respectively.

Fixed effects considered were: growing area (3 levels), maturity phase (3 stages), ripening class (2 classes) and year (3 years).

Sample collection, preparation and analysis

To prevent possible modifications resulting from respiration activity, pre-ensiling subsamples were
immediately collected after harvesting and placed in a large vessel, which could hold about 1.5–2.0 kg of fresh product. Each subsample was scanned twice using a portable NIRs system (poliSPECNIR, ITPhotonics, Breganze, Italy), and each scan was of 10 s (with an integration time of about 10 ms). Thus, the within-sample variability was mostly acquired. Subsamples \((n = 1644)\) were suddenly ensiled in vacuum-packed bags (Orved 2633040, Orved SpA, Musile di Piave, VE, Italy) as described by Andrighetto et al. (2018) and stored in a dark room at 23°C for 60 days (Marchesini et al. 2019). Post-ensiling subsamples were scanned twice by the use of a FOSS NIRSetup 5000 scanning monochromator (FossNIR-System, Hillerød, Denmark) and predicted using the calibration curve as previously reported by Andrighetto et al. (2018). From the analysis results, a fermentation quality index (FQI) was calculated according to what was reported by Andrighetto et al. (2018) for quality index I1. The FQ index is a lab-scale quality index designed for the evaluation of maize silage fermentation results. The FQI was intended to evaluate the maize silage fermentation quality upon weighted scores assigned according to the main fermentative parameters’ values. The FQI index has a range of values from 1 to 100, where all the samples higher than a cut-off limit set at 57.4 (Andrighetto et al. 2018), were classified as excellent quality (EQ), while the remaining were considered as not excellent quality (NEQ), obtaining a classification variable for FQI (FQIc).

### NIRs instruments and calibrations

A poliSPECNIR Near Infra-red system, together with a robust calibration curve was used to analyse DM, ash, crude protein (CP), ether extract (EE), neutral detergent fibre (aNDF), acid detergent fibre (ADF), acid detergent lignin (ADL), WSC and starch on fresh harvested pre-ensiling subsamples. Calibration performances are reported in Table 1. A FOSS NIRSystem 5000 scanning monochromator with the same calibration described by Andrighetto et al. (2018), built with the use of a large dataset \((n = 2098)\), was used to analyse post-ensiling subsamples. The reference methodologies used to calibrate both the NIRs instruments were: #934.01 (AOAC 2003) for DM, 2001.11 (AOAC 2005) for CP, #942.05 (AOAC 2003) for ash; #996.11 (AOAC 2000) for starch, 2003.05 (AOAC 2006) for EE; ANKOM Technology (2015a) for aNDF; ANKOM Technology (2015b) for ADF; Akon-Technology (2020) for ADL; and Charles (1981) for WSC. The ammonia was analysed according to Megazyme’s assay procedure (Megazyme 2014) and pH as proposed by Martillotti et al. (1987) methods 1.5–18, and Playne and McDonald (1966). The volatile fatty acids and lactate, ethanol and mannitol were examined according to the method proposed by Martillotti and Puppo (1985) and described in Andrighetto et al. (2018).

Table 1. Specifications of the calibration curve used for fresh harvested pre-ensiling maize \((n = 205)\), by the use of a poliSPECNIR near infrared system.

| Constituents (g/kg DM) | Mean \((X)\) | Standard deviation \((SD)\) | Standard error of calibration \((SEC)\) | \(R^2\) |
|-----------------------|--------------|-----------------------------|--------------------------------------|--------|
| DM (g/kg)             | 357          | 54.6                        | 19.8                                 | 0.93   |
| Ash                   | 39.6         | 5.88                        | 3.47                                 | 0.82   |
| Crude protein         | 72.4         | 13.0                        | 7.67                                 | 0.82   |
| Ether extract         | 24.6         | 8.99                        | 5.91                                 | 0.77   |
| aNDF                  | 424          | 75.6                        | 28.1                                 | 0.93   |
| ADF                   | 225          | 40.5                        | 16.5                                 | 0.92   |
| ADL                   | 23.4         | 5.46                        | 4.89                                 | 0.48   |
| Water-soluble carbohydrates | 65.1 | 26.3                        | 17.4                                 | 0.77   |
| Starch                | 267          | 93.3                        | 43.1                                 | 0.89   |

\(\text{X}\): Coefficient of determination.

DM: Dry matter; aNDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin.

### Statistical analysis

In this study, the authors tested many different multivariate approaches by the use of different software for data computation. Thought approaches are intended to describe the pre-ensiling dataset and the relation of the latter traits with the FQI and FQIc. The post-ensiled dataset is presented merely for the calculation of the FQI and to describe the traits of the silages. A resuming graphical scheme for multivariate approaches is reported in the Supplementary Material Figure 1.

### Preliminary investigation

A preliminary investigation was conducted on the proximate composition database of both pre-ensiling and post-ensiling subsamples, by the use of R (R version 3.5.3 (2019-03-11)). To assess the normality of the data the Shapiro–Wilk test \((>0.9 = \text{normally distributed})\) was applied. The same datasets were submitted to a descriptive analysis to evaluate Minimum, Maximum, Mean and Standard Deviation values. With the use of SAS release 9.4 (SAS Institute Inc., Cary, NC
2012), an ANOVA model was applied including hybrid ripening class (two levels: early and late), input level fields (three levels: low, medium and high), maturity phase at harvest (three levels: EH, MH, and LH) as fixed effects. Post-hoc pairwise comparisons were run between factor levels using Bonferroni correction. The assumptions of the linear model on the residuals were graphically tested.

As a preliminary investigation of the pre-ensiled traits dataset and its relations with the quality outcomes, a graphical plot of the feature space is useful to understand its structure. Principal Component Analysis (PCA) is an unsupervised technique merely considering the link between the predictors, without considering any classification factor. PCA permits, by the calculation of an orthogonal transformation, a preliminary exploration of data (Kim and Kim 2012), decreasing the original pool of correlated variables into a reduced number of uncorrelated components. PCA allows increasing the dataset interpretability with a few losses of information (Jolliffe et al. 2016). One of the significant advantages of PCA is the possibility to accomplish a biplot graphical representation of the multivariate dataset. Data were submitted to the pre-process ‘centering’ and ‘scaling.’

**Features selection**
The Authors applied a features selection to identify the most predictive variables able to discriminate between the FQIc or to estimate FQI continuous variables.

Random Forest is a popular ML algorithm characterised by a typically easy interpretability. Unlike PCA, the Random Forest considers the information between feature values and the target class. Moreover, the Random Forest does not require the assumption of normality. From another point of view, it is useful to select the relevant attributes to solve the problem of finding the only relevant ones, instead of merely the non-redundant. One of the primary objectives of RF is to avoid a decrease in accuracy when too many variables are present, and their number is significantly higher than optimal (Kursa 2010).

A Random Forest (RF) feature selection procedure, based on the Boruta algorithm (Kursa 2010), was applied to pre-ensiling corn composition parameters. Such algorithms operate through a wrapper approach built around a Random Forest classifier, in order to achieve the best discrimination for the FQIc, by maximising the distance among EQ Vs. NEQ groups.

**Regression algorithms**
The use of ‘createDataPartition’ of ‘caret’ package (Kuhn 2008) in R splits the original pre-ensiling dataset in training (training = 70%) and testing (testing = 30%), keeping the proportion of the FQIc classes, for further utilisation.

A stepwise algorithm, with the use of SAS (PROC REG), performed as a feature selection procedure applied to pre-ensiling corn composition parameters, allowed to estimate linear regression relating the predictors to the FQI variable. Such a stepwise algorithm, computed on the training dataset, has furtherly been validated with the testing dataset. The coefficient of determination ($R^2_v$) and the standard error ($SE_v$) in validation were calculated as indicators of the goodness of the prediction procedure, applying the linear regression coefficient, estimated in the training set, to the samples of the testing dataset. To evaluate the effects of harvesting conditions, a ‘reduced’ dataset with only three hybrids ($n = 1$ early, $n = 2$ late), harvested in the 3 years (2016, 2017 and 2018), and the two fields (high and medium input field), in total 108 subsamples, was evaluated for FQI in the STEPSWISE algorithm. The fixed effects were previously converted to dummy variables.

Partial Least Square regression (PLS) is a technique that combines the feature selection, as for the PCA, and the computation of a linear regression algorithm (Cui and Fearn 2017). Moreover, PLS allows good data interpretability, such as by the use of the Variable Importance in Projection (VIP) (Mehmood et al. 2012; Ottavian et al. 2015). A PLS was computed by the use of Matlab R2017a (software V9.2.0.538062, The MathWorks Inc., Natick, MA) and PLS Toolbox (PLS Toolbox V5.8.2.1, Eigenvector Research Inc., Manson WA) in the pre-ensiling dataset and evaluated by cross-validation (Venetian blinds w/10 splits and two samples per split), for the predictions of the FQI. The coefficient of determination in cross-validation ($R^2_{cv}$) and the standard error in cross-validation ($SE_{cv}$) measured the reliability of the regression method on the training dataset and, furtherly, tested on the testing dataset ($R^2_v = \text{coefficient of determination in validation; } SE_v = \text{standard error in validation}$), by comparing the Predicted Vs the Actual FQI values. The number of the latent variable used to perform the PLS was chosen by minimising the Root Mean Square Error in Cross-Validation ($RMSE_{cv}$). Variable importance in projection (VIP) index was calculated to underline the most critical parameters in the regression (Ottavian et al. 2014).
Classification algorithms
Using a preliminary stepwise discriminant analysis (PROC STEPDISC, SAS) performed as a features selection algorithm, a canonical discriminant analysis (PROC CANDISC, SAS) was applied as a classification algorithm for FQIc in the dataset (822 samples, 25 incomplete records removed). The predictive variables used were the pre-ensiling maize composition parameters. In this case, three classes for FQIc were calculated, and the thresholds were chosen as silages with FQI < 47.7 are classified as "lower" quality silage, silages whit 47.7 ≤ FQI < 57.4 are "good" quality silage, and for those with FQI ≥ 57.4 are classified as 'excellent' quality silage. The use of Wilks’ λ and approximately F evaluated performances of the algorithm, as well as the squared Mahalanobis distances (D2-Mahalanobis), showed the degree of dissimilarity among FQI classification (Bisutti et al. 2019). Outcomes of the canonical discriminant analysis (CDA) are plotted according to the two main canonical functions CAN1 and CAN2. For each sample, the Mahalanobis Distances from the coordinates of the geometric centre of each group (lower, good, excellent) were calculated, and as a result, three values per sample were obtained. The lower of the three values indicated the assigned group. In such a way, it was built a confusion matrix. As for stepwise regression, the performance of the approach in CDA for FQIc was tested in a reduced dataset (101 samples, 7 incomplete records removed).

Partial Least Square Discriminant Analysis (PLS-DA) (Barker and Rayens 2003) is a chemometric classification based on the PLS algorithm. PLS-DA is an adaptation of PLS regression methods to solve the problems of supervised clustering, used to sharpen the separation between groups of observations. Using MATLAB and PLS Toolbox, PLS-DA for FQIc, after autoscaling of data, was evaluated by cross-validation (Venetian blinds w/10 splits and 2 samples per split) on the training dataset and furtherly tested on the testing dataset. The PLS-DA model was evaluated by the sensitivity, specificity, accuracy, precision, and Matthews Correlation Coefficient (MCC) as they are defined in (Bisutti et al. 2019).

Machine learning approaches
Referring to the pre-ensiling dataset and using the aforementioned classification for FQIc, the Machine Learning technique was tested.

The Support Vector Machine (SVMs) is a powerful technique for classification (James et al. 2013). An SVMs was modelled by the use of package ‘caret’ (Kuhn 2008) on R software, through both the svmLinear and svmRadial kernel, and applied on the training dataset with repeated cross-validation (setting in number = 10 and repeats = 5). C-value (Cost) in the Linear classifier was customised by inputting values in a grid search. Even testing the Radial Basis Function sigma was customised, adopting a grid search. Before training the SVMs model, both pre-process ‘centering’ and ‘scaling’ were applied to the training dataset. After training the SVMs model, a validation was explored on the testing dataset by the use of the ‘predict method’ for calculating results. A confusion matrix allowed evaluating the accuracy, sensitivity, specificity and MCC of the method.

K-Nearest Neighbours (KNN) is a classification method whose principle is to predict a class for a given test observed by attributing the class of the K-nearest observed sample (James et al. 2013). The idea behind KNN is to identify the closest K point in the training dataset to the tested sample, where K point is a positive integer. Finally, KNN classifies the tested sample to the class with the most considerable probability. In R, with the use of the package ‘caret’, the KNN method was tested after applying ‘centering’ and ‘scaling’ to the training dataset, with repeated cross-validation (setting number = 10 and repeats = 5). After training the KNN, the model was applied to the testing dataset by the use of the ‘predict method’ to obtain the results. A confusion matrix is allowed to evaluate the accuracy, sensitivity, specificity and MCC of the method.

Results
Dataset
The traits of the pre-ensiling and post-ensiling datasets are reported in Tables 2 and 3. Datasets included the results of analyses made on whole fresh maize plants harvested in the years 2016 (Marchesini et al. 2019), 2017 and 2018 (Table 2) and on post-ensiling samples (Table 3). All parameters, except for glucose, propionic and butyric acids, have a Shapiro test (w) value higher than 0.9. To better describe the dataset, the ANOVA analysis for FQIc is reported in Table 4. The main fixed effects (years, level input field and maturity) are significant.

Preliminary investigation
The proximate composition of the fresh whole corn plant was submitted to an ANOVA including FQIc (EQ Vs. NEQ) as a fixed effect (Table 5). It appears that all chemical parameters significantly differ for the two
### Table 2. Specifications of the fresh whole corn plant, pre-ensiling dataset.

| Variables (g/kg DM)     | Shapiro test (w) | Minimum | Maximum | Mean (X) | Standard deviation (SD) | Coefficient of variation (CV) (%) |
|-------------------------|-----------------|---------|---------|----------|--------------------------|----------------------------------|
| DM (g/kg)               | 0.97            | 238     | 552     | 352      | 53.8                     | 15.3%                            |
| Ash                     | 0.98            | 30.8    | 59.3    | 38.9     | 3.70                     | 9.51%                            |
| Crude protein           | 0.98            | 59.4    | 81.8    | 67.1     | 2.86                     | 4.26%                            |
| Ether extract           | 0.98            | 13.6    | 32.9    | 23.7     | 3.09                     | 13.0%                            |
| aNDF                    | 0.99            | 376     | 515     | 438      | 22.8                     | 5.20%                            |
| ADF                     | 0.99            | 181     | 281     | 228      | 18.9                     | 8.27%                            |
| ADL                     | 0.98            | 13.0    | 34.0    | 22.3     | 3.84                     | 17.2%                            |
| Starch                  | 0.99            | 173     | 375     | 281      | 37.4                     | 13.3%                            |
| Water-soluble Carbohydrates | 1.00       | 6.90    | 92.7    | 51.9     | 12.9                     | 24.8%                            |

DM: Dry matter; aNDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin.

### Table 3. Specifications of the post-ensiled dataset.

| Variables (g/kg DM)     | Shapiro test (w) | Minimum | Maximum | Mean (X) | Standard deviation (SD) | Coefficient of variation (CV) (%) |
|-------------------------|-----------------|---------|---------|----------|--------------------------|----------------------------------|
| DM (g/kg)               | 0.97            | 230     | 544     | 342      | 53.0                     | 15.5%                            |
| Ash                     | 0.98            | 26.0    | 46.6    | 33.9     | 3.44                     | 10.2%                            |
| Crude protein           | 1.00            | 55.4    | 92.3    | 72.3     | 5.91                     | 8.17%                            |
| Ether extract           | 0.98            | 8.40    | 31.6    | 21.9     | 3.69                     | 16.9%                            |
| aNDF                    | 0.98            | 327     | 496     | 399      | 31.3                     | 7.85%                            |
| ADF                     | 0.98            | 160     | 285     | 211      | 43.6                     | 13.6%                            |
| ADL                     | 0.99            | 13.0    | 34.0    | 22.3     | 3.84                     | 17.2%                            |
| Water-soluble Carbohydrates | 0.97       | 0.20    | 13.8    | 5.24     | 2.23                     | 42.5%                            |
| pHa                     | 0.99            | 3.56    | 4.18    | 3.83     | 0.09                     | 2.25%                            |
| Ammonia                 | 0.99            | 3.93    | 7.85    | 5.68     | 0.67                     | 11.7%                            |
| Glucose                 | 0.83            | 0.83    | 11.6    | 3.00     | 1.93                     | 64.5%                            |
| Fructose                | 0.98            | 0.00    | 5.40    | 2.24     | 0.93                     | 41.3%                            |
| Mannitol                | 0.92            | 1.30    | 29.1    | 11.1     | 4.48                     | 40.2%                            |
| Ethanol                 | 0.97            | 0.40    | 32.0    | 10.3     | 5.04                     | 49.0%                            |
| Lactic acid             | 0.99            | 24.1    | 99.2    | 49.4     | 10.4                     | 21.0%                            |
| Acetic acid             | 0.96            | 2.40    | 28.4    | 11.1     | 4.34                     | 39.3%                            |
| Propionic acid          | 0.77            | 0.00    | 4.70    | 0.55     | 0.68                     | 124%                             |
| Butyric acid            | 0.62            | 0.10    | 2.50    | 0.72     | 0.41                     | 56.8%                            |

*Expressed as a pure number.

### Table 4. Fermentation quality index (FQI) of maize silage belonging to different FAO classes (class 200 vs. class 600–700), grown in different yield potential areas (low vs. medium vs. high) and harvested at different Maturity (early, EH vs. medium, MH vs. late, LH).

| Year | Level input field | 2016 | 2017 | 2018 | p |
|------|-------------------|------|------|------|---|
| FQI value | Average | 54.4b | 57.1a | 43.4c | <.0001 |
|        | Minimum | 32.2 | 28.7 | 29.7 | |
|        | Maximum | 84.6 | 81.5 | 63.0 | |
| SEM | 0.33 | 0.59 | 0.83 | |
| Interaction years | p | p | p | Interaction level input field | p | p | p |
| Year | | | | | <.0001 | <.0001 | only 2016 |
| Level input field | <.0001 | <.0001 | 0.0005 | |
| Class cultivar | 0.1039 | <.0001 | 0.0102 | |
| Maturity | <.0001 | <.0001 | <.0001 | |

| Class cultivar | Maturity | Early (200) | Late (600–700) | p |
|----------------|----------|-------------|----------------|---|
| FQI value | Average | 51.7 | 51.5 | 0.71 | |
|        | Minimum | 31.1 | 28.7 | |
|        | Maximum | 84.6 | 77.3 | |
| SEM | 0.41 | 0.42 | |
| Interaction class cultivar | p | p | Interaction maturity | p | p | p |
| Year | <.0001 | <.0001 | <.0001 | |
| Level Input Field | <.0001 | <.0001 | <.0001 | |
| Class Cultivar | <.0001 | <.0001 | <.0001 | |
| Maturity | <.0001 | <.0001 | |

*Standard error of means.
SEM: Standard Error of Means.

a,b,cMean values within a row bearing different superscripts differ significantly (p < .05).
levels of FQIc. Ash, CP, aNDF, ADF and WSC show higher values in EQ, whereas the remaining parameters show higher values in NEQ.

Figure 1 reports the biplot of the PCA loadings. By visual inspection of PCA loadings biplot and regarding FQc, EQ coordinates are nearby to ash, aNDF and WSC loadings coordinates. The total variance explained by the two main components accounts for 72%.

Features selection
In this study, as a result of applying RF for FQIc, all nine components are confirmed as necessary, and the

Table 5. Composition of the fresh whole plant maize belonging to different FQI classes (NEQ, EQ).

| Variables (g/kg DM)     | NEQ, FQI < 57.4 | EQ, FQI ≥ 57.4 | P       |
|-------------------------|-----------------|----------------|---------|
| DM (g/kg)               | 36.5a           | 32.3b          | <0.0001 |
| Ash                     | 3.79b           | 4.05a          | <0.0001 |
| Crude protein           | 6.67b           | 6.77a          | <0.0001 |
| Ether extract           | 2.42a           | 2.18b          | <0.0001 |
| aNDF                    | 43.1b           | 44.4a          | <0.0001 |
| ADF                     | 22.4b           | 23.3a          | <0.0001 |
| ADL                     | 2.20a           | 2.09b          | 0.0002  |
| Water-soluble carbohydrates | 5.01b        | 5.48a          | <0.0001 |
| Starch                  | 29.2a           | 25.8b          | <0.0001 |

Table 5. Composition of the fresh whole plant maize belonging to different FQI classes (NEQ, EQ).

| Variables (g/kg DM)     | NEQ, FQI < 57.4 | EQ, FQI ≥ 57.4 | P       |
|-------------------------|-----------------|----------------|---------|
| DM (g/kg)               | 36.5a           | 32.3b          | <0.0001 |
| Ash                     | 3.79b           | 4.05a          | <0.00001|
| Crude protein           | 6.67b           | 6.77a          | <0.0001 |
| Ether extract           | 2.42a           | 2.18b          | <0.0001 |
| aNDF                    | 43.1b           | 44.4a          | <0.0001 |
| ADF                     | 22.4b           | 23.3a          | <0.0001 |
| ADL                     | 2.20a           | 2.09b          | 0.0002  |
| Water-soluble carbohydrates | 5.01b        | 5.48a          | <0.0001 |
| Starch                  | 29.2a           | 25.8b          | <0.0001 |

*Fermentative qualitative index (FQI).
NEQ: Not excellent quality; EQ: Excellent quality; DM: Dry matter; aNDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin.
Mean values within a row bearing different superscripts differ significantly (p < .05).

Regression algorithms
The previous investigation did not identify clearly the most useful variables for predicting FQIc or FQI. Conversely, RF suggests that all features should be used.

Stepwise regression in SAS was performed within the training set (Table 6) and then validated on the testing set. FQI regression used a negative coefficient for DM, ADL and starch and a positive coefficient for Ash, EE and aNDF, \( R^2 = 0.48 \). In validation, FQI produced an \( R^2_v = 0.42 \).

Regression coefficients for the reduced dataset are reported in Table 6; fixed effects (growing area, maturity phases and years) resulted statistically significant in the regression.

Using MATLAB and PLS Toolbox, a PLS regression for FQI was calculated on the training set, X data were autoscaled. Regression results were \( R^2_{cv} = 0.44 \) and \( SE_{cv} = 7.11 \). Selecting 4 PC (captured variance for PC1 = 49.7%, for PC2 = 7.93%, PC3 = 7.93% and PC4 = 4.49%) and applying the regression to the testing set, resulted in \( R^2_v = 0.42 \) and \( SE_v = 7.26 \).

Variables with VIP > 1, considered significant, are DM (1.40), Ash (1.01), EE (1.21) and Starch (1.25).
Regression coefficients were DM = −0.53, Ash = 0.005, EE = 0.003, Starch = −0.092.

**Classification algorithms**

A CDA allowed a Can1 and Can2 bi-plot chart (Figure 2(A)). The STEPDISC procedure selected DM, Ash, CP, ADL and Starch for FQIc. The canonical functions showed a low discriminative power (Wilk’s λ = 0.60 with $p < 0.0001$, approximate $F$ value = 45.5 and 1.87, df1 = 10 and 4, df2 = 1580 and 791, $p < 0.0001$ and 0.114 for Can1 and Can2, respectively). D2-Mahalanobis distance between means = 1.35, 4.33 and 0.91, $p < 0.0001$ for comparison of classes lower vs. Good, lower vs. Excellent and good Vs. Excellent, respectively. The Can1 accounted for 98.6% and Can 2 accounted

Table 6. Results of stepwise regression of constituents for FQI: estimated regression coefficients, intercept and p ($R^2 = 0.48$ for the training dataset and 0.55 for reduced dataset).

| Constituents (g/kg DM)         | FQIa training dataset coefficients | p   | FQIa reduced dataset coefficients | p    |
|-------------------------------|-----------------------------------|-----|----------------------------------|-----|
| DM (g/kg)                     | −1.10                             | <0.0001 | 20.5                           | 0.0181 |
| Ash                           | 6.60                              | 0.001 |                                  |      |
| Ether extract                 | 19.2                              | 0.0002 |                                  |      |
| aNDF                          | 0.72                              | 0.0301 |                                  |      |
| ADL                           | −15.9                             | <0.0001 |                                  |      |
| Starch                        | −0.76                             | 0.0611 | −1.89                           | 0.0043 |
| WSC                           | 1.44                              | 0.0473 |                                  |      |
| The year = 2018               | v10.6                             | <0.0001 |                                  |      |
| Maturity phases = late (EH)   | −3.56                             | 0.0364 |                                  |      |
| Level input field = ‘high’    | 3.75                              | 0.0058 |                                  |      |
| Intercept                     | 45.1                              | 0.0004 | 47.5                            | <0.0001 |

*aFermentative qualitative index (FQI).

$R^2$: Coefficient of determination; FQI: Fermentative qualitative index; DM: Dry matter; aNDF: Neutral detergent fibre; ADL: Acid detergent lignin; WSC: Water-soluble carbohydrates.

Figure 2. Scatter plot for the outcomes of the CDA (Can1 and Can2) for the samples of the original dataset, considering the three classes for FQIc. (A) Green filled area = ‘lower’; red filled area = ‘good’; blue filled area = ‘excellent’. (B) Green filled area = ‘lower’; red filled area = ‘good or excellent’. Ellipses represent a 95% confidence interval.
For 1.40% of the variability. For FQIc 69, 46 and 69% of correct attributions were for the three-class FQI lower, good and excellent silage, respectively (Table 7).

However, as many samples are confused between 'excellent' and 'good' classes, they may be joint in one 'overall' class that can be opposed to the 'lower silage' class (Figure 2, panel B). In the latter case, correct attributions were 69 and 83% for 'lower silage' and 'overall' classes for FQIc, respectively.

The reduced dataset was used to evaluate the fixed effects in the CDA for FQIc. For FQIc, Can1 accounted for 95.7% and Can2 accounted for 4.30% of the variability (Figure 3). The selected features were Ripening Classes, Year, CP, WSC and Starch. The confusion matrix is reported in Table 7, and 95, 45 and 13% of correct attributions were found for the three-class FQIc lower, good, and excellent silage, respectively. For the reduced dataset, the correct attribution was 95%, and 57% for 'lower silage' and 'overall' classes, respectively.

**Table 7.** Confusion matrix for CDA in the original dataset (n = 797, 25 incomplete records removed) and in the reduced dataset (n = 101, 7 incomplete records removed) for FQIc, classified in three levels.

| Actual Class       | FQI$^a$ < 47.7 | 47.7 $\leq$ FQI$^a$ < 57.4 | FQI$^a$ $\geq$ 57.4 |
|--------------------|----------------|----------------------------|---------------------|
| Predicted as FQI < 47.7 | 153            | 84                         | 14                  |
| Predicted as 47.7 $\leq$ FQI < 57.4 | 59             | 148                        | 64                  |
| Predicted as FQI $\geq$ 57.4 | 9              | 93                         | 173                 |

| Sensitivity | 0.69 | 0.46 | 0.69 |
| Specificity | 0.83 | 0.74 | 0.81 |
| Accuracy    | 0.79 | 0.62 | 0.77 |
| Precision   | 0.61 | 0.55 | 0.63 |
| MCC$^b$     | 0.55 | 0.35 | 0.55 |

$^a$Fermentative qualitative index (FQI); $^b$Matthew Correlation Coefficient (MCC).

PLS-DA resulted in FQIc with VIP $> 1$ for DM (1.38), Ash (1.03), EE (1.27) and Starch (1.31). Regression coefficient for NEQ, were DM = $-0.37$, Ash = 0.02, EE = 0.06, Starch = 0.11 with selected four PCs (captured variance for PC1 = 49.5%, for PC2 = 21.3%, PC 6.53% and PC4 = 5.65%). In Table 8, are reported the confusion matrix for PLS-DA in validation (testing set): 80.9 and 78.2.0% were correctly predicted for NEQ, FQI $< 57.4$ and EQ, FQI $\geq 57.4$, respectively.

**Figure 3.** Scatter plot for the outcomes of the CDA (Can1 and Can2) for the samples of the reduced dataset, in the three classes for FQIc. (A) Green filled area = “lower”; red filled area = “good”; blue filled area = “excellent”. Ellipses represent a 95% confidence interval.

**Machine learning approaches**

Support vector machine (SVM) algorithm was tested for linear and radial basis function, by selecting the c (cost) function and sigma by the use of a grid. Best performances were reached by the linear kernel, C = 0.75 which correspond accuracy = 0.78 and Kappa = 0.45, in cross-validation. The confusion matrix was calculated in the testing set (Table 8), where the 93.0...
and 46.0% of true positive are correctly predicted for NEQ, FQI < 57.4, and EQ, FQI ≥ 57.4, respectively.

By the use of the KNN algorithm, for FQI classification, the better cross-validate choice in the training set was K-point = 13, corresponding to accuracy = 0.78 and k = 0.43. In Table 8, we report the confusion matrix for the testing set in FQI classification. The percentage of the true positive was 95 and 48% for NEQ, EQ, respectively, while MCC > 0.54.

Table 8. confusion matrix for PLS-DA, SVMs and KNN algorithms, validated on the testing set of FQIc.

| Predicted as Actual Class | NEQ<sub>c</sub>, FQI<sub>a</sub> < 57.4 | NEQ<sub>c</sub>, FQI<sub>a</sub> ≥ 57.4 |
|---------------------------|------------------|------------------|
| Predicted as NEQ<sub>c</sub>, FQI<sub>a</sub> =< 57.4 | 119 | 15 | 137 | 37 | 140 | 36 |
| Predicted as EQ<sub>d</sub>, FQI<sub>a</sub> =< 57.4 | 28 | 54 | 10 | 32 | 7 | 33 |

| Sensitivity | NEQ<sub>c</sub>, FQI<sub>a</sub> =< 57.4 | NEQ<sub>c</sub>, FQI<sub>a</sub> ≥ 57.4 |
|-------------|------------------|------------------|
| Specifity   | 0.81 | 0.78 | 0.93 | 0.46 | 0.95 | 0.48 |
| Accuracy    | 0.78 | 0.76 | 0.46 | 0.93 | 0.48 | 0.95 |
| Precision   | 0.75 | 0.81 | 0.78 | 0.76 | 0.8 | 0.83 |
| MCC         | 0.89 | 0.66 | 0.79 | 0.76 | 0.8 | 0.83 |

*Fermentative Qualitative Index (FQI); *Matthew Correlation Coefficient (MCC); *not excellent quality (NEQ); *excellent quality (EQ); Partial Least Square Discriminant Analysis (PLS-DA); Support Vector Machine (SVMs); K-Nearest Neighbours (KNN).

Stepwise regression recognises DM, ADL and starch as the predictive variables with negative coefficients, and ash, EE, and aNDF with the positive coefficient, confirming the ones obtained by PCA. Hence, pre-ensiling traits taken as indexes of the maturity stage of the plant (Ferraretto et al. 2018) are negatively related to FQI indexes. These results are underlying that the harvest at an early maturity stage leads to better ensiling processes. This finding confirms the results from other authors (Johnson et al. 2003; Neylon and Kung 2003; Arriola et al. 2012) where fresh maize plants ensiled at different DM content resulted in higher lactic acid content when harvested early (lower DM content). These responses were attributed to the combination of low WSC concentrations in late-harvested maize (Johnson et al. 2003) and reduction in the growth of lactic acid bacteria because water activity decreased along with the increasing dryness as the forage matures (Neylon and Kung 2003). The regression led to discrete performances for FQI, \( R^2_v = 0.42 \); this result could be affected by the interference of several factors influencing the ensiling processes, such as year (Kruse et al. 2008; Loućka et al. 2015). The potential influence of the above-mentioned factors is confirmed by the increase of predicting performance (\( R^2_v = 0.55 \)) attained after the introduction of some fixed factors (growing area, maturity phase, ripening class and year) in the model applied to the reduced dataset.

PLS produces results comparable to those of stepwise regression, both in the cross-validation and validation of FQI. Using classes instead of continuous variables could simplify the calculation. The CDA resumed the variance in Can1 and Can2, but \( R^2 \) between Can1 and Can2 Vs. CLASS variable was weak (Can1 = 0.39 and Can2 = 0.009 for FQI classification). The CDA performed with the use of fixed-effects confirmed the usefulness of Ripening Classes and Year on the FQIc.

On the contrary, PLS-DA performed good results, with 80.9 and 78.2% of correct predictions for NEQ.
and EQ, respectively. VIP for the PLS-DA algorithm shows as the most related variable to FQIc: DM, Ash, EE and Starch.

SVMs improved the ability of discrimination, with 93.0 and 46.0% of true positives which are correctly predicted for EQ and NEQ, respectively. Finally, the K-Nearest Neighbours confirmed to be a secure and robust classifier method with MCC > 0.54 for FQIc.

In this study, the FQI was greater for samples harvested at 1/3 milk line maturity phase than in later maturity phases. These findings confirm the relevance of the maturity phase at harvest, which is related to the plant nutrients, such as WSC content (Johnson et al. 2003) and affects silage fermentation characteristics determining silage final pH and aerobic stability, through the differentiated production of acetic and other organic acids (Filya, 2004). The FQI value (accordingly to FQIc class) is influenced by the role of some pre-ensiled traits as shown by this study (ANOVA, PCA, feature selection and CDA) with negative coefficients for DM and starch, and positive coefficients for aNDF. These traits, and their variation are indicators of the maturity stage of the maize plant (Johnson et al. 2002; Jensen et al. 2005) and contribute to the definition of silage quality in terms of DM, CP, starch, fibre content and nutrients digestibility (Kuehn et al. 1999; Addah et al. 2011; Krämer-Schmid et al. 2016). In our findings, the influence of the crop year, level input field and maturity phase in the FQI value have been shown by the ANOVA as well as by their relevance in the multiple regression or CDA algorithms. Anyhow, other several factors not considered in this study, such as genetics, plant ecophysiology, and different agro-techniques, including seeding density, fertilisation, meteorological conditions, might affect the final fermentation quality (Berzsenyi and Dang 2008; Sárvári and Pepó 2014) together with the ensiling procedures such as the mechanical processing, the use of inoculants and additives, and the packing density (Johnson et al. 2002; Johnson at al. 2003).

Conclusions

NIRs technique applied through the use of portable instruments was confirmed to be an essential tool to apply the precision feeding strategy to the assessment of the composition of maize plants intended for ensiling. The prediction of the fermentation quality, summarised through the FQI index, starting from the composition of the plant at harvest was successful, even though it was evident that the inclusion of data on growing and harvesting conditions can improve the prediction capacity. Among the traits of the fresh maize plant DM, starch and ADL appeared to be negatively related to FQI whereas aNDF and WSC were positively related to FQI. This finding, obtained by the multivariate approaches, might be relevant to focus on the desired improvement in the maize selections, agronomic techniques and ensiling process. Among several algorithms tested for the predictive or classification ability, performances were similar in overall used models but, due to its simplicity in the application and to the fewer calculation requirements, the linear regression obtained after the stepwise procedure appeared to be the most convenient method, even because it might be easily enforced on harvesting machines. Moreover, the use of a stepwise procedure before the linear regression would reduce the risk of multicollinearity of predictive variables and reduce the optimism in the results, as demonstrated by the likeness of the determination coefficient obtained in calibration and validation. Conversely, for research purposes, the tested multivariate approaches allow to a deeper description and knowledge of the dataset and of the relationships within the predictive variables and among them with the outcomes. In practical terms, the multivariate approaches would drive to better prediction performances using a larger number of predictive variables which drive to more selectable features and latent variables. Nevertheless, the unexplained variability for the better tested algorithms in regression was 45%, which let to assume that other predictors as well as the sample variability within the field must be furtherly considered for more consistent results. As the application of technologies in the precision feeding sector is in strong expansion and continuous evolution, further research on this topic is needed.

Disclosure statement

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