The Effect of Time and Method of Storage on the Chemical Composition, Pepsin-Cellulase Digestibility, and Near-Infrared Spectra of Whole-Maize Forage

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The Effect of Time and Method of Storage on the Chemical Composition, Pepsin-Cellulase Digestibility, and Near-Infrared Spectra of Whole-Maize Forage

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Featured Application: Predicting the nutritive value of forage relies on forage samples with in vivo values. Obtaining in vivo values is time-consuming because many samples are required. Sometimes, the samples used to develop prediction methods were obtained a long time ago and stored at sub-optimal conditions (temperature and humidity). Consequently, possible modifications to the sample composition under sub-optimal storage conditions can be a source of error when developing prediction methods for the forage's nutritive value. We found that changes in temperate forage samples after 29 years of storage did not modify their nutritive value. Samples stored under ambient temperatures over three decades did not modify their nutritive value compared to the samples stored and frozen at −20 °C.

Abstract: This study examined the effects of long-term storage conditions on the chemical composition, pepsin-cellulase dry matter digestibility (PCDMD), and visible (VIS)/near infrared spectra (NIR) of forage. Eighteen samples of different whole-crop maize varieties originally harvested in 1987 were used. After drying, these samples were analyzed in the laboratory for ash, crude protein (CP), structural carbohydrates, total soluble carbohydrates (TSC), starch and PCDMD, and the remaining samples were stored frozen (at −20°C) or at barn temperature (ambient temperatures ranged from −8.5 °C to 27.1 °C). In 2016, the samples were analyzed for ash, CP, structural carbohydrates, TSC, starch and PCDMD. The visible/NIR spectra of both storage methods were obtained. Chemical composition and PCDMD analyses revealed significant differences ($p < 0.05$) between the storage methods for TSC but not for the other parameters ($p > 0.05$). After sample harvesting in 1987, the analyses were compared with those in 2016. It was found that the post-harvest TSC and ash content were higher ($p < 0.05$) and lower ($p < 0.05$), respectively, during 2016. No significant differences were found for starch and PCDMD. Important differences between the VIS/NIR spectra of both storage methods were obtained in the VIS segment, particularly in the area between 630 and 760 nm. We concluded that storing dry forage samples at ambient temperature for a very long time (29 years) did not change their nutritive value compared to the values obtained before storage.

Keywords: whole-maize forage; long-term storage; storage method; chemical composition; nutritive value

1. Introduction

Ruminant performance is known to be dependent on forage nutritive value and intake. The determination of nutritive value is based on digestibility and degradability measurements, which, in combination with intake measurements, are considered to be the reference methods for determining the feed value of forage [1]. These in vivo feed evaluation methods are considered the gold standard and are generally used in research programs [2]. However, these methods are expensive, involve intensive animal studies, and, therefore, are not routinely used to evaluate the nutritive or feed values of forages for ruminants. Generally, in vivo feed evaluations are used as a reference to compare and estimate the nutritive value of forages using less expensive chemical/laboratory-based methods [3] or in vitro methods, including an estimation of in vitro digestibility [4–6] or visible (VIS)/near-infrared (NIR) spectroscopy [7,8]. Research studies using NIR spectroscopy models to predict the nutritive value and/or intake of forages require a large number of samples [7,9]. The nutritive value of these samples obtained from previous in vivo trials are needed [7,9]. However, this is a time-consuming process, and, in some cases, these values were obtained for in vivo trials carried out before the development of the model.

In vivo trials are followed by grinding, analysis, and storage of forage samples. The samples are then exposed to atmospheric oxygen, fluctuating temperatures, and certain enzymes released during storage, causing the destruction of the stored samples' nutrients [10]. It is recommended for dried forage samples to be stored at low temperatures [11], in the absence of direct heat, sunlight [12], and oxygen. However, in practice, samples are usually stored at ambient conditions because this is cheaper and freezers are sometimes not available for this function. This practice may change the chemical composition of the samples stored for a long period of time, making them unsuitable in the development of new predictive methods for their nutritive value. Landau et al. [13] found some changes in the NIR spectra of fecal samples after a 3-year storage period. However, to our knowledge, the potential changes in the chemical composition and nutritive value of samples stored under different conditions for many years have never been extensively addressed.

The objective of this study is to evaluate (i) changes in the chemical composition and nutritive value of forage samples (whole maize forage) stored at frozen or ambient temperatures for a very long time and (ii) potential changes in the VIS/NIR spectra of forage samples according to the storage method. We hypothesized that the storage environment could influence the chemical composition of the forage samples, their spectra, and, consequently, their nutritive value.

2. Materials and Methods

2.1. Samples

The samples studied correspond to an experiment carried out in 1987 on 125 samples of maize hybrids [12]. Eighteen samples cut in 1987 were selected according to the phenological stage of the cut (between the milky and vitreous stages) and the location of growth [14] to give a dry matter (DM) content between 0.22 and 0.35. After harvest, the samples were oven-dried at 80 °C for 48 h and ground in a hammer mill through a 1 mm screen. Two subsamples were then produced. The first sample of about 1 kg was placed in a plastic airtight container and stored in a barn at room temperature (minimal and maximal temperatures ranged from −8.5 °C to 27.1 °C). The second sample of about 500 g was placed in an airtight plastic bag and stored at −20 °C.

2.2. Chemical and Biological Analyses

After collection in 1987, the samples were analyzed for ash and crude protein (Kjeldahl nitrogen × 6.25) (CP) using mercuric oxide as a catalyzer, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) according to the method of Van Soest [15] and Van Soest and Wine [16]. In 2016, barn-stored and frozen-stored samples were analyzed for ash [17] (method 942.05) and CP [15] (Kjeldahl nitrogen; method 976.06 using selenium (2%), cooper sulphate (1.5%), and sodium sulphate

(96.5%) as catalyzers). For NDF, the procedure proposed by Van Soest et al. [18] was used, and we used the method proposed by Van Soest and Robertson for ADF and ADL [19]. The neutral detergent fiber was assayed with a heat-stable amylase to avoid interference between the starch and cell wall content [20]. In 1987 and 2016, the samples were also analyzed for starch as per Ewers (quoted by Radley) [21], total soluble carbohydrates (TSC) as per Somogyi [22], and pepsin–cellulase dry matter digestibility (PCDMD) following the procedure proposed by Aufrère and Michalet Doreau [6].

2.3. Acquisition of Visible/Near Infrared Spectra

In 2016, after homogenizing the samples, the ground frozen and barn-stored forages were placed in a 50 mm-diameter ring cup and scanned in reflectance mode at 2-nm intervals from 400 to 2500 nm using a Foss NIRSystems model 6500 scanning VIS/NIR spectrometer (Foss NIRSystems, Silver Spring, MD, USA). Spectra and reference values were recorded using ISIscan version 2.21 software (Infrasoft International, State College, PA, USA). Each spectrum was averaged from 32 scans. A reference scan (using the internal ceramic reference tile) was taken before each sample. The reflectance (R) values were converted into absorbance (A) values using the formula A = log (1/R). The raw NIR spectroscopy data were pre-processed using the standard normal variate and de-trending scatter correction procedure [23]. The spectra were then transformed using a mathematical second-order gap derivative (2,8,8,1), where the first digit is the order of the derivative, the second is the gap over which the derivative is calculated, the third is the number of datapoints in the first smoothing, and the fourth is the number of datapoints in the second smoothing. Then, the average spectrum for the samples under the same storage treatment (frozen or barn storage) was calculated. Finally, the difference between the average spectra of the storage methods was computed.

2.4. Statistical Analyses

Data on the forage chemical composition and PCDMD were tested by ANOVA using the mixed procedure of the SAS statistical package [24]. The model used the included storage method and sample as its factors. The storage method was considered to be a repeated measure and the sample to be a random variable. The effect of the storage method after 29 years (frozen vs. barn temperature) was studied for all determinations, whereas the effect of time was only tested for ash, TSC, starch, and PCDMD because the methods of analysis performed in 1987 differed from those performed in 2016 for all other determinations. The levels of both effects were compared by orthogonal contrasts.

3. Results

The means, standard deviation, and minimum and maximum values for PCDMD and chemical composition determinations obtained in 1987 for the 18 maize samples used in this experiment are given in Table 1. The coefficient of variation between the samples ranged from 5.25 for PCDMD to 49.5 for starch.

There were no differences between the storage methods (frozen vs. barn temperature over 29 years) for ash, CP, NDF, ADF, ADL, starch, and PCDMD. The TSC content was lower for the samples stored at barn temperature for 29 years ($p < 0.001$) (81 g/kg DM) than for the samples stored frozen for 29 years (93 g/kg DM). The ash content was significantly different between the samples analyzed in 1987 and 2016 (55 vs. 56.5 g/kg DM respectively; $p < 0.01$), but no difference was obtained between the storage methods (56 vs. 57 g/kg DM for samples stored at barn temperature or frozen, respectively; $p > 0.05$). The TSC significantly differed (113 vs. 87 g/kg DM) between 1987 and 2016 ($p < 0.001$). The TSC stored at barn temperature decreased by 28% in relation to the TSC content obtained in 1987 just after harvesting the samples, whereas the TSC of the samples stored frozen for 29 years decreased by only 18% in relation to the original value measured in 1987. The starch content ($p > 0.05$) did not vary over time in storage nor between frozen vs. barn-temperature samples (Table 2).

Table 1. Descriptive statistics (standard deviation (SD), minimum value (min), maximum value (max), and coefficient of variation (CV) for the ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), total soluble carbohydrates (TSC), starch contents, and pepsin-cellulase dry matter digestibility (PCDMD) within the set of samples (n = 18) analyzed in 1987.

	Mean	SD [2]	Min [3]	Max [4]	CV [5]
Ash (g/kg DM [1])	55	6.5	45	67	11.8
CP (g/kg DM)	84	8.2	69	99	9.8
NDF (g/kg DM)	488	39.8	432	575	8.2
ADF (g/kg DM)	235	32.5	188	302	13.8
ADL (g/kg DM)	26	4.9	20	37	18.7
TSC (g/kg DM)	113	44.6	55	185	39.3
Starch (g/kg DM)	226	111.8	39	388	49.5
PCDMD	0.67	0.004	0.59	0.71	5.25

[1] DM = dry matter; [2] SD = Standard deviation; [3] min = minimum value; [4] max = maximum value; [5] CV = coefficient of variation.

The averaged second-derivative NIR spectrum for frozen and barn-stored whole-maize forage samples is presented in Figure 1a. Whole-maize forage samples presented bands with peaks at 660, 728, 750, 1420, 1920, 2022, 2248, 2314, and 2412 nm. The different spectra between the second-derivative NIR spectra for the frozen and barn-stored samples is presented in Figure 1b. The average spectra for both storage methods showed substantial differences at 660, 698, 728, 1420, 1920, and 2066 nm.

Table 2. Contents of ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), total soluble carbohydrates (TSC), starch and pepsin–cellulase digestibility (PCDMD), and standard error of the mean (SEM) for the whole-maize forage samples analyzed in 1987 and in 2016 (stored frozen or at barn temperature).

	1987	2016		Significance		
		BT [2]	Frozen	SEM	SM [3]	Time
Ash (g/kg DM [1])	55	57	56	1.7	ns [4]	**
CP (g/kg DM)	84	83	82	2.2	ns	– [5]
NDF (g/kg DM)	488	457	458	11.4	ns	–
ADF (g/kg DM)	235	231	233	7.9	ns	–
ADL (g/kg DM)	26	21	21	1.0	ns	–
TSC (g/kg DM)	113	81	93	10.4	***	***
Starch (g/kg DM)	226	225	233	32.2	ns	ns
PCDMD	0.67	0.67	0.68	0.008	ns	ns

[1] DM = dry matter; [2] BT = barn temperature; [3] SM = storage method; [4] ns = non-significant ($p > 0.05$); ** = $p < 0.01$; *** = $p < 0.001$; [5] – = not tested.

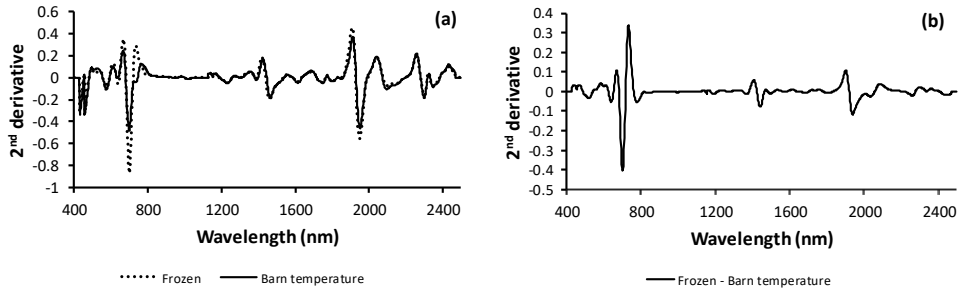

Figure 1. Visible and near/infrared second-derivative spectra for the average whole-maize forage samples stored frozen or at barn temperature over 29 years (**a**) and the differences between the average second-derivative spectra of the average frozen or barn-dried whole-maize forage samples (**b**).

4. Discussion

The samples used in this experiment were chosen to represent a broad range of the variability in the nutritive value of temperate forages (PCDMD between 0.59 and 0.71) reported in the literature [3]. It is common practice for research centers to store samples at room temperature after in vivo trials, and several studies using samples stored under these conditions have been published [6,25,26]. However, studies that used stored freeze-dried samples have also been published [27]. In this study, we used whole-maize forage samples to investigate the changes over time for a number of chemical and biological determinants related to the nutritive value of forages for ruminants. The chemical composition of the samples used in the current study is characterized by high TSC, starch, and structural carbohydrates contents, and moderate ash and CP contents [3]. The samples used in this study were dried at 80 °C, which is not an optimal drying condition to preserve the chemical composition of fresh forages [28] because of the potential formation of Maillard reaction products. These drying conditions could influence the extent of the changes during very-long-term storage time, as Maillard products remain very stable over the long-term [20].

Other studies have investigated the effects of storage time on the nutritive value of hays [29,30], fresh forages, and silages [31,32], but, to our knowledge, this is the first work to investigate changes in the nutritive value of ground forages after a long storage period in contrasting conditions. Earlier studies [29,30] found that in-storage changes in forage chemical composition and nutritive value are mainly related to the moisture content of the samples. In our study, the DM content of the samples in 2016 was higher than 0.88, regardless of the storage method (0.91 ± 0.005 for barn-stored samples and 0.89 ± 0.010 for frozen samples), which means that the extent of changes in the chemical composition and nutritive value of the forage may be limited.

The TSC content was lower in the samples stored at barn temperatures for 29 years than in the samples frozen during the same interval. Soluble carbohydrates are sensitive to storage conditions. Greenhill et al. [33] reported that the loss of DM (mainly nonstructural carbohydrates) increased along with high storage temperatures, high moisture content, and time in storage. Smith [10] claimed that TSC is likely to show less change when dried samples are stored at low temperatures (which fits with the results found here), as heat drying does not inactivate all the enzymes related to the degradation of nonstructural carbohydrates.

Our results indicate no influence of the storage method on ash, CP, structural carbohydrate, and starch contents. Other authors, however, have reported differences in these parameters when forage samples were submitted to different drying procedures before analysis [34,35]. Changes in the structural carbohydrate content are related to the formation of Maillard reaction products, which interfere with these assays. Maillard reaction products are affected by the type, amount of nonstructural carbohydrates [28], heat [36], water activity [37], and pH [38]. In our experiment, although differences in the temperatures between storage methods were produced over time, the barn temperatures were not high enough to produce Maillard products in the forage samples and thus increased the structural carbohydrate contents of the barn-stored samples relative to the frozen samples. The drying conditions of the fresh samples (80 °C) in 1987 could have modified the chemical composition of the samples and made them less sensitive to the effects of ambient temperature in subsequent years. Blackman and Templeman [39] reported the presence of active starch-hydrolyzing enzymes after drying samples at 95 °C. However, in the current study, the storage methods did not affect the starch content. Blackman and Templeman [39] found that, although starch-hydrolyzing enzymes were not inactivated when samples were dried at 95 °C, most of the starch disappeared in 3 weeks. Therefore, samples prepared under the same conditions were not expected to show in-storage changes in starch content. Changes in ash and CP contents were explained by the modifications produced in other components, mainly TSC [30,34]. The current study found that the decrease in the TSC content of barn-stored samples was not sufficient to increase the ash and CP contents. Finally, these changes in the chemical compositions of the samples were not able to modify the PCDMD of samples stored differently.

The comparison between the average NIR spectra of the frozen and barn-stored samples showed that the bands with the highest differences were obtained in the VIS segment (660–700 nm). This might be due to the potential loss of color, mainly because of chlorophyll pigment degradation, in samples stored at barn temperature. The differences observed between the frozen and barn-stored second-derivative spectra at around 1420 and 1920 nm could be related to the different moisture contents of both preservation methods (i.e., 0.911 for the barn-stored samples and 0.890 for the frozen samples). The absorbance values at 2028 and 2250 nm might be related to the –C=O- and –OH molecular groups, respectively [40]. The carbonyl and hydroxyl molecular groups are characteristic of TSC, confirming that the main storage method-related changes in the forage samples are due to TSC changes.

The storage duration effect was analyzed only for ash, TSC, starch, and PCDMD, as the analytical method used for CP and structural carbohydrates was different in 1987 and 2016 [18,41,42], and this may cause a confounding effect on the storage duration. The TSC and ash content for the samples across 29 years of storage changed, whereas the starch content did not vary between 1987 and 2016. The two methods (frozen and barn-dried) showed similar results, which could be explained by the fact that TSC-hydrolyzing enzymes are not fully heat-inactivated [10]. However, the possibility of presence of starch-hydrolyzing enzymes after drying in 1987 [39] would be negligible or they could be biologically inactivated. Finally, it needs to be noted that all changes produced by prolonged storage of the whole-maize forage samples did not affect their digestibility.

5. Conclusions

This study suggests that the nutritive value of whole maize samples oven-dried at 80 °C, ground down to 1 mm, and stored under barn-temperature conditions, is similar to that of the samples stored frozen at −20 °C for a very long period of time. The results of this study show that the chemical composition of the samples stored over the long term changed, especially the TSC fraction and other components related to NIR absorption in the visible segment (400–700 nm). However, these changes are not sufficient to modify the nutritive value of forages in comparison with their post-harvest and pre-storage values.

Author Contributions: D.A. carried out the experimental design, data interpretation, manuscript writing, and editing. F.P. was involved in the experimental design, data recovery, data analysis, and manuscript revision. C.B. was involved in the data analysis and manuscript revision. V.M. was involved in the data analysis and manuscript revision. C.G. was involved in the data analysis and manuscript revision. G.M. contributed to the interpretation of results, manuscript writing, and manuscript revision. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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