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FERMENTATION PROPERTIES AND NUTRITIVE VALUE OF SUNFLOWER ENSILED WITH DIFFERENT MIXING RATIOS OF SILAGE MAIZE, SWEET CORN AND SWEET SORGHUM

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Abstract: In recent years, with the effect of global warming, researches on the potential of plants that are more resistant to drought as forage crops and their ensiling properties have become increasingly widespread. Current study aimed to investigate the ability of ensiling, fermentation quality and nutritive value of a sunflower variety, which is more drought resistant than many forage crops, with silage corn, sweet corn and sweet sorghum at different mixing ratios. Plant species used as silage material in present study were grown simultaneously in separate plots. All plants were harvested by hand in the range of about 25-32% dry matter and these harvested crops were theoretically chopped with 2-3 cm electric shredding machine for silage. In addition to pure silages of all crop materials, mixed silages of 25%+75%, 50%+50% and 75%+25% were made in 3 replications. Besides some properties of silage beginning materials, some fermentation properties and feed quality parameters of resulted silages were investigated. As a result of this study, 50%+50% mixed silage of the sunflower variety used as silage material with maize, sweet corn and sweet sorghum increased the silage fermentation quality compared to the pure sunflower silages and improved the silage feed quality compared to the pure cereal (corn, sweet corn and sweet sorghum) silages.

Keywords: Silage, Mixed silage, Fermentation, Nutritive value, Sunflower

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1. Introduction

Ensiling is described as preserving technology of fresh crops and their by-products based on lactic acid fermentation process under anaerobic conditions (Ertekin and Kızılşimşek, 2020; Ertekin et al., 2022). Preserved materials can be presented to livestocks when attainability to fresh forages is difficult especially in winter period. Lactic acid bacteria found on ensilaged fresh material surface produce the organic acids mainly lactic acid by using water soluble carbohydrates (WSC) and it is preserved fresh crops (Ertekin and Kızılşimşek, 2020) in the silo. Silage fresh materials which has a low water soluble carbohydrates and not produce effectively suitable fermentation end products can be ensiled with sugar sources like molasses, glucose and crops with high WSC etc. (Kung et al., 2018). Crops with high WSC have been commonly used as sugar source in mixed silages.

Sunflower (H*elianthus annus* L.) is a mainly important oil crop around the World due to its physical, chemical and nutritive properties (Souza et al., 2005). This plant has become substantial today, when we feel the effect of global warming even more in terms of using the water in the soil efficiently and being more resistant to drought (Tomich et al., 2003). It is inevitable to have an alternative plant, especially in agricultural lands and marginal areas where water is limited. On the other hand, many studies have been carried out to evaluate this plant as silage and to include it as an alternative forage plant (Ozduven et al., 2009; Cruvinel et al., 2017; Temür et al., 2021; Yıldız et al., 2022). As a result of these studies, it has been reported that the plant can be an alternative forage source, especially in areas where irrigation is limited or no irrigation possibility is available. In addition, it has been suggested to ensilage the plant with various WSC sources to be used as silage.

In recent years, silage of plants as a mixture has become a practical method for silage fermentation quality and nutritional value. In this context, there are many current studies in the literature (Wang et al., 2019; Wang et al., 2020; Zeng et al., 2020; Mu et al., 2021; Wang et al., 2021 Li et al., 2022). It is accepted as a practical method to ensilage the plants with high WSC content as a source of WSC during ensiling, by mixing them with plants which have various cultivation and feeding advantages. Silage maize, sweet sorghum and sweet maize have adequate WSC content to obtain good quality silage (Kizilsimsek et al., 2017; Wang et al., 2019; Ertekin 2021; Ertekin and Yilmaz 2022). Ensiling these crops with crops containing low WSC can create a quality fermentation process.

Present study was carried out to determine the effect of mixtures of sunflower with silage maize, sweet maize and sweet sorghum on silage fermentation quality and nutritive value.

2. Materials and Methods

Cultivars of hybrid snack sunflower (cv. F400), hybrid silage maize (cv. BATEM 7255) sweet maize (cv. BATEM tatli) and sweet sorghum (cv. Erdurmuş) were cultivated on separate parcels in Field 49 area of Hatay Mustafa Kemal University in 2022 growing season. Cultivated all these crops were harvested at same date (July 30, 2022). Cultivars of silage maize, sweet maize and sweet sorghum were harvested about 30±2% dry matter content while hybrid sunflower cultivar was harvested in dough stage with about 25% dry matter. Harvested crops were chopped theoretically 2-3 cm theoretical lenght via chopping machine and silage maize, sweet maize and sweet sorghum crops were ensilaged with snack sunflower by mixing at rate of 25%+75, 50%+50% and 75%+25%. In addition, pure silages of all component crops were also made to compare the pure silages with mixture. Before ensiling, 500 g fresh material was taken from both mixture and pure samples to obtain dried samples for chemical analysis of the initial materials. Chopped fresh material was ensiled to vacuum bag as 300±50 g via vacuum packaging machine. The laboratory type plastic silos were stored in dark conditions at room temperature during 75 days. At the end of fermentation, silage samples were opened and about 100±20 g of samples were taken for chemical composition analysis. According to Yan et al. (2019), 20 g fresh silage was weighted and 180 ml ringer solution was added to weighted silages. Samples mixed with silage and ringer solution were blended during 1 minutes. It was obtained filtered samples from blended samples. These filtered samples were used for some analysis.

At the end of fermentation process, the silages obtained mixtures and pure fresh material and initial materials were analyzed to determine dry matter (DM), crude ash (CA) and crude protein (CP) according to procedure of AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined with ANKOM technology according to methods of Van Soest et al. (1991). Acid detergent lignin (ADL) contents of the silage and fresh materials were evaluated baker method using 72% sulfuric acid. Water soluble carbohydrates (WSC) of fresh materials were determined according to phenol-sulfuric acid method described by Dubois et al. (1956). Contents of lactic acid (LA), acetic acid (AA), propionic acid (PA) butyric acid (BA) and ethanol (ETOH) were analyzed by using high pressure liquid chromatography at 42°C, 0.6 mL min^{-1} flow rate and by using refractive index detector described by de Quiros et al. (2009) after the sample cleaning procedure. The silage pH was determined via table typed pH meter in filtered liquid samples isolated from fresh silages according to the Yan et al., (2019). Ammonia nitrogen (NH_3-N) contents of silages were analyzed with distillation unit of Kjeldahl apparatus using 100 mL filtered silage liquid samples.

Opened silage samples and fresh silage material were investigated in terms of silage microbial content. In this contexts, Filtered samples isolated from both fresh material and silage material were diluted from 10^{-1} to 10^{-10}. Diluted samples were spread in disposable sterile plastic petri dishes and to determine the lactic acid bacteria and yeast and mold, it was used MRS agar (De Man Ragosa and Sharpe) and MEA (Malt Extract Agar) nutrient media, respectively. Prepared petri dishes with filtered samples and nutrient media were put in an incubator. These samples were allowed to proliferate for lactic acid bacteria and yeast and mold during 48 hours at 37°C.

All data obtained from current study were evaluated according to general linear model using SAS JMP 13.0 statistical package programme. Traits found significant in 0.05 probability level were compared Tukey pairwise test.

3. Results and Discussion

The dry matter (DM), chemical compositions and microbial counts of silage beginning materials are given in Table 1. Investigated properties for DM, chemical compositions and microbial counts expect for mold were significant among plant species. These results indicated that there are vital differences among plant species in terms of silage beginning characteristics. The most quality crop in terms of nutritive value was sunflower. The highest WSC content and LAB count were determined in sweet sorghum.

Results given for NDF, ADF, ADL, CA and CP properties of silages evaluated in terms of nutritive value are in Table 2. All characteristics were found to be significant due to effects of pure and mixture silage treatments. The NDF contents of the silages ranged from 50.02 to 65.16 %. The highest NDF value was obtained from 25SF+75SS treatment while the lowest was in 50SF+50M treatment. It has been reported that excessively high NDF content in feeds can cause various ailments in animals fed with these feeds (Beauchemin, 1996). On the other hand, NDF content of less than 32% is known to be a disadvantage in terms of animal nutrition (Broderick, 2003). The NDF contents of pure M, SM and SS were higher than pure SS treatment. However, as the sunflower ratio increased, the NDF content did not increase. Presumably, the presence of microorganisms that degrade cell wall substances in pure sunflower silages may have caused this situation. The ADF content ranged from 20.21 to 40.81 % (Table 2). The highest ADF value was detected in 75SF+25M treatment, whereas the lowest ADF was found in 25SF+75M treatment. It has been stated that as the ADF content in the feeds decreases, the digestibility of the feeds increases (Ball et al., 2001). Therefore, it can be said that 25SF+75M treatment has a more suitable ADF content in terms of animal nutrition. The ADL contents of the silages obtained from pure and mixture silage materials ranged from 3.31 to 9.20 % (Table 2). The highest ADL value was obtained from pure SF while the lowest ADL content was in pure M. High ADL content is undesirable since it negatively affects the digestion in terms of animal nutrition and does not contribute energy to the fed animals (Manaye et al., 2009). As the proportion of sunflower in mixed silages increased the ADL content of the silages increased. The CA contents of pure and mixed silages ranged from 16.01 and 26.02%. The highest CA was obtained from 50SF+50SS treatment while the lowest value was in Pure SC silage. The crude ash content for feeds is very important trait because of its nutritional value (Quirino et al., 2023). Nurk et al. (2017) reported that the CA of mixed silages changes according to mixing ratios in a research used as silage material of maize and common bean similar to results of current study. The CP ratio significantly affected from the treatments and ranged from 5.43 and 10.56%. The highest CP content was determined in 75SF+25SC treatment whereas the lowest CP was in Pure M. The CP content in feeds is one of the most important nutrients that meet the basic nutritional needs of animals fed with feed (Rezende et al., 2023). Nurk et al. (2017) stated that the CP of mixed silages made from maize and common bean improve similar to results obtained from present study.

Table 1. Dry matter, chemical compositions and microbial counts of fresh material before ensiling

Properties	Plant species				P values
	Maize	Sunflower	Sweet Corn	Sweet Sorghum	
DM (%)	31.03±0.68 a	25.69±0.40 b	32.16±0.75 a	30.17±0.46 a	0.0003
NDF (% DM)	50.62±0.49 c	53.59±0.64 bc	57.09±0.95 b	62.20±1.73 a	0.0003
ADF (% DM)	24.48±0.20 d	36.74±0.37 a	27.10±0.46 c	34.35±0.74 b	<.0001
ADL (% DM)	2.08±0.08 c	7.92±0.15 a	1.69±0.13 c	3.77±0.20 b	<.0001
CA (% DM)	6.41±0.06 d	11.08±0.09 a	7.19±0.06 b	6.80±0.05 c	<.0001
CP (% DM)	5.62±0.18 c	12.45±0.16 a	9.03±1.35 b	5.09±0.08 c	0.0002
WSC (% DM)	19.15±0.54 b	5.25±0.12 c	22.27±0.58 a	23.38±0.63 a	<.0001
LAB (log_{10} cfu g^{-1} DM)	3.18±0.09 b	2.68±0.12 c	3.11±0.03 bc	3.83±0.14 a	0.0004
Yeast (log_{10} cfu g^{-1} DM)	3.86±0.07 b	2.22±0.03 c	4.12±0.06 a	4.26±0.05 a	<.0001
Mold (log_{10} cfu g^{-1} DM)	1.12±0.07	1.23±0.03	1.24±0.07	1.10±0.07	ns

DM= dry matter, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, CA= crude ash, CP= crude protein, WSC= water soluble carbohydrate, LAB= lactic acid bacteria, ns: not significant

Table 2. Chemical compositions of pure and mixed silages obtained from different plant species

Treatments	Properties				
	NDF (% DM)	ADF (% DM)	ADL (% DM)	CA (% DM)	CP (% DM)
Pure M	55.79±0.84 cd	30.26±0.59 de	3.31±0.08 e	21.95±5.25 abc	5.43±0.03 f
Pure SC	58.89±0.05 bc	33.67±0.57 cd	3.63±0.49 e	16.01±0.50 c	8.80±0.09 c
Pure SF	51.65±0.08 ef	38.97±0.02 ab	9.20±0.04 a	16.98±2.92 bc	9.58±0.20 abc
Pure SS	65.13±1.19 a	39.73±0.92 a	4.93±0.04 cde	16.13±0.61 c	5.67±0.03 f
25SF+75M	50.91±0.31 ef	29.21±0.23 e	3.61±0.24 e	25.55±0.14 a	6.34±0.12 ef
25SF+75SC	56.92±0.71 cd	33.42±0.36 cd	4.66±0.13 de	22.85±2.06 abc	9.29±0.06 bc
25SF+75SS	65.16±0.27 a	37.75±0.29 ab	5.70±0.12 cd	23.08±3.59 ab	7.75±0.24 d
50SF+50M	50.02±0.45 f	30.23±0.70 de	4.67±0.14 de	24.61±1.17 a	7.39±0.09 d
50SF+50SC	54.50±1.18 de	34.14±1.06 c	6.38±0.38 bc	23.46±1.48 ab	10.33±0.11 a
50SF+50SS	56.91±0.81 cd	36.15±0.68 bc	6.57±0.36 bc	26.02±0.72 a	8.98±0.10 c
75SF+25M	61.81±1.27 ab	40.81±1.14 a	8.70±0.47 a	16.99±2.91 bc	6.94±0.51 de
75SF+25SC	56.59±0.19 cd	37.76±0.75 ab	7.92±0.66 ab	23.14±1.37 ab	10.56±0.18 a
75SF+25SS	57.40±0.16 cd	38.01±0.54 ab	7.66±0.18 ab	22.68±1.96 abc	10.20±0.23 ab
P values	<.0001	<.0001	<.0001	0.0312	<.0001

DM= dry matter, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, CA= crude ash, CP= crude protein, M= maize, SC= sweet corn, SF= sunflower, SS= sweet sorghum

Parameters of silage pH, ammonia nitrogen (NH_3-N), lactic acid bacteria (LAB), yeast and mold counts significantly affected from treatments (Table 3). The silage pH was between 3.54 and 4.22. As sunflower ratio increased in mixed silages, silage pH ascended. Many studies informed that the silage pH in mixed silages increased as the ratio of effortless ensilaged crops increased (Wang et al., 2019; Zeng et al., 2020; Mu et al., 2021). The NH_3-N content of silages obtained from pure and mixed treatments was between 4.22 and 7.09%. The highest NH_3-N were found in Pure SF while the lowest value was in Pure M. As the SF ratio in mixed silages increased the NH_3-N content increased. It was stated that the NH_3-N content of silage materials with high N content is higher than that of low N content similar to results obtained from present study (Zeng et al., 2020). The LAB count ranged from 3.32 to 5.54 log_{10} cfu g^{-1} DM. While the lowest LAB count was obtained from pure SF treatment the highest LAB was detected in Pure SC. As the sunflower ratio in the mixed silages decreased, the LAB number of the silages increased. Similar to results obtained from current study, some researchers reported LAB count in pure and mixed silages (Wang et al., 2019; Zeng et al., 2020). Yeast and mold count of pure and mixed silages were between 1.43-2.44 log_{10} cfu g^{-1} DM and 1.25-1.70 log_{10} cfu g^{-1} DM. For both yeast and mold count, the highest value was obtained from pure SS the lowest value was in pure SF. As the sunflower ratio in the mixed silages increased, both the yeast and mold counts of the silages decreased. Similar to the results obtained from this study, it was reported that both yeast and mold numbers decreased as the ratio of other plants in the cereal silage increased (Pursiainen and Tuori 2008).

Table 3. Silage pH, microbial counts and ammonia nitrogen contents of the silages obtained from pure and mixed of different plant species

Treatments	Properties				
	pH	NH_3-N (% DM)	LAB (log_{10} cfu g^{-1} DM)	Yeast (log_{10} cfu g^{-1} DM)	Mold (log_{10} cfu g^{-1} DM)
Pure M	3.73±0.01 h	4.22±0.19 e	4.37±0.08 cd	2.02±0.03 bcd	1.54±0.06 abc
Pure SC	3.65±0.02 i	4.39±0.21 de	4.75±0.06 bc	2.10±0.04 bc	1.46±0.09 abc
Pure SF	4.22±0.01 a	7.09±0.33 a	3.32±0.15 g	1.43±0.05 g	1.25±0.04 c
Pure SS	3.54±0.01 j	4.29±0.26 de	5.54±0.16 a	2.44±0.05 a	1.70±0.13 a
25SF+75M	3.93±0.02 f	5.39±0.22 cde	3.99±0.08 def	2.02±0.02 bcd	1.47±0.04 abc
25SF+75SC	3.86±0.01 fg	5.52±0.13 cd	4.75±0.08 bc	1.90±0.04 b-e	1.41±0.07 abc
25SF+75SS	3.73±0.01 h	5.74±0.21 bc	5.24±0.12 ab	2.40±0.05 a	1.59±0.09 ab
50SF+50M	4.09±0.02 cd	6.11±0.26 abc	3.83±0.05 ef	1.87±0.01 c-f	1.40±0.03 abc
50SF+50SC	4.00±0.01 e	6.19±0.14 abc	4.41±0.08 cd	1.82±0.06 def	1.36±0.06 bc
50SF+50SS	3.85±0.00 g	6.44±0.21 abc	4.75±0.06 bc	2.11±0.06 b	1.48±0.05 abc
75SF+25M	4.16±0.01 ab	6.82±0.30 ab	3.53±0.07 fg	1.63±0.06 fg	1.33±0.03 bc
75SF+25SC	4.12±0.01 bc	6.87±0.22 ab	3.86±0.11 ef	1.74±0.06 ef	1.30±0.05 bc
75SF+25SS	4.03±0.01 de	6.89±0.36 ab	4.18±0.08 de	2.02±0.05 bcd	1.37±0.01 abc
P values	<.0001	<.0001	<.0001	<.0001	0.0032

DM= dry matter, pH= power of hydrogen, NH_3-N= ammonia nitrogen, LAB= lactic acid bacteria, M= maize, SC= sweet corn, SF= sunflower, SS= sweet sorghum

The lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) and ethanol (ETOH) properties were affected by pure and mixture silages obtained from maize, sweet corn, sunflower and sweet sorghum crops (Table 4). When the all silage end products were investigated, the highest LA, AA, PA, BA and ETOH were obtained from Pure SS treatment whereas the lowest value for all silage end products were determined in Pure SF. As the proportion of the sunflower in mixed silages increased, Values for all silage end product characteristics decreased. Such results in studied on mixture silages stated and it was emphasized that plants that promote lactic acid production should be used to improve lactic acid content in silages (Pursiainen and Tuori 2008; Kennedy et al., 2018; Di Miceli et al., 2023).

5. Conclusion

This study was carried out to determine the nutritive value and silage fermentation quality of pure and mixed silages of maize, sweet corn, sunflower and sweet sorghum plants. Compared to pure cereal silages, the nutritive value of all mixture silages improved because sunflower crop offered a higher quality nutritive value. On the other hand, as the proportion of cereal in mixed silages increased, silage fermentation quality improved. When all the results obtained from the current study are evaluated, the use of 50%+50% sunflower and corn, sweet corn or sweet sorghum in silages may provide a better quality silage in terms of both nutritive value and silage fermentation quality.

Table 4. Lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) and ethanol (ETOH) contents of the silages obtained from pure and mixed of different plant species

Treatments	Properties				
	LA (% DM)	AA (% DM)	PA (% DM)	BA (% DM)	ETOH (% DM)
Pure M	4.23±0.06 abc	1.33±0.04 bcd	0.54±0.03 b-f	0.36±0.02 cd	0.88±0.02 cde
Pure SC	4.34±0.16 ab	1.48±0.02 ab	0.69±0.04 ab	0.42±0.03 abc	1.05±0.04 bc
Pure SF	2.00±0.08 j	0.72±0.05 i	0.29±0.02 g	0.25±0.01 d	0.53±0.05 f
Pure SS	4.49±0.19 a	1.56±0.06 a	0.77±0.06 a	0.53±0.05 a	1.43±0.11 a
25SF+75M	3.77±0.05 cde	1.23±0.04 cde	0.50±0.03 def	0.35±0.02 cd	0.86±0.01 cde
25SF+75SC	3.86±0.10 bcd	1.35±0.02 bcd	0.62±0.03 a-d	0.40±0.02 bc	0.99±0.04 cd
25SF+75SS	3.97±0.16 abc	1.40±0.05 abc	0.68±0.04 abc	0.48±0.04 ab	1.28±0.09 ab
50SF+50M	3.21±0.05 fgh	1.08±0.04 e-h	0.44±0.03 efg	0.33±0.01 cd	0.78±0.02 c-f
50SF+50SC	3.27±0.05 efg	1.15±0.03 d-g	0.52±0.02 c-f	0.36±0.01 cd	0.86±0.04 cde
50SF+50SS	3.35±0.13 def	1.19±0.04 c-f	0.56±0.03 b-e	0.41±0.03 abc	1.05±0.07 bc
75SF+25M	2.66±0.06 i	0.93±0.04 hi	0.38±0.02 fg	0.30±0.01 cd	0.69±0.03 ef
75SF+25SC	2.68±0.03 hi	0.97±0.04 gh	0.42±0.02 efg	0.31±0.01 cd	0.73±0.05 def
75SF+25SS	2.72±0.10 ghi	0.98±0.04 fgh	0.44±0.02 efg	0.34±0.02 cd	0.82±0.06 cde
P values	<.0001	<.0001	<.0001	<.0001	0.0032

DM= dry matter, LA= lactic acid, AA= acetic acid, PA= propionic acid, BA= butyric acid, ETOH= ethanol, M= maize, SC= sweet corn, SF= sunflower, SS= sweet sorghum

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	İ.E.	E.C.
C	50	50
D	100	
S		100
DCP	90	10
DAI	60	40
L	80	20
W	50	50
CR	20	80
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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