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Araştırma Makalesi

Comparison of Different Laboratory-Scale Ensiling Methods for Evaluating the Influence of Silage Additives on Maize Silage

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Abstract

The current study aimed to compare the nutritional, chemical, and fermentative changes of maize silages with some additives prepared in standard glass jars (SGJ) and vacuum-packed model polyethylene bags (VP, Rostock model silages). The treatment groups were control group (no addition), barley group (grinded barley addition 20 and 40 g kg^{-1}), lactic acid bacteria (LAB) group, and LAB+enzyme mixture group. The ensiling method had no effect on the pH of the silage. (P=0.974) but the pH was increased with LAB addition (P=0.030). The dry matter (DM) contents of silages were increased by barley addition (P=0.030). Silages hemicellulose (HEM) content was decreased by ENZ inoculation (P=0.017). Silages total carbohydrates (TC) and metabolizable energy (ME) concentrations were highest in 40 g kg^{-1} barley group (P<0.01). The silages Fleig Point (FP) was decreased by LAB inoculation (P=0.016). Silage ether extract (EE), ADF, NDF and crude cellulose (CC), total digestible nutrients (TDN), and fermentation parameters (acetic, butyric, lactic and propionic acids) were not impacted by the ensiling methods or silage additives (P>0.05). These results showed that vacuum-packed polythene bags do provide practical, flexible, and cost-efficient alternative to fixed-capacity glass containers for laboratory scale silage experiments.

Key words: Barley, enzyme, glass jar, LAB, vacuum-packed

Silaj Katkı Maddelerinin Mısır Silajına Etkisinin Değerlendirilmesinde Laboratuar Ölçekli Farklı Silolama Yöntemlerinin Karşılaştırılması

Öz

Bu çalışmada, standart cam kavanozlarda (SGJ) ve vakumlu model polietilen torbalarda (VP, Rostock model silajlar) farklı katkı maddeleri ile yapılan mısır silajlarının kimyasal, besinsel ve fermentatif değişimlerinin karşılaştırılması amaçlanmıştır. Muamele grupları kontrol (ilavesiz), arpa (öğütülmüş arpa ilaveli 20 ve 40 g kg^{-1}), laktik asit bakterileri (LAB) ve LAB+enzim karışımı gruplardan oluşturulmuştur. Silaj pH'sı silolama yönteminden etkilenmemiş (P=0.974) ancak LAB ilavesi ile pH değerleri artmıştır (P=0.030). Silajların kuru madde (KM) içerikleri arpa ilavesi ile artmıştır (P=0.030). Silajların hemiselüloz (HEM) içeriği, ENZ aşılaması ile azalmıştır (P=0.017). Silajların toplam karbonhidrat (TC) ve metabolik enerji (ME) konsantrasyonları en yüksek 40 g kg^{-1} arpa grubunda bulunmuştur (P<0.01). Silajların Fleig Skorları (FS) LAB inokülasyonu ile azalmıştır (P=0.016). Silajların ham yağ (EE), ADF, NDF ve ham selüloz (HS), toplam sindirilebilir besin maddeleri (TSBM) ve fermentasyon parametreleri (laktik, asetik, propiyonik ve butirik asit) silolama yönteminden veya silaj katkı maddelerinden etkilenmemiştir (P>0.05). Bu sonuçlar, vakumla paketlenebilen polietilen torbaların, laboratuvar ölçekli silaj çalışmaları için sabit hacimli cam kavanozlara göre daha uygun, esnek ve daha az maliyetli bir alternatif olduğunu göstermiştir.

Anahtar kelimeler: Arpa, enzim, cam kavanoz, LAB, vakumlu paket

Introduction

The ensiling processing methods at the laboratory scale has potential effects on forage preservation, determining the efficacy of additives and the quality of silage material. Current methods are also effective in terms of the process costs, labor and ensiling equipment needs. Although there are only few studies about the ensiling processing methods at the laboratory scale that accepted as a potential method. Among the current methods, glass preserving jars recommended by DLG (German Agricultural Society) are the most preferred, but in recent years laboratory-scale test tubes, porcelain containers and polyethylene bags produced in different volumes have been used for ensiling process. Standard glass jar model silages are widely used but it has some disadvantages such as time consuming and costs (Hoedtke and Zeyner, 2011), also where silage additives are examined under though ensiling condition with an extremely low packing density. This state, however, does not accurately indicate silo quality. Silage or silo models generally allow to air ingress and can affect the effectiveness of additives or inoculants to the silage material (Pauly and Hjelm, 2015).

Therefore, the objective of this research was to compare the ensiling method with glass jar or vacuum-packed polyethylene bags based with some silage additives to improve silage quality.

When the current literature is examined, it is very difficult to identify the impacts of silos on silage quality. Obtaining thorough information on the ensiled in silage material research is quite challenging. The volume of the material, the air permeability, the density of the packing and the applied pressure are very effective in determining the silo quality. For this reason, it is impossible to evaluate the existing literature without a new study. While planning the current work, the studies that give details about the silo in the previous studies are based on. For example, Robinson and Swanepoel (2016) produce some fodder silages using polyethylene silage stack underlayment plastic with or without raised oxygen barrier, Weatherly et al. (2018) used a vacuum sealer for sealing polyethylene bag, also Ke et al. (2018) used a vacuum-packaging machine for sealing polyethylene bag for ensiling. Nevertheless, Johnson et al. (2005) compared that vacuum-packed plastic bag silos versus glass tube silages with different initial vacuum settings and varying packing densities. These researchers concluded that vacuum-packed plastic bag silos a highly flexible method compared with glass tube model silage. After all this assessment, plastic bags provide such a suitable alternative for glass containers, but needs some revisions such determine the responses to some different additives into silage.

Therefore, the objective of this research was to compare standard glass jar model silages (SGJ) versus the vacuum-packed plastic bag silages (VP) under same laboratory conditions (standardized method).

Material and Method

Maize was harvested in milk-dough stage from Agricultural Research and Practical Center fields in autumn season. Maize dry matter (DM) was 317 g kg^{-1}. Forages after harvest, were chopped using a forage harvester (Çelikel Challenger, Turkey) to about 1.5 - 3 cm. Then sampled material were filled in standard glass jars (SGJ, 2L) and vacuum-packed model plastic bags (VP, 2L) with 6 replicates and stored for 90 days at room temperature (between 20-26 °C). SGJ and VP silages were carried out under equivalent circumstances, with particular attention to identical packing densities (2L). Silages were prepared without additives (control) and with the addition of 20 g kg^{-1} grinded barley (B20), the addition of grinded barley 40 g kg^{-1} (B40), lactic acid bacteria (LAB) additive (LAB; 1.5 g ton^{-1}, a mixture of LAB consisting of Lactobacillus plantarum and Enterococcus faecium applied at a rate of 6.00 log10 cfu LAB g^{-1} of fresh material (Pioneer 1174, USA), and LAB+enzyme mixture additive (ENZ; 2 g ton^{-1}, inoculant and enzyme mixture (Lactobacillus plantarum, Enterococcus faecium bacterium and cellulose, amylase and pentosanase enzymes), Silaid WSTM, Global Nutritech Co., USA) in six replications each. Mash was made from ground barley, then LAB and EZM were dissolved in 20 mL water and sprayed over chopped corn samples.

Two laboratory-scale ensiling methods were designed for the ensiling process.

Standard glass jar silages (SGJ)

Before usage, standard glass jars were cleaned and sterilized (180 °C, 8 h). In a 100 g sample, the jar was filled with maize and crushed by hand with the use of a rod. The same procedure was repeated until the final weights were two kg in all samples (6 standard glass jar). Finally, glass jars were closed and fixed by a rubber lid than after fixed by metal clips. All silages were kept in 20-26 °C room temperature and opened on day 90.

Vacuum-packed silages (VP)

A vacuum sealer was used to seal VP plastic bag silages (DZ-260/PD, SELES). About one kg of plant materials were weighted and placed in

gas permeable polyethylene bags (600 mm × 900 mm). The silage was manually put into the bags before they were heat-sealed and air-vacuumed. In order to prevent the punching of the bags from the silage material, the same method was repeated using a second plastic bag. All silages were kept under the same circumstances and opened on the 90th day.

Chemical analyses and calculations

The silages were sampled at the conclusion of the 90-day ensiling period for nutritional, chemical, and fermentation parameter assessments. pH measurements were taken for 25 g of silage samples in a beaker by mixing 100 mL of distilled water in a blender for 5 minutes and then decomposing with silage. A pH meter was used to measure the pH (WTW Inc., Weilheim, Germany). The DM of the fresh material and silages was assessed by drying them in an oven at 60 °C for 72 hours. The AOAC (2019) techniques were used to determine crude ash (CA), crude protein (CP), crude cellulose (CC), and ether extract (EE). The sodium sulphite addition technique with residual ash was used to assess neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991). The difference between NDF and ADF estimates hemicellulose (HEM). The total digestible nutrition (TDN) values were obtained using Chandler's (1990) Equation 1;

$$\% \text{ TDN} = 105.2 - 0.68 \times \% \text{ NDF} \quad \text{(Eq.1)}$$

The non-fiber carbohydrates (NFC) were estimated using Weiss et al. (1992) Equation 2;

$$\% \text{ NFC} = 100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ EE} + \% \text{ CA}) \quad \text{(Eq.2)}$$

Total carbohydrates (TC) were calculated using Equation 3 by Sniffen et al. (1992);

$$\text{TC} = 100 - (\% \text{ CP} + \% \text{ EE} + \% \text{ CA}) \quad \text{(Eq.3)}$$

Metabolizable energy (ME) was computed using Kirchgessner et al. (1997) Equation 4;

$$\text{ME} = 14.03 - (0.01386 \times \% \text{ EE}) - (0.1018 \times \% \text{ CA}) \quad \text{(Eq.4)}$$

The silages were sampled as a liquid extraction at the conclusion of the ensiling phase for water-soluble carbohydrate (WSC) and volatile fatty acid (VFA) analyses. The liquid extractions were made by placing 40 g of silage in a beaker and adding 360 mL of distilled water before mixing with a blander. This liquid was centrifuged after being filtered via 54 No Whatman filter paper. Until they were examined, the samples were stored at -20 °C. The phenol sulphuric acid technique was used to determine WSCs (Dubois et al., 1956). Lepper's techniques (Akyildiz, 1986) were used to detect lactic acid (LA). VFA (Propionic, Acetic, and Butyric acid) analysis was performed in a Shimadzu GC-2010 (Kyoto, Japan) gas chromatograph equipped with FID, using a capillary column (30 m 0.25 mm 0.25 m, Restek) over a temperature range of 45–230 °C.

Statistical analysis

The statistical analysis system's general linear model (GLM) approach was used to examine the data (SPSS, 2017). With a 5% level of probability, Duncan's multiple range tests were used to examine the differences between additive treatment group means using the Ensiling technique as a fixed model. The results of statistical analysis were shown as treatment group and ensiling method means also total means were showed with standard deviation in the tables.

Based on the chemical composition, nutritional content, and VFA variables, a principal component analysis (PCA) was done using the XLSTAT software statistical and data analysis solution (Addinsoft; Boston, MA, USA). The eigenvalue similarity and significance levels were both set at 0.05.

Results and Discussion

Due to the high content of water-soluble carbohydrates (WSC) and buffering capacity (BC), maize has a good potential for silage. The aim of this study was to compare the ensiling techniques with some inoculants of glass jars and vacuum-packed model as an alternative for commonly used silo vessels. The effects of ensiling method and some additives on DM, pH, CP and EE of maize silage is given in Table 1. The ensiling technique had no significant influence on the DM ($P>0.05$), however barley addition (B20 and B40 groups) increased the DM ($P<0.05$).

High-quality silage should have 250–350 g kg^{-1} dry matter (Oliveira et al., 2018). In a recent research, the effects of the ensiling technique on DM were deemed unimportant since only occasional significant differences between the ensiling procedures were seen (Hoedtke and Zeyner, 2011). The pH value of the silages (4.03 - 4.48) were close to the optimal silage pH value (3.8 - 4.2). The pH of silages was not affected by the ensiling method ($P>0.05$), 4.23 for SGJ and 4.13 for VP, but was influenced by inoculation ($P<0.05$). The pH showed a rise in LAB group (4.47), this may be due to a decrease in fermentable carbohydrates levels that can be used by the LAB at the end of 90 days. Along with the high carbohydrate content and limited buffer capacity of maize, according to Oliveira et al. (2018), pH does not easily increase in silage that has a high maize concentration. Also, the DM content can affect the silage pH which are ensiled too wet (Guyader et al., 2018).

Table 1. The effects of some additives (A) and ensiling method (EM) on pH; dry matter (DM); crude protein(CP); ether extract (EE) and crude ash (CA) concentration of maize silage

Additives	Ensiling Method	pH	DM	CP	EE	CA
			g kg^{-1} DM			
Control	SGJ	4.19^{b}	308.1^{bc}	87.4^{b}	11.6	71.7^{a}
	VP	4.05^{b}	310.4^{bc}	90.0^{b}	10.1	90.7^{a}
B20	SGJ	4.31^{ab}	333.0^{a}	89.3^{b}	11.3	82.6^{a}
	VP	4.17^{ab}	324.3^{a}	87.0^{b}	10.8	75.8^{a}
B40	SGJ	4.17^{b}	331.9^{ab}	105.9^{a}	11.1	48.3^{c}
	VP	4.05^{b}	320.3^{ab}	103.5^{a}	12.5	52.5^{c}
LAB	SGJ	4.48^{a}	289.8^{c}	85.2^{b}	11.7	83.9^{a}
	VP	4.46^{a}	299.8^{c}	93.4^{b}	11.6	85.6^{a}
EZM	SGJ	4.03^{b}	311.3^{ab}	90.6^{b}	11.8	67.2^{b}
	VP	4.06^{b}	318.2^{ab}	83.1^{b}	11.8	73.6^{b}
Total	SGJ	4.23	318.4	93.7	11.5	70.6
	VP	4.13	314.5	92.9	11.2	77.5
	SD	0.19	16.6	8.5	1.0	14.5
P	A	*	*	**	NS	***
	EM	NS	NS	NS	NS	NS
	A×EM	NS	NS	NS	NS	NS

Control: no additive; B20: 20 g kg^{-1} grinded barley; B40: 40 g kg^{-1} grinded barley; LAB: a mixture of *Lactobacillus plantarum* and *Enterococcus faecium;* 1.5 g ton^{-1}; and EZM: LAB+enzyme mixture (cellulose; amylase and pentosanase enzymes); 2 g ton^{-1}; SGJ: standard glass jar; VP: vacuum packed; $^{a;b;c}$: values with different superscript in the same column differ significantly : P < 0.05; P: probability; SD: standard deviation.

Interactions were identified for ensiling method × additives (P>0.05) for silage DM and pH. Both SGJ and VP (P<0.01) indicated an increase in CP in B40 silages, as predicted. The EE ratio was not influenced with method or additive. The effects of ensiling method and some additives on CC, ADF, NDF and HEM of maize silage is given in Table 2. Although the CC, ADF and NDF contents were not showed a significant difference between treatment groups, lower HEM value was observed in EZM group. Differences in the quantity of DM losses that occur throughout the fermentation process can explain increases in fiber concentration (Konca et al., 2018). Enzyme addition of silage has previously been used to breakdown cell walls and hence enhance the digestibility of silage fiber (Yang et al., 2019). Also, in a study was showed that changes in fiber content by bacterial enzymes activity with degradation of cell wall in silage (Sarıçiçek et al., 2016).

Table 2. The effects of some additives (A) and ensiling method (EM) on the crude cellulose (CC); acid detergent fiber (ADF); neutral detergent fiber (NDF) and hemicellulose (HEM) concentration of maize silage

Additives	Ensiling Method	CC	ADF	NDF	HEM
		g kg^{-1} DM			
Control	SGJ	250.2	318.5	475.5	157.1^{a}
	VP	249.1	276.2	453.6	177.4^{a}
B20	SGJ	275.0	300.7	433.9	133.2^{a}
	VP	257.2	300.4	446.4	146.0^{a}
B40	SGJ	273.2	268.6	448.0	179.4^{a}
	VP	273.2	270.8	427.4	156.7^{a}
LAB	SGJ	270.0	289.6	417.2	127.5^{a}
	VP	257.0	287.7	448.0	160.3^{a}
EZM	SGJ	255.3	359.2	398.8	139.6^{b}
	VP	269.9	301.9	433.3	131.5^{b}
Total	SGJ	266.2	307.3	433.0	125.6
	VP	260.7	287.9	442.2	154.4
	SD	15.8	38.8	29.0	50.1
P	A	NS	NS	NS	**
	EM	NS	NS	NS	NS
	A×EM	NS	NS	NS	NS

Control: no additive; B20: 20 g kg^{-1} grinded barley; B40: 40 g kg^{-1} grinded barley; LAB: a mixture of *Lactobacillus plantarum* and *Enterococcus faecium;* 1.5 g ton^{-1}; and EZM: LAB+enzyme mixture (cellulose; amylase and pentosanase enzymes); 2 g ton^{-1}; SGJ: standard glass jar; VP: vacuum packed; $^{a.b;c}$: values with different superscript in the same column differ significantly: p<0.05; P: probability; SD: standard deviation.

The effects of ensiling method and some additives on TDN, OM, NFC, TC, ME and FP of maize silage is given in Table 3. Although the TDN and NFC contents were not showed a significant difference between treatment groups, lower FP value was observed in LAB group. The pH and DM were used to determine the Fleig point, which provides information on the quality of maize silage. The silages Fleig point was lowest in LAB group (P<0.05). However, in this experiment all Fleig point of silage treatments were categorized as "excellent class". The total carbohydrates (TC) contents of silage was highest in B40 group (P<0.05), related to the barley addition.

Table 3. The effects of some additives (A) and ensiling method (EM) on the total digestible nutrient (TDN); organic matter (OM); non-fiber carbohydrate (NFC); total carbohydrate (TC); metabolisable energy (ME) and fleig point (FP) concentration of maize silage

Additives	Ensiling Method	TDN	OM	NFC	TC	ME	FP
		g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	Mj kg^{-1} DM	
Control	SGJ	835.5	928.3^{c}	343.8^{b}	819.4^{c}	12.95^{c}	99.01^{a}
	VP	864.2	909.3^{c}	345.6^{b}	799.2^{c}	12.76^{c}	105.09^{a}
B20	SGJ	847.5	917.4^{c}	382.9^{ab}	816.8^{bc}	12.81^{c}	99.41^{a}
	VP	847.7	924.2^{c}	380.0^{ab}	826.4^{bc}	12.90^{c}	103.05^{a}
B40	SGJ	869.3	951.7^{a}	386.7^{ab}	834.6^{a}	13.16^{a}	104.45^{a}
	VP	867.9	947.5^{a}	404.1^{ab}	831.5^{a}	13.12^{a}	107.07^{a}
LAB	SGJ	855.1	916.1^{c}	402.1^{ab}	819.3^{c}	12.80^{c}	83.75^{b}
	VP	856.4	914.4^{c}	361.5^{ab}	809.5^{c}	12.80^{c}	86.56^{b}
EZM	SGJ	807.7	932.8^{b}	431.6^{a}	830.4^{ab}	12.99^{ab}	106.18^{a}
	VP	846.7	926.4^{b}	398.2^{a}	831.5^{ab}	12.91^{ab}	106.45^{a}
Total	SGJ	843.0	929.4	391.3	824.3	12.94	99.65
	VP	856.2	922.5	376.2	818.4	12.88	102.82
	SD	26.4	14.5	35.0	12.4	1.4	8.82
P	A	NS	***	NS	**	***	**
	EM	NS	NS	NS	NS	NS	NS
	A×EM	NS	NS	NS	NS	NS	NS

Control: no additive; B20: 20 g kg^{-1} grinded barley; B40: 40 g kg^{-1} grinded barley; LAB: a mixture of *Lactobacillus plantarum* and *Enterococcus faecium;* 1.5 g ton^{-1}; and EZM: LAB+enzyme mixture (cellulose; amylase and pentosanase enzymes); 2 g ton^{-1}; SGJ: standard glass jar; VP: vacuum packed; ^{a,b,c}: values with different superscript in the same column differ significantly: p<0.05; P: probability; SD: standard deviation.

The effects of ensiling method and some additives on WSC and fermentation parameters of maize silage is given in Table 4. It is generally understood that the initial packing density of silage effects the fermentation process (Muck and Holmes, 2000), however, in this study the fermentation parameters did not influenced with ensiling method, that probably due to the same weight of silage material. The primary acid was acetic acid not affected to ensiling method or additive (P>0.05).

Interactions between the ensiling method × addition were observed not significantly (P>0.05). Acetic acid concentration in well-preserved silages should be 10-40 g kg^{-1} DM (Jones et al., 2004), in current experiment the acetic acid content found 45.9, 70.74 g kg^{-1} DM in SGJ and VP silages respectively. While the ensiling method had no effect on the lactic acid concentration (P=0.608), the silage material (P<0.001) and addition (P<0.001) had no effect on lactic acid generation. To guarantee adequate acidification, high-moisture maize silages (700-750 g kg^{-1} DM) should include 5-20 g kg^{-1} lactic acid (Kung and Shaver, 2001). In this study the lactic acid concentration was ranged as 17-55.6 g kg^{-1} DM for the 90th day. The propionic acid content was not different significantly (P>0.05) between SGJ and VP. Propionic acid is reported to be abundant in wet silages (<250 g kg^{-1} DM) (Kung and Shaver, 2001), but in this study the propionic acid was changed as 1.10–3.70 g kg^{-1} DM, it can be mostly related to the silage DM (289–333 g kg^{-1}) content. Silages butyric acid concentration was not influenced by ensiling method and addition (P>0.05). However, in the vacuum-packed model silages mostly not detected butyric acid, so it can be suggested as an ensiling method for good fermentation quality of silage. In all treatments, no significant variations in WSC were found between SGJ and VP silages (P>0.05). WSC concentration of silages ranged as 303 - 523 g kg^{-1} DM for the 90th day. Weinberg et al. (2010) observed that at the end of 5 months, the WSC content of corn silage in small silos was 135.7 g kg^{-1} DM. Table 5 shows the Eigenvalues, variability, and factor loadings values of variance for the PCA parameters that were studied.

Table 4. The effects of some additives (A) and ensiling method (EM) on the water-soluble carbohydrate (WSC); lactic; acetic; propionic and butyric acid concentration of maize silage

Additives	Ensiling Method	WSC	LA	AA	PA	BA
		g kg^{-1} DM				
Control	SGJ	162.8	29.0	46.9	2.8	0.10
	VP	338.8	55.0	107.1	3.7	0.00
B20	SGJ	361.9	17.0	24.1	2.1	2.90
	VP	523.5	20.2	31.2	1.1	0.00
B40	SGJ	421.0	30.2	44.5	1.7	00.4
	VP	378.4	45.7	75.0	2.0	00.0
LAB	SGJ	303.0	55.6	96.3	1.2	14.6
	VP	370.9	42.5	75.4	1.1	11.1
EZM	SGJ	403.1	27.4	42.2	1.9	0.80
	VP	344.1	40.4	73.1	2.1	9.10
Total	SGJ	346.5	29.3	45.9	1.9	3.20
	VP	376.9	42.7	77.4	2.2	4.20
	SD	114.5	18.2	34.5	1.4	7.00
P	A	NS	NS	NS	NS	NS
	EM	NS	NS	NS	NS	NS
	A×EM	NS	NS	NS	NS	NS

Control: no additive; B20: 20 g kg^{-1} grinded barley; B40: 40 g kg^{-1} grinded barley; LAB: a mixture of *Lactobacillus plantarum* and *Enterococcus faecium;* 1.5 g ton^{-1}; and EZM: LAB+enzyme mixture (cellulose; amylase and pentosanase enzymes); 2 g ton^{-1}; SGJ: standard glass jar; VP: vacuum packed; $^{a,b;c}$: values with different superscript in the same column differ significantly : p<0.05; P: probability; SD: standard deviation.

Table 5. Eigenvalues; variability and factor loadings values of variance for investigated parameters of PCA analysis

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue and Variability values						
Eigenvalue	6.771	4.396	3.443	2.017	1.543	1.018
Variability (%)	33.854	21.980	17.214	10.087	7.716	5.090
Cumulative %	33.854	55.834	73.047	83.134	90.851	95.941
Factor loadings values						
pH	0.568	-0.371	0.405	-0.482	-0.316	-0.050
DM	-0.709	0.317	-0.154	-0.408	0.250	0.352
CP	-0.615	0.376	0.529	-0.004	-0.091	-0.276
EE	-0.351	-0.602	0.286	0.358	-0.377	0.145
CA	0.920	-0.068	-0.256	-0.150	0.224	0.054
CC	-0.421	-0.346	0.601	-0.176	0.182	0.494
ADF	-0.076	-0.506	-0.800	0.185	-0.165	-0.151
NDF	0.233	0.738	-0.049	-0.280	-0.471	0.171
HEM	-0.123	0.871	0.197	0.018	-0.120	-0.380
TDN	0.077	0.506	0.800	-0.184	0.163	0.152
OM	-0.920	0.068	0.256	0.150	-0.224	-0.054
NFC	-0.531	-0.728	0.073	0.231	0.323	-0.100
TC	-0.856	-0.391	0.083	0.040	-0.096	0.078
ME	-0.916	0.105	0.209	0.174	-0.243	-0.096
FP	-0.686	0.396	-0.380	0.255	0.339	0.162
WSC	-0.417	-0.093	0.056	-0.479	0.630	-0.395
LA	0.496	0.102	0.565	0.599	0.177	-0.132
AA	0.626	0.146	0.483	0.519	0.288	0.019
PA	0.158	0.671	-0.349	0.509	0.088	0.308
BA	0.576	-0.605	0.451	-0.011	-0.030	0.062

DM: dry matter; CP: crude protein; EE: ether extract; CA: crude ash; CC: crude cellulose; ADF: acid detergent fibre; NDF: neutral detergent fibre; HEM: hemicellulose; TDN: total digestible nutrients; OM: organic matter; NFC: non-fibre carbohydrates; TC: total carbohydrates; ME: metabolizable energy; FP: fleig point; WSC: water soluble carbohydrates; LA: lactic acid; AA: acetic acid; PA: propionic acid; BA: butyric acid.

PCA analysis results are shown in Figure 1. PCA analysis, which has recently been applied to assess silage quality, is an excellent approach for identifying discriminative metabolites or markers following experimental intervention (Gallo et al., 2016). Larrigaudiere et al. (2004) found that variance disclosure rates of more than 70% were sufficient in PCA analysis. As a result, graphics up

to PC3 are provided in this study for better comprehension of the results (Figure 1). PC1, PC2, and PC3 explained 73.047% of the total variance. Furthermore, the total variance alone explained 33.854% of PC1, 21.980% of PC2 and 17.214% of PC3. In present study, eigenvalues were PC1: 6.771, PC2: 4.396, PC3: 3.443, PC4: 2.017, PC5: 1.543 and PC6: 1.018. In datasets, eigenvalue values larger than 1.0 are regarded to determine variance, according to the Kaiser rules (1960). As a consequence, the eigenvalue values of the first 6 PCs in this investigation seemed to be more than 1.0 (Table 5). Considering the factor loading values, pH, DM, CP, CA, OM, TC, ME, FP and AA from variation in PC1, EE, NDF, HEM, NFC, PA and BA from variation in PC2, CC, ADF and LA appears to be responsible for TDN, PC4 variation, and WSC for variation in PC5 (Table 5).

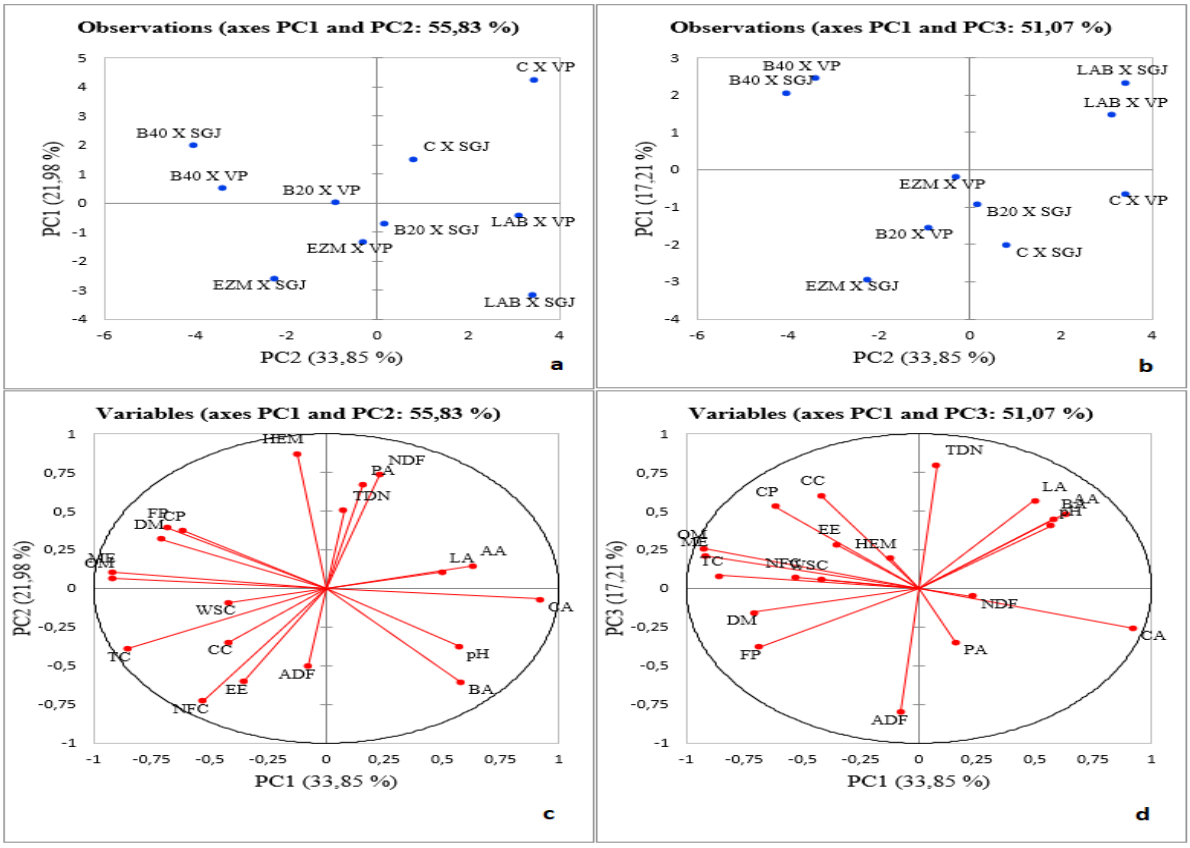

Figure 1. Scatter plots of the principal component analysis of the maize silages ensiled with different methods and additives.

((a; b) observations plots; (c; d) variables plots. C: no additive; B20: 20 g kg^{-1} grinded barley; B40: 40 g kg^{-1} grinded barley; LAB: a mixture of *Lactobacillus plantarum* and *Enterococcus faecium*; 1.5 g ton^{-1}; and EZM: LAB+enzyme mixture (cellulose; amylase and pentosanase enzymes); 2 g ton^{-1}; SGJ: standard glass jar; VP: vacuum packed; DM: dry matter; CP: crude protein; EE: ether extract; CA: crude ash; CC: crude cellulose; ADF: acid detergent fibre; NDF: neutral detergent fibre; HEM: hemicellulose; TDN: total digestible nutrients; OM: organic matter; NFC: non-fibre carbohydrates; TC: total carbohydrates; ME: metabolizable energy; FP: fleig point; WSC: water soluble carbohydrates; LA: lactic acid; AA: acetic acid; PA: propionic acid; BA: butyric acid)

Conclusion

The present study provided that maize silage chemical composition, nutritional content and fermentation parameters are similar in vacuum-packed and standard glass jar silages. Considering that the greatest difference between these two model methods is the ease of silencing, the vacuum method should be considered as an advantageous method. However, the most important condition to be questioned in the silo is that the same method was obtained with the glass method even though there was no procedure for vacuuming. In this case, it is considered that the compression applied to the samples is not important. And it can be suggested that other methods in which samples can be stored without air can also be used in ensiling. In addition, the differences between the two methods of different particle size should be determined in future studies.

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