



Valorization of Citrus Pulp Wastes on Improving the Silage Quality of Alfalfa (*Medicago sativa* L.): Effects on Chemical Composition, Fermentation, Gas Production, and Aerobic Stability Properties

Yonca (*Medicago sativa* L.) Silaj Kalitesinin İyileştirilmesinde Narenciye Posası Atıklarının Değerlendirilmesi: Kimyasal Bileşim, Fermantasyon, Gaz Üretimi ve Aerobik Stabilitate Özellikleri Üzerindeki Etkileri

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Abstract: This study was carried out to evaluate the effect of the pulps obtained from citrus on the quality properties of alfalfa (*Medicago sativa* L.) silage, which has low water-soluble carbohydrates (WSC) and high protein content. The alfalfa was harvested in the pre-blooming period and chopped into 1-3 cm lengths, added 0%, 5% and 10% fresh orange, tangerine, grapefruit and lemon pulps, then siled in vacuum bags for 90 days with 3 replications. At the end of the ensiling period, chemical analyses, gas production (GP), and other calculations were performed. Citrus pulp addition increased the dry matter (DM), crude ash (CA), crude protein (CP), and Flieg Score (FS) of the silages, and reduced the pH, net GP, and methane (CH₄). The addition of 10% tangerine pulp increased the FS of silages more than 50%. While the control group silages were graded "low", the addition of 10% tangerine pulp increased the quality grades of the silages to "good". With the addition of 5% grapefruit pulp, a 27% decrease in the CH₄ on a per-mL basis of the silages was determined. When silages were compared with the control group, only the silages with %5 tangerine pulp addition had anti-methanogenic properties due to their percentage. With the addition of citrus pulp, excessive degradation of proteins occurring in alfalfa silage was prevented. Silage quality and environmental pollution can be addressed to some extent by using citrus pulp waste to ensile alfalfa, which improves both the nutritional and fermentation characteristics of the silage.

Keywords: Alfalfa silage, Citrus pulp, Fermentation, Gas production, Aerobic stability

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Öz: Bu çalışma, farklı narenciye posalarının düşük suda çözünür karbonhidrat (SÇK) ve yüksek protein içeriğine sahip yonca (*Medicago sativa* L.) silajlarının kalite özellikleri üzerindeki etkisini belirlemek amacıyla gerçekleştirilmiştir. Çiçeklenme öncesi dönemde hasat edilen yonca, 1-3 cm uzunluğunda doğranmış ve %0, %5, %10 oranlarında taze portakal, mandalina, greyfurt ve limon posaları eklenerek, üçer tekerrürlü olarak vakumlu torbalarda 90 gün boyunca silolanmıştır. Silolama süresinin sonunda kimyasal analizler, gaz üretimi (GP) ölçümleri ve diğer hesaplamalar yapılmıştır. Narenciye posası ilavesi, silajların kuru madde (KM), ham kül (HK), ham protein (HP) ve Flieg Skoru (FS) değerlerini artırırken, pH, net gaz üretimi (GÜ) ve metan (CH₄) seviyelerini düşürmüştür. %10 oranında mandalina posası ilavesi, silajların FS'sini %50'den fazla yükseltmiştir. Kontrol grubu silajları "düşük kalite" olarak sınıflandırılırken, %10 mandalina posası ilavesi ile silajlar "iyi kalite" seviyesine yükselmiştir. Ayrıca, %5 oranında greyfurt posası ilavesi ile silajların mililitre başına CH₄ üretiminde %27 oranında azalma tespit edilmiştir. Kontrol grubu ile karşılaştırıldığında, yalnızca %5 mandalina posası ilaveli silajlar gaz üretimi açısından anti-metanojenik özellik göstermiştir. Narenciye posası ilavesi, yonca silajında meydana gelen aşırı protein yıkımını önlemiştir. Yoncanın silolanmasında narenciye posası atıklarının kullanılması, silajın hem besin değerini hem de fermantasyon özelliklerini iyileştirerek silaj kalitesini artırılabilir ve çevresel kirliliği belirli ölçüde azaltılabilir.

Anahtar Kelimeler: Yonca silajı, Narenciye posası, Fermantasyon, Gaz üretimi, Aerobik stabilite

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INTRODUCTION

The demand for livestock products rises as a result of various factors, including but not limited to the growth of human population, increasing income levels, and urban development (Thornton, 2010). This increase in demand leads to the growth of the livestock production sector and therefore, an increase in feed requirements. In addition to meeting the increasing feed needs of animals, it is not easy to provide this rationally and sustainably at all times of the year. For this reason, feeds must be stored and stored in different ways.

Silage is a fermented feed made from fresh forage that has been stored under anaerobic conditions. This process preserves the forage by preventing spoilage and decay, allowing it to be stored for long periods of time without losing its nutritional value. Silage not only aids in preserving crops, but it also enhances their nutritional value and digestibility (Sarwar et al., 2002). Animals find high-quality silage very appetizing, and minimal feed wastage occurs during consumption (Denek et al., 2017). The fermentation quality of silage plays a crucial role in enhancing dairy animal feed intake, nutrient digestibility, and milk production (Huhtanen et al., 2002; Ferraretto et al., 2015; Lim et al., 2015). Preserving the nutritional value of harvested forage until it is consumed by animals holds significant economic importance in animal production, and successful ensiling is built upon effective management practices (Kung, 2009). By acidifying biomass, silage is a straightforward and effective method for preserving moist forages, as it inhibits the growth of microorganisms that cause spoilage, leading to safe and long-lasting storage (McDonald et al., 1991).

Alfalfa (*Medicago sativa* L.) is a perennial leguminous plant from the family *Fabaceae*, originally native to Central Asia but now widely cultivated in many regions of the world for forage and hay production. Despite its benefits as a legume crop, ensiling alfalfa can be challenging due to its high buffering capacity and low concentration of water-soluble carbohydrates (WSC) (Coblentz et al., 2014; Ni et al., 2018). During the ensiling process of alfalfa, a common issue that arises is proteolysis, which refers to the degradation and breakdown of proteins to the point where they become unusable (Winters et al., 2000). In order to promote the rapid production of lactic acid and subsequent pH reduction during fermentation, additives are frequently incorporated prior to ensiling alfalfa (Ke et al., 2017; Yuan et al., 2018).

Silage additives are specialized substances that are added during the silage making process to enhance the quality of the resulting silage. These additives have numerous benefits, such as improving the nutritional value of the silage, promoting healthier feeding for animals, controlling the fermentation process, and preventing spoilage. By preserving the nutrients and preventing spoilage, silage additives help maintain the quality of proteins, sugars, and other nutrients in the silage (Kung and Muck, 1997). Preventing the entry of oxygen into the silage material serves the purpose of inhibiting the growth of undesirable microorganisms like *Clostridium* and *Enterobacter* species, which can spoil the silage and release harmful fermentation products that can negatively affect animal health (Ergün et al., 2016). Additionally, these additives are utilized to control and reduce unpleasant odors associated with silage. Silage additives come in various types, including yeast, bacteria, enzymes, acids, and preservatives (Muck, 1993; Weinberg et al., 1993). The selection of additive type depends on the type of plant used for silage, the region where it is grown, and the time of harvest. Ultimately, the use of silage additives is an important practice for ensuring the production of high-quality silage for animal consumption (Kung and Stokes, 2003; Weinberg and Muck, 2007; Kung, 2018). Silage additives can be derived from by-product wastes such as those generated in the food industry or from agricultural processing. These wastes typically have a high moisture content, which makes them suitable for use in silage production. Incorporating by-product wastes into silage production can offer several benefits, including reduced feed costs for farms, a more environmentally friendly approach to waste management, and a more sustainable approach to production. By using these wastes as silage additives, farmers can help to maximize the nutritional value of their animal feed while minimizing waste and reducing their environmental footprint.

Citrus refers to a group of fruit-bearing trees and shrubs that are economically valuable, including species such as oranges (*Citrus sinensis* L.), lemons (*Citrus lemon* L.), tangerines (*Citrus reticulata*), and grapefruits (*Citrus paradisi* L.). The most important production areas are in China, Brazil, the USA, Mexico, Argentina,

Spain, Italy, Egypt, and Turkey (FAO, 2022). In 2022, the global citrus production was around 105 million metric tons, with oranges accounting for 50, tangerines for 37.8, grapefruits for 6.9, and lemons for 9.9. Out of the total citrus production, 76.77% was consumed fresh while the remaining 22.42% was used for various food processing purposes (USDA, 2023). Citrus pulp is typically composed of the residues left over after processing oranges, grapefruits, tangerines and lemons. These residues typically contain 60-65% shell, 30-35% internal tissues, and 10% seeds (Crawshaw, 2003). Citrus pulp is also rich in chemical compounds such as phenolic compounds, phenolic acids, flavonoids, coumarins, and limonoids (Justesen, 1998). The quantity of citrus pulp produced can fluctuate depending on several factors, including the processing techniques utilized, the intended use of the pulp, and the type and amount of citrus fruit processed. Nevertheless, the Food and Agriculture Organization of the United Nations reports that the worldwide production of citrus pulp reached approximately 29 million tonnes in 2020 (FAO, 2020). Due to their high water content, pulp is susceptible to deterioration during storage. As a result, a significant portion of pulp is typically used fresh as animal feed, particularly in areas where fruit juice factories are located. Unfortunately, a substantial amount of pulp is also discarded without being utilized, which can contribute to environmental pollution (Yalçinkaya et al., 2012). Citrus pulp is a suitable alternative to other ingredients like corn, grasses, and legumes in silage production, as it can be used partially or completely. Due to its high moisture and sugar content, citrus pulp is an ideal substrate for lactic acid bacteria, which play a crucial role in the fermentation process during silage production. Citrus pulp typically contains 5-10% crude protein (CP), 6.2% ether extract (EE), 54% soluble sugar, 10-40% soluble fiber, as well as 0.1% phosphorus and 1-2% calcium (Bakshi and Wadhwa, 2013). The fermentation of citrus pulp in silage produces lactic acid, which can help to lower the pH of the silage and improve its preservation. In addition, citrus pulp contains abundant fermentable carbohydrates and easily digestible nutrients (Scerra et al., 2001; Bampidis and Robinson, 2006).

Although the potential for utilizing citrus pulps as agro-industrial by-products in ruminant livestock nutrition is enormous, it is currently underutilized (Tayengwa and Mapiye, 2018). Recycling citrus waste and utilizing its components in various applications can be an effective solution to reducing waste and preventing environmental pollution. This approach not only helps address environmental concerns but can also have economic benefits. The objective of this study was to assess the nutritional quality, fermentation properties, and resistance to aerobic deterioration of alfalfa silage supplemented with citrus pulp, and to explore the potential use of this industrial by-product as a silage additive.

MATERIAL AND METHOD

Study Materials and Design

The silage material used in this study consisted of a "Magnum 5" alfalfa (*Medicago sativa* L.) variety, which was grown and harvested by farmers in the Iğdır province of Türkiye on June 19, 2022. The field (located at 39° 58' 35.38" N and 43° 56' 18.35" E) is situated at an altitude of 879 meters above sea level, with an annual rainfall of 256 mm and an average temperature of 11.6°C. The Magnum 5 alfalfa had an average dry matter (DM) content of 20.36%, CP content of 19.50%, acid detergent fiber (ADF) content of 36.80%, and neutral detergent fiber (NDF) content of 44.85% (Avci et al., 2018; Harmanlıoğlu and Kaplan, 2020). Citrus pulp obtained from waste products of orange, tangerine, grapefruit, and lemon was used as an additive, with moisture levels ranging from 76 to 84%.

The alfalfa, which was cut with a scythe during the pre-blooming period (at 28.50% DM), was transported to the Feeds and Animal Nutrition Laboratory of the Animal Science Department. The alfalfa was then cut into approximately 2 cm pieces using a grass chopper and placed on a clean floor. The citrus pulp was weighed according to the predetermined ratios (5% and 10%) (DM basis), sprinkled homogeneously on the alfalfa, and mixed well. This mixture was then transferred to 23x26 cm 120-micron vacuum bags. For each group, 1.500 g of fresh chopped alfalfa was mixed thoroughly with 5% and 10% of citrus pulp and divided into three portions. The control group (0%) did not receive any citrus pulp. The prepared vacuum bags were placed in a vacuum machine, and the air was removed with the help of a sudden pressure change. The mixtures were then ensiled, with three replications per group, and the silage process was set to last for 90 days. After the 90-day ensiling period, the samples were opened and subjected to physical and chemical

analyses. Crude ash (CA), DM, pH, and aerobic stability were determined for fresh samples. To determine EE, CP, ADF, NDF, and gas production (GP), the silages were left to dry in paper bags at 70°C in a ventilated oven for 96 hours. The silages were then ground to a particle size of 1 millimeter using a grinding mill and prepared for analysis.

Chemical Composition and GP

The DM, CA, EE, and CP contents of the silages were analyzed using the method described by AOAC (1990). ADF and NDF contents were determined using the method reported by Van Soest et al. (1991). Dry silage samples were subjected to fermentation as described by Menke et al. (1979). No animals were directly used in this study. Rumen fluid (2 L) was obtained immediately after the slaughter of two cattle at the slaughterhouse for the GP test. The rumen fluid was filtered through four layers of cheesecloth while being flushed with CO₂. Buffered rumen fluid was then obtained by combining buffer solution with rumen fluid at a ratio of 1:2. Triplicate samples of approximately 200 mg of each sample were subjected to fermentation with a 30 mL rumen fluid-buffer mixture at 39°C for 24 h. The net GP of silage samples was calculated after correction for blank and hay standards (University of Hohenheim, Germany), with a predicted GP of 49.61 mL per 200 mg. The methane (CH₄) content of gas produced after 24 h of incubation was determined using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) based on the following equation (Goel et al., 2008):

$$\text{CH}_4 \text{ production (mL)} = \text{Total GP (mL)} \times \text{Percentage of CH}_4 (\%) \quad (1)$$

Determination of pH, Flieg score (FS), Required pH (RpH), Metabolizable energy (ME), Organic matter digestibility (OMD), and aerobic stability.

The pH of the fresh silage samples was measured using an electronic pH meter with a sensitivity of 0.01 and a digital display, after homogenizing 20 g of the sample with 180 mL of distilled water for 5 minutes.

The FS required to determine the quality of silages were calculated according to the formula given below (Kılıç, 1986).

$$\text{FS} = 220 + (2 \times (\text{DM}\% - 15)) - (40 \times \text{pH}) \quad (2)$$

Based on the DM and pH values, the RpH values that should be used to determine the quality of the silages were calculated according to the formula given below (Meeske, 2005).

$$\text{RpH} = (0.00359 \times \text{DM}) + 3.44 \quad (3)$$

The ME and OMD of silage samples were calculated using net GP, CP, EE, and CA of silage samples (Menke and Steingass, 1988).

$$\text{ME (MJ kg}^{-1} \text{ DM)} = 2.20 + (0.1357 \times \text{GP}) + (0.057 \times \text{CP}) + (0.002859 \times \text{EE}^2) \quad (4)$$

$$\text{OMD (\%)} = 15.38 + (0.8453 \times \text{GP}) + (0.595 \times \text{CP}) + (0.675 \times \text{CA}) \quad (5)$$

GP: gas production (mL) from a 200 mg sample following 24 hours of incubation

The temperature of the silage samples was measured every hour using temperature probes (TMC6-HD) to monitor their response to exposure to air. The recorded data was then transferred to a computer and used to determine the aerobic stability of the silage materials, following the method described by Kung et al. (2000).

Statistical Analysis

The data set was statistically evaluated with a one-way analysis of variance (one-way ANOVA), which was evaluated using the Levene homogeneity test and the Kolmogorov-Smirnov normality test (SPSS, 2017). These tests confirmed that the assumptions of homogenous distribution and normality were achieved ($p>0.05$). A Duncan multiple comparison test was used to evaluate differences between means, and significance was set at 0.05.

RESULTS AND DISCUSSION

Tables 1, 2, and 3 present the nutrient content, fermentation parameters, GP, ME, and OMD values of alfalfa silages supplemented with citrus pulps. The aerobic stability test results are shown in Table 4 and Figure 1.

Chemical Composition

Incorporation of citrus pulp additive resulted in increased DM, CA, and CP contents in the silages, but caused alterations in the cell-wall components ADF and NDF. Although no significant difference was observed in the EE values, they were found to be higher compared to the control group. The addition of citrus pulp to alfalfa silage resulted in a significant increase in the DM content ($p<0.05$).

Table 1. Nutrient content of silage mixtures (Mean $\pm$ Std. Error).

Çizelge 1. Silaj karışımlarının besin içerikleri (Ortalama $\pm$ Standart hata).

Treatments	DM	CA	CP	EE	ADF	NDF
Alfalfa Control	22.91 $\pm$ 0.034 ^c	10.09 $\pm$ 0.296 ^b	19.56 $\pm$ 0.539 ^b	2.27 $\pm$ 0.233	32.97 $\pm$ 1.419	39.46 $\pm$ 0.387
Alfalfa + OP5	23.51 $\pm$ 0.153 ^{ab}	10.39 $\pm$ 0.030 ^b	19.65 $\pm$ 0.218 ^b	2.57 $\pm$ 0.266	31.61 $\pm$ 0.529	38.69 $\pm$ 0.305
Alfalfa + TP5	23.14 $\pm$ 0.180 ^{bc}	10.95 $\pm$ 0.133 ^a	20.34 $\pm$ 0.606 ^b	2.59 $\pm$ 0.355	33.40 $\pm$ 0.707	38.65 $\pm$ 0.537
Alfalfa + GP5	23.32 $\pm$ 0.272 ^{abc}	10.55 $\pm$ 0.089 ^{ab}	19.67 $\pm$ 0.159 ^b	2.71 $\pm$ 0.092	35.37 $\pm$ 0.995	40.19 $\pm$ 0.830
Alfalfa + LP5	23.30 $\pm$ 0.255 ^{abc}	10.57 $\pm$ 0.121 ^{ab}	20.85 $\pm$ 0.390 ^{ab}	2.55 $\pm$ 0.169	33.14 $\pm$ 0.463	40.09 $\pm$ 0.620
Alfalfa + OP10	23.83 $\pm$ 0.184 ^a	10.11 $\pm$ 0.206 ^b	22.01 $\pm$ 0.185 ^a	2.81 $\pm$ 0.183	32.03 $\pm$ 0.564	38.30 $\pm$ 0.596
Alfalfa + TP10	23.79 $\pm$ 0.011 ^a	10.19 $\pm$ 0.040 ^b	20.59 $\pm$ 0.306 ^b	2.86 $\pm$ 0.428	31.06 $\pm$ 0.743	37.73 $\pm$ 0.471
Alfalfa + GP10	23.75 $\pm$ 0.171 ^a	10.16 $\pm$ 0.171 ^b	19.79 $\pm$ 0.652 ^b	2.80 $\pm$ 0.308	32.31 $\pm$ 1.348	38.18 $\pm$ 0.658
Alfalfa + LP10	23.61 $\pm$ 0.052 ^{ab}	10.38 $\pm$ 0.054 ^b	20.17 $\pm$ 0.185 ^b	2.73 $\pm$ 0.058	32.40 $\pm$ 0.258	37.41 $\pm$ 1.284
Sig.	0.015 [*]	0.014 [*]	0.009 ^{**}	0.846 ^{ns}	0.095 ^{ns}	0.100 ^{ns}

^{abc} Column means with common superscripts do not differ ($p>0.05$). DM: Dry matter (%), CA: Crude ash (% of DM), CP: Crude protein (% of DM), EE: Ether extract (% of DM), ADF: Acid detergent fiber (% of DM), NDF: Neutral detergent fiber (% of DM), OP: Orange pulp, TP: Tangerine pulp, GP: Grapefruit pulp, LP: Lemon pulp, Sig: Significant level, *: $p<0.05$, **: $p<0.01$, ns: Not significant.

The DM values ranged between 22.91% and 23.83% for the silages, with the highest value observed in the alfalfa silage containing 10% orange pulp. The addition of citrus pulp to the silage mixtures resulted in a significant increase in the CA content, which varied between 10.09 and 10.95%. The CP contents of silage mixtures with citrus pulp varied between 19.56 and 22.01%, and the addition of pulp significantly increased the CP ($p<0.01$). The lowest CP value was found in the control group, and the highest was found in silage with 10% orange pulp. The EE content of the silage mixtures with citrus pulp varied between 2.27 and 2.86%, and the addition of citrus pulp had no statistically significant effect on the EE. The ADF and NDF contents of the silage mixtures with citrus pulp varied between 31.06% and 35.37% and 37.41 and 40.19%,

respectively. The addition of citrus pulp to alfalfa silage had no statistically significant effect on the ADF and NDF. While the lowest ADF and NDF values were found in silages with 10% tangerine and lemon pulp, respectively, the highest values for both were found in the silage with 5% grapefruit.

Fermentation Properties

Citrus pulp added to alfalfa silage significantly affected the pH, FS ($p<0.01$). Table 2 shows that the addition of citrus pulp reduced the pH of silages and increased the FS. The addition of citrus pulp to alfalfa silage changed the pH of the silage to between 4.41 and 4.88. The pH of the silages decreased with an increasing dose of the additive, and the lowest value was found in the group with 10% tangerine pulp. Citrus pulp added to alfalfa silage significantly affected the FS, used as a quality classification for silages ($p<0.01$). The FS ranged between 40.36 and 61.18. The lowest value was found in the control group, while the highest value was found in silage with 10% tangerine pulp. The differences between the RpH values were small, and not statistically significant. The pH values (4.41-4.88) of the silages in the study were higher than the RpH values (3.52-3.53).

Table 2. Fermentation parameters of silage mixtures (Mean $\pm$ Std. Error).

Çizelge 2. Silaj karışımlarının fermentasyon parametreleri (Ortalama $\pm$ Standart Hata).

Treatments	pH	FS	RpH
Alfalfa Control	4.88 $\pm$ 0.023 ^f	40.36 $\pm$ 0.883 ^f	3.52 $\pm$ 0.000
Alfalfa + OP5	4.65 $\pm$ 0.013 ^{bc}	50.89 $\pm$ 0.560 ^c	3.52 $\pm$ 0.003
Alfalfa + TP5	4.73 $\pm$ 0.025 ^{de}	47.09 $\pm$ 1.155 ^e	3.52 $\pm$ 0.000
Alfalfa + GP5	4.75 $\pm$ 0.012 ^e	46.37 $\pm$ 0.528 ^e	3.52 $\pm$ 0.003
Alfalfa + LP5	4.71 $\pm$ 0.008 ^{de}	48.12 $\pm$ 0.857 ^{de}	3.52 $\pm$ 0.000
Alfalfa + OP10	4.60 $\pm$ 0.012 ^b	53.40 $\pm$ 0.713 ^b	3.52 $\pm$ 0.003
Alfalfa + TP10	4.41 $\pm$ 0.005 ^a	61.18 $\pm$ 0.207 ^a	3.53 $\pm$ 0.000
Alfalfa + GP10	4.64 $\pm$ 0.017 ^{bc}	51.77 $\pm$ 0.360 ^{bc}	3.52 $\pm$ 0.003
Alfalfa + LP10	4.68 $\pm$ 0.020 ^{cd}	50.03 $\pm$ 0.690 ^{cd}	3.52 $\pm$ 0.003
Sig.	0.000 ^{**}	0.000 ^{**}	0.256 ^{ns}

abc Column means with common superscripts do not differ ($p>0.05$), FS: Flieg score, RpH: Required pH value, OP: Orange pulp, TP: Tangerine pulp, GP: Grapefruit pulp, LP: Lemon pulp, Sig: Significant level, **: $p<0,01$, ns: Not significant.

Gas Production, ME, and OMD

The addition of citrus pulp significantly affected the net GP, CH₄ content (ml and %), ME, and OMD of the silages (Table 3) ($p<0.05$). Compared to the control group, the addition of citrus pulp resulted in a decrease in the net gas and CH₄ production on a per-mL basis, and a difference in the amount of CH₄ percentage. The net GP, CH₄ (mL), and CH₄ (%) of silages ranged from 28.15 to 39.27 mL, 3.50 to 6.65 mL, and 12.32 to 20.98%, respectively. The lowest net GP (28.15 mL) was observed in the group with 5% added grapefruit pulp, while the highest was observed in the control group (39.27 mL). The ME and OMD values ranged from 7.16 to 8.65 MJ kg⁻¹ DM, and 48.82 to 58.62%, respectively. The lowest values for both parameters were observed in the group with 5% added grapefruit pulp, while the highest values were observed in the control group.

Aerobic Stability

The impact of citrus pulp addition to alfalfa silage on aerobic stability was assessed by subjecting the silage to a temperature change test over seven days. The degradation times and temperature changes of the silages are displayed in Table 4 and Figure 1. Additives led to variations in the degradation time of silages.

The temperature readings from the control group and the silages with 5% and 10% orange, tangerine, grapefruit, and lemon pulp were higher than the ambient temperature at certain points in time. The first silage to reach the degradation point was the 10% orange pulp silage, which rose 2°C above ambient temperature in the fifth hour. The 10% orange pulp silage also exhibited the largest temperature increase, reaching 24.51°C at the 152nd hour. The silages that showed degradation, the groups with 10% additive exceeded ambient temperature sooner than the silages with 5% additive. The temperature of all silage groups was 2 degrees higher than the ambient temperature, indicating that all silages underwent theoretical degradation after a certain period of time upon exposure to oxygen.

Table 3. Gas production, metabolic energy and organic matter digestibility of silage mixtures (Mean±Std. Error).

Çizelge 3. Silaj karışımlarının gaz üretimi, metabolik enerjisi ve organik madde sindirilebilirliği (Ortalama±Standart Hata).

Treatments	Net Gas	CH ₄ (ml)	CH ₄ (%)	ME	OMD
Alfalfa Control	39.27±0.799 ^d	6.65±0.387 ^c	16.93±0.645 ^{bcd}	8.65±0.091 ^a	58.62±0.558 ^a
Alfalfa + OP5	31.38±1.222 ^{abc}	6.01±0.378 ^{bc}	19.13±0.536 ^{de}	7.60±0.177 ^{cde}	51.68±1.181 ^{cd}
Alfalfa + TP5	31.38±2.010 ^{abc}	4.91±0.583 ^b	15.58±0.999 ^b	7.64±0.271 ^{cde}	52.00±1.744 ^{cd}
Alfalfa + GP5	28.15±1.383 ^a	3.50±0.495 ^a	12.32±1.104 ^a	7.16±0.181 ^e	48.82±1.188 ^d
Alfalfa + LP5	30.91±0.799 ^{abc}	5.03±0.360 ^b	16.29±1.161 ^{bc}	7.60±0.093 ^{cde}	51.82±0.622 ^{cd}
Alfalfa + OP10	35.53±1.220 ^{cd}	6.64±0.035 ^c	18.73±0.563 ^{cde}	8.30±0.173 ^{ab}	56.44±1.169 ^{ab}
Alfalfa + TP10	34.14±1.846 ^{bc}	6.42±0.371 ^c	18.80±0.183 ^{cde}	8.03±0.260 ^{bcd}	54.57±1.756 ^{bc}
Alfalfa + GP10	29.99±1.661 ^{ab}	6.29±0.381 ^c	20.98±0.300 ^e	7.42±0.192 ^{de}	50.51±1.220 ^d
Alfalfa + LP10	35.53±1.220 ^{cd}	6.58±0.167 ^c	18.57±0.920 ^{cde}	8.19±0.169 ^{abc}	55.61±1.222 ^{abc}
Sig.	0.001 ^{**}	0.000 ^{**}	0.000 ^{**}	0.001 ^{**}	0.000 ^{**}

^{abc} Column means with common superscripts do not differ ($p>0.05$), CH₄: Methane, ME: Metabolizable energy, OMD: Organic matter digestion, OP: Orange pulp, TP: Tangerine pulp, GP: Grapefruit pulp, LP: Lemon pulp, Sig: Significant level, **: $p<0.01$.

Table 4. Aerobic stability parameters of of silage mixtures.

Çizelge 4. Silaj karışımlarının aerobik stabilite parametreleri.

	Degradation Time (Hours)	Max Temperature(°C)	Max Temperature Time (Hours)
Alfalfa Control	16	24.14	137
Alfalfa + OP5	11	24.26	79
Alfalfa + TP5	13	24.39	77
Alfalfa + GP5	20	24.08	36
Alfalfa + LP5	15	24.19	46
Alfalfa + OP10	5	24.51	152
Alfalfa + TP10	11	24.25	125
Alfalfa + GP10	9	24.45	125
Alfalfa + LP10	10	24.35	120

OP: Orange pulp, TP: Tangerine pulp, GP: Grapefruit pulp, LP: Lemon pulp.

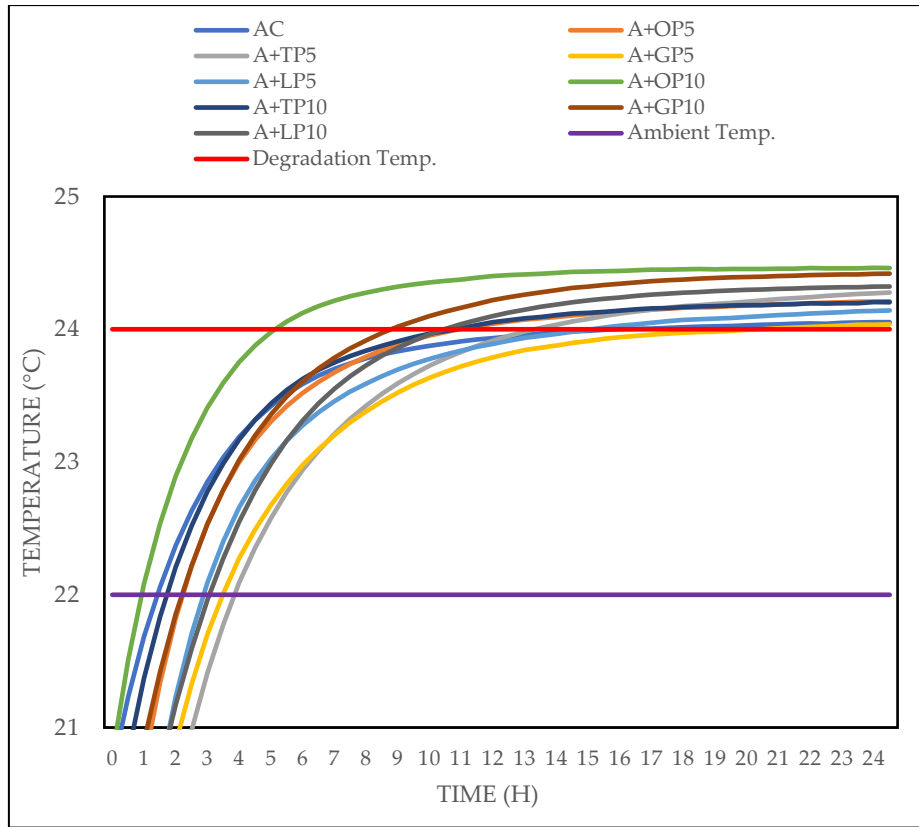


Figure 1. Effect of citrus pulps on aerobic stability of alfalfa silages.

Şekil 1. Narenciye posalarının yonca silajlarının aerobik stabilitesi üzerine etkisi.

DISCUSSION

The enhancement of nutritional quality and digestibility in citrus by-products, for use in ruminant nutrition, has garnered increased attention in recent times. This is likely due to the global abundance of citrus production, as well as its prevalence as an agro-industrial waste (Bambidis and Robinson, 2006; Mamma and Christakopoulos, 2014). In this study, the effects of the valorization of citrus pulp waste on improving the silage quality of alfalfa were evaluated by analyzing their effects on chemical composition, fermentation, gas production, and aerobic stability properties.

The nutritional value of forages and alternative feed resources is determined by several vital nutrients, including DM, CA, CP, EE, ADF, and NDF contents. These nutrients play a crucial role in determining the overall nutritive value of the feed. Adding citrus pulp as a supplement to silage increased the DM by potentially reducing the water activity in the stored silage. This can result in a better fermentation process, as the lowered water activity creates an optimal environment for beneficial fermentation bacteria to grow and enhance the quality of the silage. The increase in the CA content of silages with additives is thought to be due to the higher level of inorganic matter in the additive compared to alfalfa. Olowu (2021) reported that dry citrus pulp has CA contents between 3.57 and 5.10%. The CA results of silages with the addition in this study are generally higher than other results in the literature (Canbolat et al., 2010; Besharati et al., 2021).

Proteolysis is a process encountered during silage formation. In this process, the proteins in the silage are broken down into amino acids by bacterial enzymes, resulting in the structural integrity of the proteins being compromised (Kung, 2018). Proteolysis can have a negative impact on the quality of the silage because the resulting amino acids can react with other nutrients in the silage and reduce its nutritional value. Additionally, some compounds produced as a result of proteolysis can create an unpleasant odor or taste in the silage (Albrecht, 1991). The higher content of CP observed in the silages with citrus pulp

addition, as compared to the control group, may be attributed to the potential ability of certain compounds present in citrus pulp to inhibit proteolysis, the enzymatic breakdown of proteins. Earlier studies have also suggested that tannin polyphenols might hinder proteolysis in ensiled forage by deactivating proteolytic enzymes and/or by creating protein-polyphenol complexes (Guo et al., 2008; Lee et al., 2008).

NDF, as the structural component of plants, contributes to their bulk and serves as a predictor of voluntary intake. On the other hand, ADF includes the least digestible parts of plants, such as lignin and cellulose, resulting in feeds with lower ADF content generally having higher energy levels. Citrus pulp is a highly nutritious feed ingredient owing to its abundant supply of readily available fructose and glucose, as well as its diverse array of oxidative substrates for ruminal microorganisms, including fermentable sugars and a high proportion of easily digestible neutral detergent fiber (NDF) (Tariq, 2021). The study revealed variations in the ADF and NDF ratios among different citrus species, which were attributed to their fiber content. And the decrease observed in ADF and NDF values could be attributed to the breakdown of hemicelluloses, cellulose, and lignin by acid solubilization during the ensiling process (McDonald et al., 1991). Previous research has suggested that these differences in ADF and NDF content can have a direct impact on the quality of citrus pulp silage (Lashkari and Taghizadeh, 2013; Palangi et al., 2013; Beyzi et al., 2018).

Due to its high protein content and low levels of soluble carbohydrates and DM, alfalfa presents a challenge in terms of producing enough lactic acid to effectively reduce the pH value (Santos et al., 2016). Generally, pH values higher than 4.2 have been found to adversely affect the aerobic stability and long-term storage potential of silage (McDonald et al., 1991). Although there was a decrease between 2.66 and 9.63% in pH values compared to the control group, the achieved values remained above the desired levels (Table 2). Stallings et al. (1981) reported that the pH values of alfalfa silage after fermentation were between 4.50 and 4.70 without the use of additives. Accordingly, it was observed that the inclusion of 10% tangerine pulp was effective in achieving the desired pH levels in alfalfa silage. Recent studies have demonstrated that roughage can be improved in quality for use as ruminant feed through various processing methods such as fermentation, silage production, and the addition of additives (Zhang et al., 2017). The addition of citrus pulp into silage has been shown to enhance both the quantity and quality of the resulting feed, resulting in a lower pH and higher levels of sugars and acidic bacteria. This reduces the need for additional acid supplements (Crawshaw, 2003). The FS is a reference value used to determine the quality of silage, and it is based on measuring the pH and DM values of the silage. The addition of 10% tangerine pulp increased the FS of silages by more than 50%. While the control group silages were graded "low", the addition of 10% tangerine pulp increased the quality grades of the silages to "good". A high FS, which means a lower pH value, is associated with better silage quality. Conversely, higher pH values indicate lower quality silage, which is less nutritious. Therefore, measuring the FS is an important tool for evaluating the quality of silage and ensuring that it meets the nutritional needs of livestock. The RpH value is a parameter that describes the relationship between the DM values and pH levels in silages (Meeske, 2005). However, in this study, the additives did not lead to the desired or expected reduction in silage pH levels. According to Kamalak et al. (2009), a dose of 4.50-6.00% honey locust pods is required in alfalfa silage to achieve the desired RpH values. Many researchers have found it challenging to reduce pH levels to RpH values by adding additives to alfalfa silage (Sakalar and Kamalak, 2016). Additionally, the linear increase in DM resulting from the additives directly affects the RpH values, which are calculated based on the DM and pH values of the silage.

In vitro rumen fermentation is a rapid analysis method commonly utilized to study feed degradation, detect subtle variations in fermentation characteristics among supplements, and allow for more frequent testing (Makkar, 2004). In both in vivo and in vitro feeding studies, citrus flavonoids have been demonstrated to be safe and non-toxic (Li et al., 2014). Alfalfa, when combined with orange molasses and pulp, has been shown to improve silage quality, GP, and in vitro DM digestibility (Besharati et al., 2017). By-products derived from grapes have been found to reduce methane production by up to 20% in ruminants (Moate et al., 2014). Lopez et al. (2010) have classified feeds based on their CH₄ production percentages. Feeds with 14% or more CH₄ are considered to lack antimethanogenic properties, while values

between 11 and 14% are defined as having low antimethanogenic effects. Moderately antimethanogenic feeds fall within the range of 6 to 11%, and values less than 6% are classified as having high antimethanogenic properties. In the current study found that alfalfa silage does not possess antimethanogenic properties, as evidenced by the CH₄ percentage of 16.93% in the control group. However, the addition of citrus pulp to the silage resulted in varying antimethanogenic effects. While there was a change in the percentage of CH₄ production when citrus pulp silages were compared with the control group, only silages with 5% grapefruit exhibited antimethanogenic properties (12.32%). The GP results in this study are generally compatible with other results in the literature (Beshatari et al., 2017). While the addition of citrus pulp did not yield the desired effect, using pulp directly during the silage-making process resulted in a silage that produced less methane during digestion. This finding highlights the potential of using direct pulp in the production of silage as a way to reduce methane emissions in livestock farming (Başar and Atalay, 2020). Although this reduction in CH₄ percentage is insufficient in terms of global warming and energy loss of animals, a reduction of 27% can be considered a positive result. When GP values are considered as a whole, the antimethanogenic properties and the effect on preventing the negative effects of global warming of these silage additives will be limited.

Provision of feed is crucial for animal productivity, which in turn is heavily reliant on an adequate energy supply. The amount of energy that animals consume to meet their requirements can be used for growth, milk production, wool production, work, or other activities. The higher the percentage of OMD, the higher the proportion of organic matter content in the feed that can be digested by the animal. In order to increase OMD, it is known that excessive protein degradation needs to be prevented. Uçar (2016) reported a decrease in both ME and OMD values when pomegranate peel was added to alfalfa silages. This was due to the presence of tannin and phenolic compounds in the additive, which limited the amount of gas produced in in vitro tests. The reason for the decrease in ME and OMD values obtained as a result of the study is due to the decrease in the net GP of silages due to the additive. Additionally, Atalay (2009) found that adding a mixture of molasses and bay leaves to alfalfa silage resulted in a decrease in ME content and OMD, depending on the dose of the additive. Therefore, in animal feeding, the ME amount is an important factor for the healthy and efficient development of animals.

Silage aerobic stability refers to the ability of silage to maintain its quality during exposure to air. When silage is exposed to air, oxidation and decay processes caused by microorganisms begin (Wilkinson and Davies, 2013). The aerobic stability test measures how long silage can maintain its quality under conditions where it is exposed to air, providing information about the quality of the silage (Filya, 2004). Aerobic stability is also a crucial aspect to consider because it can cause not only nutrient and DM losses, but also pose health hazards to both animals and humans due to mycotoxins generated by unwanted microorganisms (Schmidt and Kung, 2010). In this study, the rate of degradation in the silages was found to be positively correlated with the dose of the additive, such that silages treated with higher doses of the additive exhibited faster rates of degradation. Although degradation was detected in all of the silage groups, none of them showed any signs of mold formation during the seven-day aerobic stability test. Meanwhile, Kamalak et al. (2012) observed that the addition of fodder carob as a source of water-soluble carbohydrates to alfalfa silage had no significant impact on its quality over an extended period of time.

CONCLUSION

This study has demonstrated a significant contribution of citrus pulp waste to eliminate potential negative effects on alfalfa silage during ensiling. The addition of waste improved the nutritional and fermentation quality of the silages while inhibiting proteolysis, which is one of the most important gains in feeding. This study has presented a different way of utilizing thousands of tons of citrus waste generated annually and concluded that this approach can prevent environmental pollution.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

DECLARATION OF AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

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