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The Effect of Cultivar and Stage of Growth on the Fermentation, Aerobic Stability and Nutritive Value of Ensiled Quinoa

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ABSTRACT

Quinoa has the potential to be an important alternative source of silage as a forage crop. However, there is limited information on the ensiling of quinoa in the literature. This study investigates the silage fermentation quality, nutritive value and aerobic stability of quinoa cultivars harvested at different plant growing stages. The experiment was carried out in the experimental area of the Hatay Mustafa Kemal University, Faculty of Agriculture in the 2019 and 2020 growing seasons. The experiment was laid out in a split plot with a randomized block design with three replications, the three main plots were based on harvesting times (flowering, milky and dough stages) and the five sub-plots were based on cultivars (Mint Vanilla, Cherry Vanilla, French Vanilla, Red Head and Titicaca). Traits such as pH, ammonia nitrogen, lactic acid bacteria (LAB), LA, acetic acid (AA), butyric acid (BA), propionic acid, ethanol (EtOH), dry matter (DM), neutral detergent fiber, acid detergent fiber, acid detergent lignin, crude protein, ash, ether extract, water soluble carbohydrate (WSC) and relative feed value were analyzed in order to determine silage fermentation quality and nutritive value. In addition, all silages were evaluated in terms of aerobic stability. In reference to the interaction effects, pH, ammonia nitrogen, LAB, AA, BA and EtOH, the silage fermentation quality parameters were between 3.83-4.16, 5.57-14.83%, 4.69-5.80 $\log_{10}$cfu/g DM, 1.37-2.10%, 0.32-0.51% and 0.79-1.63, respectively. On the other hand, DM, ADF, ash and WSC changed between 21.95-33.36%, 22.39-28.36%, 15.41-17.70% and 2.35-9.50%, respectively, as silage nutritive composition features. The carbon dioxide production values of silages exposed to air were between 5.49 g/kg and 10.26 g/kg according to interactions. Among the evaluated quinoa cultivars, cv. Titicaca and cv. French Vanilla provided superior results in terms of fermentation quality compared to other cultivars. It was also determined that it would be more appropriate to harvest these superior quinoa cultivars during the dough stage for quality silage. Among the silages, the cv. Titicaca had the best aerobic stability. As a result of this study, it was concluded that cv. French Vanilla and cv. Titicaca should be harvested during the dough stage in order to obtain better silage quality. According to the results of this study, it was deduced that the quinoa plant could be an alternative ensiling crop.

Keywords: Aerobic stability, Alternative forage crops, Fermentation quality, Harvesting time, Quinoa cultivars, Silage

1. Introduction

Ensiling is one of the most widely used roughage storage techniques in many parts of the world (Silva et al. 2020). The loss of nutrients is relatively lower in silages obtained in accordance with silage making rules, when compared to the hay-making method. In many countries, silage production tends to be increasing in comparison to hay-making (Wilkinson & Taivonen 2003). Ensiling is a method applied to reduce nutrient loss, improve feed intake and to provide long-term preservation through the use of lactic acid (LA) fermentation under anaerobic conditions (Ertekin et al. 2022). An effective fermentation in the silo can be achieved thanks to sufficient dry matter (DM) (30-35%), water-soluble carbohydrates and epiphytic lactic acid bacteria (LAB) on chopped crop material (Kızılsimsek et al. 2016). With the proliferation of LAB in the silo, the water soluble carbohydrates (WSC) in the ensiled material are converted into various organic acids, mainly LA (Khota et al. 2016). Thus, there is a rapid pH drop in the silo and the growth of undesirable microorganisms is prevented. Finally, conditions for the conservation of the roughage are stabilized (Bao et al. 2016). The presence of sufficient fermentable carbohydrates among plant material is crucial in producing LA which is necessary in order to reduce pH and increase the silage quality during the fermentation process (Bai et al. 2011).

The quality of the silage depends plant genotypic characteristics, chopping length, DM content, crop digestibility and silage additives. The stage of maturity at harvest is another immensely significant factor in terms of the nutritional value of silage. The maturing process is highly complicated and contains numerous changes in the distribution and structure of plant organs which affect the fermentation process (Atis et al. 2013). The DM content of the crops at the time of harvest is also an important factor in obtaining better quality silage. The DM content of the plants is directly related to harvest time (Carmi et al. 2006).

The increasing importance of silage has revealed the need to investigate the ensiling properties of alternative forage plant sources. Quinoa (*Chenopodium quinoa*) is a crop basically produced for its seed. In recent years, it has been suggested that quinoa has also a high nutrient content, and its potential to be used as a whole-plant forage crop has been evaluated worldwide (Galwey 1992; Peiretti et al. 2013). On the other hand, it has been reported that using the quinoa plant a as a silage material in dairy farms would provide more protein and produce a better milk yield (Darwinkel 1997). Many studies have evaluated quinoa plants being an alternative feed source in animal nutrition in recent years and its superiority of feed efficiency and quality has been emphasized in various research articles (Fuentes & Bhargava 2011; Peiretti et al. 2013; Liu et al. 2017; Asher et al. 2020; Kaya & Kizil-Aydemir 2020; Tan & Temel 2020; Temel & Yolcu 2020; Shah et al. 2020; Liu et al. 2021). However, there only a few examples of research on quinoa silage (Erdogan & Koca 2020). There is no comprehensive study however on the preservation of the quinoa plant by ensiling. This suggests that the determination of the appropriate harvesting time is a very important factor for successful quinoa ensiling.

Quinoa is an important plant with the potential to be used as an alternative forage crop yet there is no comprehensive study on the preservation of quinoa by ensiling in the current literature. Therefore, the aim of the present study is to evaluate the effect of cultivar and stage of growth on the fermentation, aerobic stability and nutritive value of ensiled quinoa.

2. Material and Methods

2.1. Experimental area and its climatic conditions

This study was carried out in the Experimental Area of Hatay Mustafa Kemal University Faculty of Agriculture located in Reyhanlı, Hatay (36°15'13.56"N 36°30'7.96"E, altitude 96 m above sea level) in the 2019 and 2020 growth seasons. The experimental soil was clay loam with a pH of 7.12 (slightly alkaline), and an organic matter reading of 1.93% (low), P 7.41 mg/kg soil (moderate), lime 6.45% (moderate) and total salt 0.0078% (low). When climatic data (Figure 1) are investigated, the means of the temperature values of the growing seasons of 2019 and 2020 were recorded as 21.9 °C and 22.2 °C, and the total precipitation amounts were recorded as 162.4 mm and 101.8 mm, respectively.

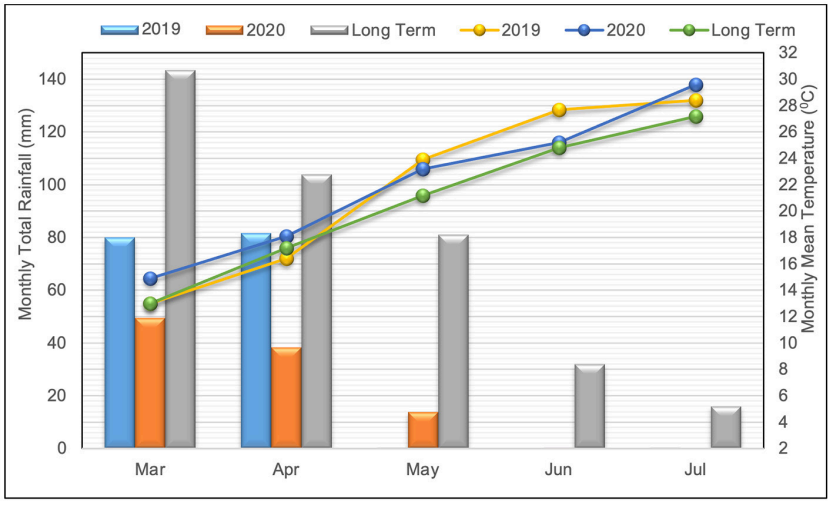

Figure 1- Monthly means of air temperature and total rainfall during the period from March to July of 2019 and 2020, as well as the long-term averages (1940-2019) in Hatay, Turkey. In the figure, the bars and lines show the monthly total precipitation and the monthly average temperature, respectively

2.2. The experimental design, cultivation and harvesting times

The experiment was laid out on a split plot in a randomized block design with three replicates, three main plots treatments aligned with harvesting times (flowering, milky and dough stages) and five sub-plots treatments were made up of the cultivars (mint vanilla, cherry

vanilla, french vanilla, red head and titicaca). Each sub-plot had 7 rows with 20 cm row spacing and 4 m row length. The sowing was performed by hand on 25 March 2019 and 27 March 2020. The seed rate was 10 kg/ha. The seeds were sown at a depth of 1-2 cm. When the seeds were sown, the soil was fertilized with 60 kg/ha N, P and K and then when the plants reached 50 cm in height, they were fertilized with 60 kg/da N as urea (Tan & Temel 2020). The plants were irrigated three times on 15 May and 30 May and 15 June in both years at field capacity with a drip irrigation system. The plants were harvested with a hand harvester at the flowering, milky and dough stages of quinoa plants.

2.3. The silage making, storage conditions and opening procedures

The quinoa plants were harvested according to the harvesting times, were chopped into 2-3 cm size for ensilaging via a chopping machine (CAN SP255, CANTEK MAKINA, Sinop, Turkey) and the chopped materials were ensilaged into 25 cm x 35 cm polyvinyl bags via an industrial vacuum packaging machine (CromPack VM 42 D. Istanbul, Turkey) with five replications for each treatment containing 400 g of fresh material. All silages were stored at 25 °C in a conditioning chamber for 90 days. The mini silos (bags) were opened after 90 days of fermentation.

2.4. Microorganism counting

The quinoa silages from all treatments were homogenized in 180 mL of Ringer's solution. Serial dilutions (from 10^{-1} to 10^{-10}) were prepared from this extract and inoculated into disposable sterile Petri dishes (90x15 mm) containing De Man Ragosa and Sharpe (MRS) agar for LAB, Malt Extract Agar (MEA) for yeast and Violed Red Bile Agar Glucose (VRBA-G) for *Enterobacteria* (Santos et al. 2014). These were incubated at 37 °C for 48 h for MRS and MEA and at 33 °C for 18 h for VRBA-G. The petri dishes presenting colonies' proliferation between 10 and 300 CFU (colony forming unit) were counted and recorded. The numbers of colonies of LAB, *Enterobacteria* and yeast were given as $\log_{10}$cfu/g DM.

2.5. Silage pH and fermentation end products

To determine pH changes of quinoa silages, a homogenized Ringer's solution obtained from quinoa silages was tested via a table-type pH meter (Inolab 8F93, Weilheim, Germany). Ammonia nitrogen (NH_3-N) was detected using distillation (Behrotest S2 KAS20, Dusseldorf, Germany) and titration methods based on the Kjeldahl procedure. The organic acids and alcohol such as lactic acid (LA), acetic acid (AA), butyric acid (BA), propionic acid (PA) and ethanol (EtOH) were detected using the methodology described by Siegfried et al. (1984) in a Shimadzu high-performance liquid chromatography system (Shimadzu KC-811, Kyoto, Japan) at a 42 °C, 0.6 mL/min flow rate via refractive index detector following a cleaning of the samples.

2.6. The aerobic stability

After a 90-day ensiling period, the laboratory silages and the bottle system described by Asbell and Stenson (1982) were opened, which is one of the most commonly used methods (Filya 2003; Filya 2004; Koç et al. 2021). This bottle system is based on trapping CO_2 gas in the KOH solution, and was used to determine the aerobic stability of quinoa silages. With this method, the silages were exposed to air for 5 days, and the aerobic stability of the silages was evaluated in terms of CO_2 production, pH level, number of *Enterobacteria* and yeast and mold.

2.7. The dry matter of the silages

Samples dried at 65 °C for 48 hours in an oven-drying cabinet were milled to pass a 1 mm sieve for the preparation for a chemical analysis. The DM contents of samples from quinoa silages were determined by a drying-oven cabinet at 105 °C for 24 hours (AOAC 2019).

2.8. The nutrient content

The crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and WSC properties of quinoa silages were investigated in order to determine their nutrient content. The CP and EEs were determined according to the Kjeldahl method and an extraction method using diethyl ether solvent, respectively (AOAC 2019). The ash content was detected by burning the samples in an ash furnace at 550 °C for 4 hours. The cell-wall components such as NDF, ADF and ADL were analyzed with an ANKOM Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA) according to the method described by Van Soest et al. (1991). WSC were analyzed according to the phenol-sulfuric acid method described by Dubois et al. (1956).

2.9. The nutrient compositions of fresh quinoa

The nutrient content data of the fresh quinoa were presented in our previously published article (Yilmaz et al. 2021). This data can be reviewed from this article if necessary.

2.10. Statistical analysis

The statistical analysis of the data obtained from the current study was performed using the statistical program of JMP 13 software. The general linear model was used to determine the differences among the means of cultivar, harvesting time and their interactions according to the split plot in the randomized complete blocks model. The Tukey pairwise ($p<0.05$) test was used to group factor levels.

3. Results

3.1. The silage pH, lactic acid bacteria and fermentation end-products

The effects of cultivar, harvesting time and interaction on pH, ammonia nitrogen and LAB were determined to be statistically significant (Table 1). The pH levels of the interactions are given in Figure 2a. The pH level among the interactions varied between 3.83 and 4.16. The lowest pH was obtained from cv. Titicaca, harvested at the flowering stage. However, the cv. Titicaca harvested at the flowering stage was statistically in the same group as the cv. Red Head harvested also at the flowering stage. The highest pH level was found in cv. Mint Vanilla harvested at also flowering stage. The ammonia nitrogen content among the interactions ranged from 5.57% to 14.83% (Figure 2b). The highest ammonia nitrogen content was obtained from cv. Red Head harvested at the dough stage, whereas the lowest value was found in the cv. French Vanilla harvested at flowering stage. The LAB numbers among the interactions ranged from 4.69 $\log_{10}$cfu/g DM to 5.80 $\log_{10}$cfu/g^{-1} DM (Figure 2c). While the highest LAB number was obtained from cv. Mint Vanilla harvested at milky stage, the lowest value was recorded in cv. French Vanilla harvested at dough stage.

Table 1- Anova test results of all the characteristics examined in this study

Items	*Characteristics*	*Cultivar*	*Harvesting time*	*Interaction*
Fermentation quality	**pH**	<0.0001	0.0001	<0.0001
	Ammonia nitrogen	0.0001	<0.0001	<0.0001
	Lactic acid bacteria	<0.0001	0.0405	0.0001
	Lactic acid	0.0035	0.0002	ns
	Acetic acid	<0.0001	0.0034	0.0125
	Butyric acid	ns	<0.0001	0.0325
	Propionic acid	ns	0.0010	ns
	Ethanol	<0.0001	0.0002	0.0007
Aerobic stability	**Carbon dioxide**	<0.0001	ns	<0.0001
	pH	<0.0001	<0.0001	<0.0001
	Enterobacteria	<0.0001	<0.0001	<0.0001
	Yeast and Mold	<0.0001	<0.0001	<0.0001
Nutritive value	**Dry matter**	<0.0001	<0.0001	<0.0001
	NDF	<0.0001	0.0247	ns
	ADF	<0.0001	0.0036	0.0002
	ADL	ns	0.0029	ns
	Crude protein	ns	0.0008	ns
	Ash	<0.0001	0.0017	<0.0001
	Ether extract	0.0008	0.0021	ns
	WSC	<0.0001	0.0020	<0.0001
	Relative feed value	<0.0001	0.0460	ns

pH: Power of hydrogen, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, WSC: Water soluble carbohydrate

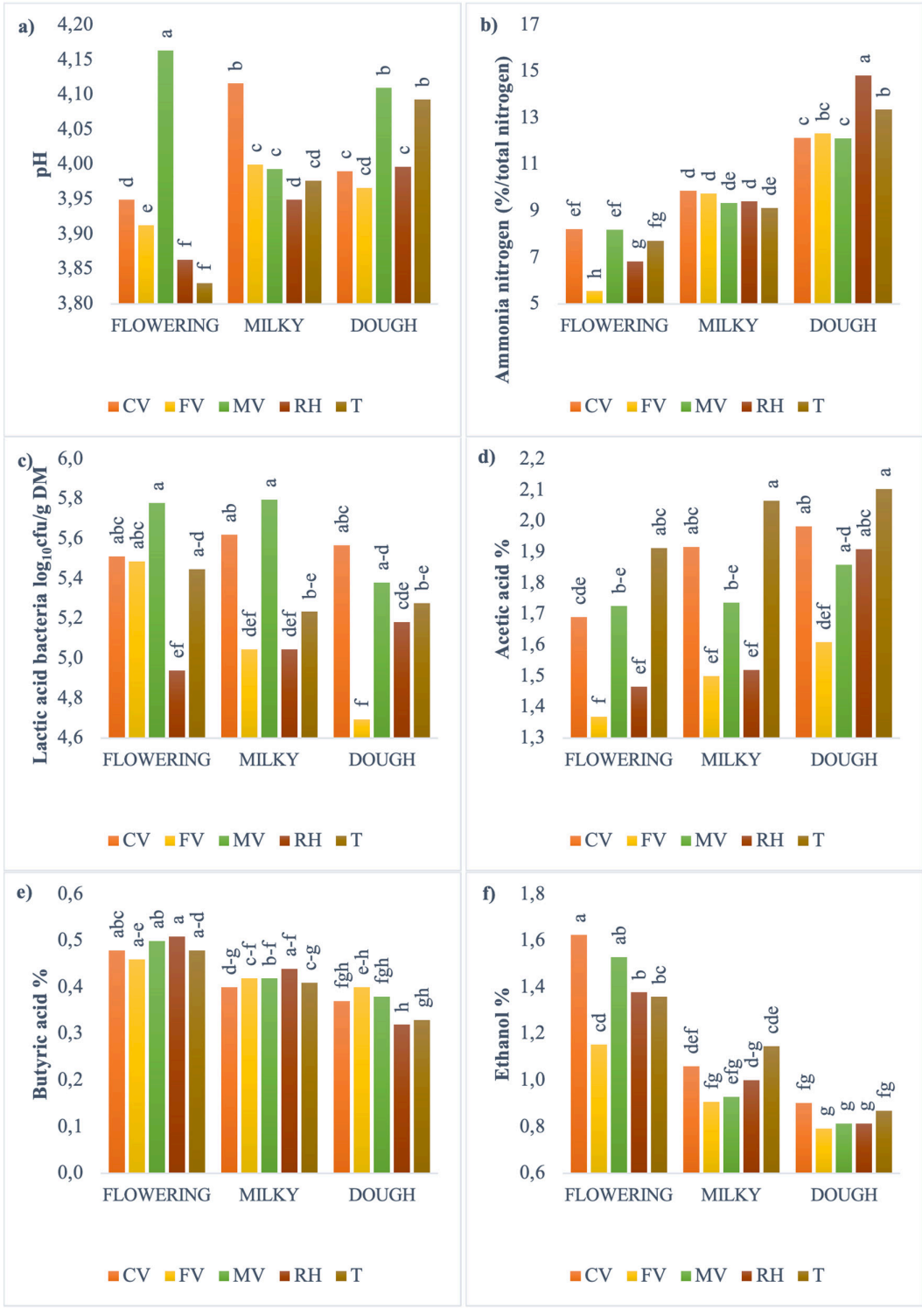

Figure 2- pH, ammonia nitrogen, lactic acid bacteria, acetic acid, butyric acid and ethanol characteristics of the evaluated silages according to the interactions

The cultivar and harvesting time changed the LA content of the silages while their interactions did not (Table 1). The LA ratio among the cultivars varied between 4.28% and 4.85%. The highest LA content was obtained from cv. French Vanilla (Table 2). Moreover, except for cv. Cherry Vanilla, other cultivars were statistically similar with cv. French Vanilla. The LA content of cultivars which had varying harvesting times increased as the harvesting time was delayed. The dough stage gave a very high LA content (5.48%). The effects of the harvesting time on PA were significant yet the cultivar and interaction were insignificant (Table 1). The PA values varied

between 0.67% and 0.86% among the harvesting times (Table 2). The highest PA was obtained from the flowering stage whereas the lowest was at the dough stage. The effects of cultivar, harvesting time and their interactions on AA content were significant (Table 1). The AA contents in the interactions were determined to be between 1.37% and 2.10% (Figure 2d). The highest AA content was obtained from cv. Titicaca, harvested at the dough stage, whereas the lowest value was found in cv. French Vanilla, harvested at the milky stage. The harvesting time and interaction changed the BA content of silages while cultivar did not (Table 1). As the harvesting times were delayed, the BA contents of all the cultivars decreased (Figure 2e). The highest BA content in the interaction was recorded in cv. Red Head harvested at the flowering stage, while the lowest was in the same cultivar harvested at the dough stage. There was a significant effect of cultivar, harvesting time and their interaction on the EtOH content of quinoa silages (Table 1). It was found that the EtOH contents in interactions were between 0.79% and 1.63% (Figure 2f). The highest EtOH content was obtained from cv. Cherry Vanilla harvested at flowering stage while the lowest EtOH content was recorded in cv. French Vanilla, harvested at the dough stage.

3.2. The aerobic stability of the silages

The carbon dioxide (CO_2), pH, *Enterobacteria* and yeast and mold properties were used as silage deterioration indicators in order to evaluate the aerobic stability of the silages, and these were significantly affected by genotype, harvesting times and interaction (Table 1). The CO_2 production among the interactions varied between 5.29 and 10.26 g/kg (Figure 3a). The highest CO_2 production was obtained from cv. Mint Vanilla, harvested at the flowering stage, while the least CO_2 production was in cv. French Vanilla harvested also at the flowering stage. The pH level of the silages exposed to air ranged from 4.15 to 6.24 (Figure 3b). The highest pH level was determined in

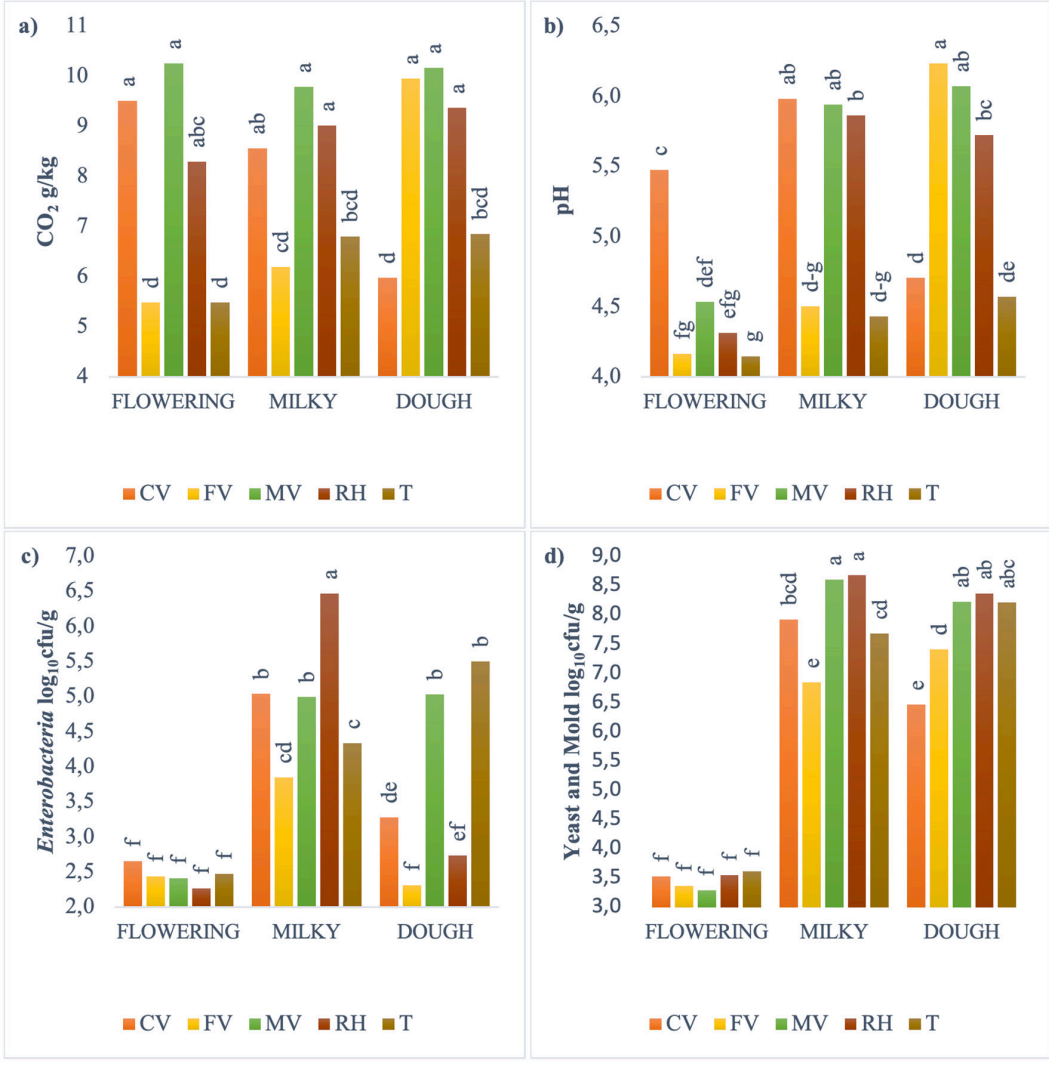

Figure 3- CO_2, pH, *Enterobacteria* and yeast and mold characteristics of the evaluated silages assessed for aerobic stability according to the interactions

cv. French Vanilla harvested at the dough stage, whereas the lowest pH level was found in cv. Titicaca harvested at the flowering stage. The *Enterobacteria* numbers of the silages under the interaction effects varied between 2.27 and 6.47 $\log_{10}$cfu/g (Figure 3c). The least and the highest *Enterobacteria* numbers were obtained from the cv. Red Head genotype, yet the highest and the lowest were found in the plants harvested at the milky stage and at the flowering stage, respectively. The yeast and mold numbers of the interactions ranged from 3.29 to 8.68 $\log_{10}$cfu g^{-1} (Figure 3d). The highest yeast and mold numbers were detected in cv. Red Head harvested at milky stage. The lowest yeast and mold numbers were obtained from cv. Mint Vanilla harvested at the flowering stage, in addition, the yeast and mold numbers of the other cultivars harvested at the flowering stage were statistically similar to those of cv. Mint Vanilla.

3.3. The nutrient contents of silages

The effects of cultivar, harvesting time and interaction on the DM of the silages were significant (Table 1). The DM contents of the interactions are given in Figure 4a. Among the interactions, the DM contents varied between 21.95% and 33.36%. The highest DM was obtained from Red Head harvested at the dough stage while the lowest DM was recorded in cv. Cherry Vanilla harvested at the flowering stage. The NDF content was affected by the cultivars and the harvesting times significantly (Table 1). The effects of the interaction on NDF content were not significant (Table 1). The NDF contents of the cultivars varied between 38.62% and 44.22% (Table 2). The lowest NDF content was obtained from cv. Titicaca. The NDF concentrations of cv. Cherry Vanilla, cv. Cherry Vanilla, cv. Mint Vanilla and cv. Red Head were statistically in the same group. The lowest NDF content among the harvesting times was recorded at the flowering stage. The NDF contents at the milky and dough stages were similar to each other.

The cultivar, harvesting time and their interaction on the ADF content of silages all had an effect on the results (Table 1). The ADF concentrations of interactions varied between 22.39% and 28.36% (Figure 4b). The lowest ADF content was obtained from cv. Titicaca harvested at the dough stage whereas the highest value was determined in cv. Mint Vanilla harvested at the milky stage. The ADL content was affected by harvesting time, yet not affected by cultivar and interaction (Table 1). The ADL contents of harvesting times were recorded between 4.07% and 4.79% (Table 2). It was observed that the ADL content of the silages decreased as the harvesting time was delayed.

Neither cultivar nor interaction had a significant effect on the CP contents of the silages (Table 1). However, the harvesting time significantly influenced the contents of the CP of the silages evaluated (Table 1). The highest CP (10.98%) among the harvesting times was recorded at the flowering stage (Table 2). As the harvesting time was delayed, the CP content of the silages decreased.

Table 2- Averages and mean comparison test results of lactic acid, propionic acid, NDF, ADL, crude protein and ether extract properties of silages depending on the main effects

Cultivars	***Lactic acid (DM%)***	***Propionic acid (DM%)***	***NDF (DM%)***	***ADL (DM%)***	***Crude Protein (DM%)***	***Ether Extract (DM%)***
Cherry Vanilla	4.28±0.25^{B}	0.75±0.03	44.21±0.50^{A}	4.53±0.11	9.70±0.32	2.43±0.06^{A}
French Vanilla	4.85±0.27^{A}	0.74±0.03	42.38±0.82^{A}	4.36±0.17	10.16±0.35	2.39±0.05^{A}
Mint Vanilla	4.61±0.28^{AB}	0.77±0.04	44.22±0.84^{A}	4.52±0.13	9.78±0.32	2.45±0.06^{A}
Red Head	4.64±0.29^{AB}	0.75±0.03	41.91±0.47^{A}	4.56±0.19	9.68±0.37	2.32±0.05^{B}
Titicaca	4.41±0.29^{AB}	0.77±0.04	38.62±0.45^{B}	4.32±0.11	10.01±0.28	2.30±0.04^{B}
Harvesting times						
Flowering	3.69±0.09^{C}	0.86±0.02^{A}	40.70±0.45^{B}	4.07±0.06^{B}	10.98±0.13^{A}	2.20±0.02^{C}
Milky	4.50±0.10^{B}	0.74±0.02^{B}	43.44±0.57^{A}	4.52±0.09^{A}	9.61±0.13^{B}	2.42±0.02^{B}
Dough	5.48±0.08^{A}	0.67±0.01^{C}	42.67±0.89^{A}	4.79±0.09^{A}	9.01±0.12^{C}	2.52±0.03^{A}

^{A,B,C}Means in the same column with different superscripts are significantly ($p<0.05$) different from each other. NDF: Neutral detergent fiber, ADL: Acid detergent lignin, DM: Dry matter

The effects of the cultivar, harvesting time and interaction on the ash content of the silages were significant (Table 1). The ash contents of the interactions were recorded between 15.41% and 17.70% (Figure 4c). The highest ash concentration was obtained from cv. Mint Vanilla harvested at the flowering stage while the lowest value was found in cv. French Vanilla harvested at the milky stage.

The cultivar and harvesting time had an effect on the EE contents of the silages, while the EE content was not influenced by interactions (Table 1). The EE values among the cultivars ranged from 2.30% to 2.45% (Table 2). The highest EE content was obtained from cv.

Mint Vanilla whereas the lowest value was recorded in cv. Titicaca. There was an increase in the EE content of the silages from the flowering stage to the dough stage (Table 2). The highest EE content was determined to be 2.52%.

The effects of the cultivar, harvesting time and their interactions on the WSC content of the silages were significant (Table 1). The WSC concentrations among the interactions ranged from 2.35% to 9.50% (Figure 4d). While the highest WSC content was found in cv. Titicaca harvested at the dough stage, the lowest WSC amount was determined to be in cv. Cherry Vanilla harvested at the flowering stage.

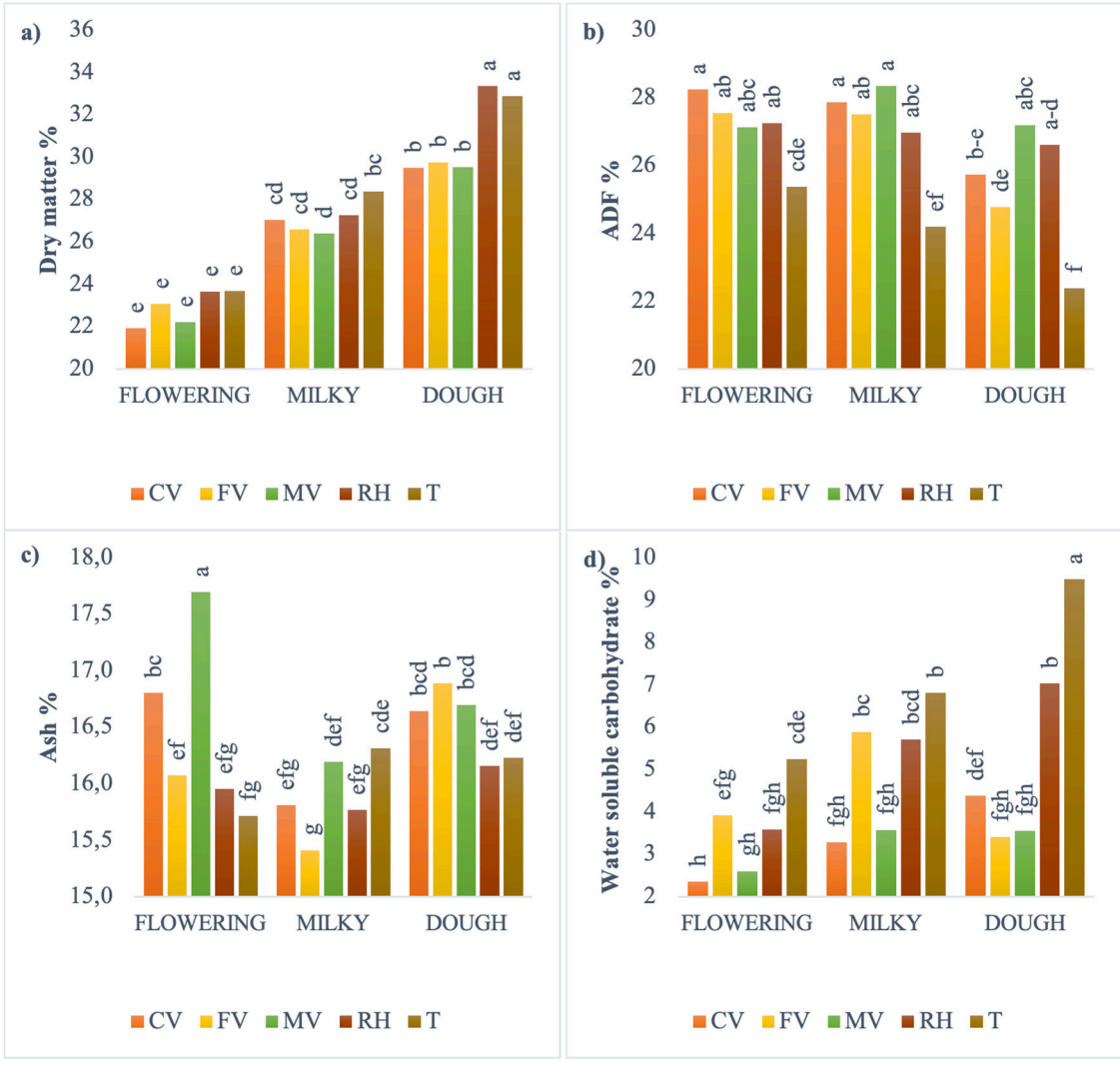

Figure 4- Dry matter, ADF, ash and water soluble carbohydrate characteristics of the evaluated silages according to the interactions

4. Discussion

4.1. The silage pH, lactic acid bacteria and fermentation end products

The difference in the response of the cultivars in terms of pH during the harvest period caused the interaction to be significant. A similar situation was reported for Kenaf by Ryu et al. (2016). Generally, the pH level of silages made from quinoa cultivars interestingly increased as the harvesting time was delayed. This result was probably due to a low DM content at the early stage and the high ash content (Table 1) of the fresh plants during the dough stage (Kung & Shaver 2001; Kung et al. 2018). In previous studies, it was determined that the silage pH levels changed according to the genotypic characteristics of different plant species (Ryu et al. 2016; Tolentino et al. 2016). Also, it was stated that the pH level of the two wheat cultivars changed with a slight fluctuation from early to late harvesting time (Filya 2003). Filya (2004) and Demirel et al. (2006) reported that the pH levels of the various silages increased as the harvesting time was delayed, similar to the pH results obtained from the present research.

The pH level of the silages is the most basic feature in explaining the fermentation quality. Generally, most plants have a pH level of 5.5-6.0 when chopped for silage. The final pH of the silages is affected by many factors, but the most important factors are the LA concentration of silages and the buffering capacity of the forage crops (Kung et al. 2018).

The amounts of LAB in quinoa silages at early harvesting times were higher than those of later harvesting times. The amounts of LAB in quinoa cultivars were affected by cultivar variation. The numbers of LAB obtained from the current study decreased as the harvesting time was delayed. The number of LAB in the plant epiphytic flora and the number of LABdetected in the silo are inversely related (Table 1). Probably the environmental conditions in the silo changed the proliferation of LAB. As a matter of fact, Whiter and Kung (2001) observed that the amount of LAB may be low in silages with high DM. During the fermentation process, LA is produced by LAB, especially homo-LAB, which is the major acid indicator for silage quality (Kung et al. 2018). Generally, the LA contents of silages vary between 2% and 4% (Kung & Shaver 2001). The LA contents of the quinoa cultivars changed according to both the cultivars and harvesting times in this study (Table 1). As the water-soluble carbohydrate content of fresh plants increased, the LA contents of the silages increased as expected. Podkówka et al. (2018) reported that the LA content of quinoa silages was between 1.83% and 1.92%. In addition, Salama et al. (2021) reported that the LA content of their quinoa silage was 3.02%. The LA contents obtained from the present study were higher than those of the previous literature reports (Table 2). Although the DM of the silages was lower (<30%), the LA contents obtained from the silages were higher (>4%) than the reported limit values. Liu et al. (2021) reported that the LA content was 5.69% in silage made from barley crops containing approximately 30% DM. LAB produce some organic acids, mainly LA, using the water-soluble carbohydrates in the plant (Kung & Shaver 2001). Depending on the epiphytic flora content of forage crops, these bacterial species may vary and the fermentation end products occurring in silage may differ (Kung et al. 2018).

The AA content varied between 1.37% and 2.1% among all treatments. These values were within the values reported for silages (Demirel et al. 2006). In general, the AA content tends to increase with the advancement of plant maturity. AA is the acid found in the second higher fermentation end product in the silages, and AA concentrations of the silages change from 1% to 3%. The moderate amount of AA in the silages delays their deterioration when they are exposed to air (Kung et al. 2018). Higher than normal concentrations (3-4%) of AA are usually found in silages dominated by *L. buchneri* bacteria (Kung 2010). Silages with high buffering capacity (high ash and protein content) may contain higher AA (Kung et al. 2000).

The BA contents of the cultivars decreased as the harvesting times were delayed (Figure 2e) and the BA contents of the quinoa silages were higher than 1.0 g/kg DM, as reported by Pinho et al. (2016). The BA contents found in the silages in this study may be considered to be high (Figure 3b). This may be due to the high moisture (Table 1) content of the silage materials of the quinoa cultivars, and may indicate that there was a *clostridial* activity in the silo during the fermentation process. The presence of BA in the well-fermented silages is indicative of the metabolic activity of undesirable *clostridial* microorganisms, which causes a large loss of DM and low energy recovery (Pahlow et al. 2003). Especially in the wetter silages, the activity of *clostridial* microorganisms is likely and they can convert the LA in the silage into BA (Kung et al. 2018).

The silage PA contents obtained from this study were higher than the values reported within optimistic limits (Table 2). The decrease in the PA content due to the delay in harvesting indicates that a late harvest may be more suitable for the purposes of this study due to increased silage quality. This can be explained by the low DM content. A high PA (0.3-0.5% DM) was found in wetter silages associated with poor fermentations (Kung & Shaver 2001). This may be due to the PA bacteria activity in the silo, as the crops used as silage material in the study were wetter (>70%). PA is generally absent or very little (<0.01%) in good silages and this acid may be undetectable in the silages with higher concentrations of DM (35-45% DM) (Kung 2010). The PA in the silages is formed by the conversion of LA and this acid is created by PA bacteria (Kung et al. 2018). Although the PA content varied depending on the cultivars, the EtOH content decreased with the delayed harvesting time (Figure 2f). As expected, the EtOH content of the silages decreased as the DM of the silage materials (fresh crops) increased. The highest EtOH content value detected in the current study was 1.63% and this value is within the acceptable limits for silage. Normal amounts of EtOH in the silages range from 1% to 2% DM (Kung 2010). A small amount of EtOH like this (<2% DM) is converted to AA in the rumen by the animal and this acid can be absorbed in the rumen wall of the animal (Bruning & Yokoyama 1988). AA can be converted into milk fat or used for body metabolism or growth (Kung et al. 2018). EtOH is the most common alcohol in silages and a high EtOH content in silages is usually associated with the activity of hetero-LAB, *Enterobacteria* and yeasts in the silo or the water soluble content of the silage material (Kung & Shaver 2001).

4.2. The aerobic stability of the silages

After 120 h of exposure to the air, the CO_2 emissions and the pH of the quinoa silages were harvested at different harvesting times, and this amount increased as the harvesting time was delayed (Figure 3a and Figure 3b) Also, as the harvesting time was delayed, the

number of *enterobacteria* (Figure 3c) and yeast and mold (Figure 3d) increased in the silages. It was determined that silages were more prone to aerobic deterioration as the harvest time was delayed. This may be due to the higher yeast count and water-soluble carbohydrate content in the fresh plants harvested later (Table 1). The aerobic stability of the cv. Titicaca genotype was found to be better at the flowering stage. The presence of oxygen during the storage and opening of the silage supports the growth of aerobic microorganisms in the silage and the growth of these microorganisms on the silage surface reduces the quality of the end product, and causes high nutrient loss (Pozza et al. 2011). Yeasts and molds grow on substrates such as sugars, as well as LA, both of which are important for silage. In the most cases, yeasts and molds are the first community of microorganisms to develop when silage comes into contact with oxygen (Muck 2010). AA has a powerful antifungal activity against aerobic deterioration (Kung & Ranjit 2001) and this acid was found to be higher in cv. Titicaca than that of the other cultivars (see Figure 2d). Therefore, the aerobic stability in cv. Titicaca was better than others.

4.3. The nutrient contents of silages

The DM of the silages is very important in obtaining a high silage nutrient quality (Borreani et al. 2018). As the harvesting times were delayed, the DM contents of the silages increased (Figure 4a). The DM contents of the silages varied significantly among the cultivars. Moreover, the DM contents of the silages were similar to those of the fresh plants (Table 1). Tolentino et al. (2016) determined that the DM contents of the silages obtained from different sorghum cultivars changed according to the cultivars. It has been reported that the silage DM content increases with the delay in the harvest time of the plants for silages made with different plants (Filya 2004; Demirel et al. 2006; Atis et al. 2013). As a matter of fact, the DM contents obtained from the present study were similar to that which was reported in the previous literature. The NDF and ADF contents of forages were fairly significant since cellulose and hemicellulose are digested to a certain extent by ruminant animals (Canpolat & Karaman 2009). The NDF content of the silages fluctuated as the harvesting times of the cultivars were delayed (Table 2). The NDF and ADF contents of the silages were slightly lower than those of the fresh plants (Table 1). This result can probably be explained by the presence and diversity of some enzymes that can exist in silages (McDonald et al. 1991). Also, the loss in the NDF and ADF content during fermentation process is minimal (McDonald et al. 1991), and this loss generally does not constitute a disadvantage in animal nutrition for most forage crops (Khota et al. 2016). Shah et al. (2020) reported that the NDF contents of different quinoa cultivars varied depending on the harvesting time. The ADF content decreased as the harvesting time was delayed in the different quinoa cultivars (Figure 4b). Shah et al. (2020) reported that the ADF content in the anthesis and grain filling stages of different quinoa genotypes ranged from 17.5% to 26.8% and 21.8% to 30.6%, respectively. Peiretti et al. (2013) determined that the ADF content of quinoa in six different harvesting times varied significantly. The ADF values in this study were within the limits reported in the above mentioned literature. As the harvesting time was delayed, the ADL content of the different quinoa cultivars decreased (Table 2). It was reported that the cell wall components in the feeds are directly affected by the cutting time of the harvested plants (Yavuz 2005; Tekce & Gül 2014). The CP content of quinoa genotypes decreased as the harvesting time was delayed (Table 2). The CP values of ensilaged quinoa cultivars were not significant. Similar results were reported for different forage plants by Butler and Muir (2003) and Nabi et al. (2006), and for different quinoa cultivars by Uke et al. (2017) and Liu et al. (2021). The results of the research indicated that quinoa had a higher protein content compared to forage maize and sorghum (Atis et al. 2013; Uke et al. 2017). The ash content of the silages fluctuated as the harvesting time was delayed in the different quinoa genotypes (Figure 4c). Liu et al. (2021) reported that the crude ash content of quinoa forage varied according to the phonological stages. In the present study, the ash content of the quinoa silages was higher than silages of many forage crops. The higher ash content in the feeds reduces the metabolizable energy content (Kung et al. 2018). The EE content of the silages increased as the harvesting time was delayed (Table 2). On the contrary, Uke et al. (2017) found that the EE contents of quinoa decreased with the delayed harvesting time. As the harvest time was delayed, the seed formation rate in the plants increased and, accordingly, the EE contents of silages (Table 2) increased. As a matter of fact, Liu et al. (2021) reported that the EE contents of quinoa plants increased as the harvest time was delayed. In addition, the high ether EE increases the total digestible nutrients and, as a result, the metabolizable energy content of the forage is increased (Khota et al. 2016). During the ensiling process, the WSC plays a critical role in silage fermentation, and it is used as a fermentable substrate in the silo in fermentation's the early stages (Silva et al. 2020). Feeds such as silages rich in water-soluble carbohydrates are an ideal energy source during early lactation, as they provide both the energy for milk production and the structural fiber needed to support chewing and rumen buffering (Klevenhusen et al. 2019). Therefore, it is very important to know the water-soluble carbohydrate content of the silages. The WSC content varied according to the cultivar (Table 1), and increased as the harvesting time was delayed in the different quinoa cultivars (Figure 4d).

5. Conclusions

This study was intended to address the silage fermentation quality, aerobic stability and nutritive value of different quinoa cultivars (harvested at varying times), which may have the potential to help address deficits in roughage production, especially in developing countries. Among the evaluated quinoa cultivars, cv. Titicaca and cv. French Vanilla gave superior results in terms of fermentation quality than the other cultivars. Among the quinoa cultivars, cv. Titicaca was the cultivar with the highest silage nutritive value. It was determined that it would be more appropriate to harvest these superior quinoa cultivars (cv. French Vanilla and cv. Titicaca) during the dough stage for a better silage quality (for fermentation quality, as well as the nutritive value and aerobic stability). Among the silages' forms, the cv. Titicaca that were harvested at the flowering stage had the best aerobic stability. As a result of this study, it was determined that cv. French Vanilla and cv. Titicaca should be harvested during the dough stage to obtain better silage quality.

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