

# Assessing the nutritional value, fermentation quality, and *in vitro* degradability of mulberry pomace silage ensiled with sumac additive

## Evaluación del valor nutricional, la calidad de la fermentación y la degradabilidad *in vitro* del ensilaje de orujo de morera ensilado con aditivo de zumaque

Pınar Tatlı Seven<sup>1</sup> , Miray Sıla Çiçek<sup>1</sup>   
Seda İflazoğlu Mutlu<sup>1\*</sup> , Furkan Melih Dadan<sup>1</sup> , Numan Yusuf Aktaş<sup>1</sup> 

<sup>1</sup>Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Firat University, Elazığ 23119, Türkiye

\*Corresponding Author: [siflazoglu@firat.edu.tr](mailto:siflazoglu@firat.edu.tr)

### ABSTRACT

This study investigates the effects of sumac addition on the nutrient composition, *in vitro* degradability, and fermentation quality of mulberry pomace silage. Mulberry pomace is rich in protein, making it a valuable feed resource for livestock production. However, its high protein content may lead to increased proteolysis during silage fermentation, resulting in elevated pH levels and undesirable butyric acid production. Sumac, which is rich in tannins and organic acids, has the potential to inhibit proteolysis and enhance the fermentation process, thereby improving silage quality. Therefore, investigating the effects of sumac addition on the nutritional composition and fermentation characteristics of mulberry pomace silage is of significant importance. Mulberry pomace was ensiled in vacuum-sealed jars as an untreated control group (M) and in triplicate with the following treatments: 5% sumac group (MS-5) and 10% sumac group (MS-10). The silos (n=18) were stored for 45 days. After the ensiling period, the jars were opened, and physical and chemical analyses were conducted on the silage samples. The addition of sumac to mulberry pomace silage had a significant effect on dry matter (DM) content ( $P < 0.001$ ), with the highest DM level observed in the silage group containing 10% sumac. Sumac addition (10%) increased the crude protein content (12.96%) ( $P < 0.05$ ) while decreasing pH (3.69) and ammonia nitrogen (3.85%) levels ( $P < 0.05$ ). Compared to the control group, the levels of neutral detergent fiber (23.92%) ( $P < 0.01$ ), acid detergent fiber (15.41%) ( $P < 0.05$ ), and acid detergent lignin (7.24%) ( $P < 0.05$ ) were significantly lower in the 10% sumac group. In conclusion, the addition of sumac to mulberry pomace silage positively enhanced silage quality, contributing to improved fermentation by inhibiting proteolysis due to its tannin content.

**Key words:** *Morus alba* L.; silage; sumac; proteolysis degradability; fermentation

### RESUMEN

Este estudio investiga los efectos de la adición de zumaque en la composición de nutrientes, la degradabilidad *in vitro* y la calidad de la fermentación del ensilaje de orujo de morera. El orujo de morera es rico en proteínas, lo que lo convierte en un recurso alimenticio valioso para la producción ganadera. Sin embargo, su alto contenido proteico puede provocar un aumento de la proteólisis durante la fermentación del ensilaje, lo que resulta en un incremento del pH y en la producción de ácido butírico no deseado. El zumaque, rico en taninos y ácidos orgánicos, tiene el potencial de inhibir la proteólisis y mejorar el proceso de fermentación, contribuyendo así a una mayor calidad del ensilaje. Por lo tanto, es de gran importancia investigar los efectos de la adición de zumaque sobre la composición nutricional y las características fermentativas del ensilaje de orujo de morera. El orujo de morera se ensiló en frascos sellados al vacío como un grupo de control no tratado (M) y en triplicado con los siguientes tratamientos: 5% de zumaque (MS-5) y 10% de zumaque (MS-10). Los silos (n=18) se almacenaron durante 45 días. Tras el período de ensilaje, se abrieron los frascos y se realizaron análisis físicos y químicos en las muestras de ensilaje. La adición de zumaque al ensilaje de orujo de morera tuvo un efecto significativo sobre el contenido de materia seca (MS) ( $P < 0,001$ ), observándose el nivel más alto de MS en el grupo de ensilaje con 10% de zumaque. La adición de zumaque (10%) aumentó el contenido de proteína bruta (12,96%) ( $P < 0,05$ ) y redujo el pH (3,69) y los niveles de nitrógeno amoniacal (3,88%) ( $P < 0,05$ ). En comparación con el grupo de control, los niveles de fibra detergente neutra (23,92%) ( $P < 0,01$ ), fibra detergente ácida (15,41%) ( $P < 0,05$ ) y lignina detergente ácida (7,24%) ( $P < 0,05$ ) fueron significativamente menores en el grupo con 10% de zumaque. En conclusión, la adición de zumaque al ensilaje de orujo de morera mejoró positivamente la calidad del ensilaje, favoreciendo la fermentación al inhibir la proteólisis debido a su contenido de taninos.

**Palabras clave:** *Morus alba* L.; ensilado; zumaque; degradabilidad por proteólisis; fermentación

## INTRODUCTION

Ensuring the production of high quality feed is crucial for the progress of animal husbandry. Feed in the form of silage, obtained from crop residues and various agricultural or industrial by-products, serves as a valuable feed for animals and fills shortages in the feed supply. Thanks to the anaerobic fermentation process facilitated by epiphytic microorganisms in the feed, silage converts water-soluble carbohydrates into organic acids. The accumulation of these organic acids reduces the pH, prevents the growth of undesirable microorganisms and ensures the preservation of the nutrient content of the silage material [1]. It is possible to produce silage from various green forage plants and residues [2, 3, 4].

Mulberry (*Morus alba*) is a woody plant belonging to *Morus* genus of *Moraceae* family in *Urticales* order. Besides being consumed fresh, mulberry can also be utilised as molasses, jam, pastille, mulberry paste, etc. It is also widely used in the production of various processed products [5]. In the production of these foods, mulberry pulp, which is a by-product rich in protein (9-21 %), is obtained after the mulberry juice is extracted. Mulberry pulp, which has the potential to be an alternative feedstuff in animal nutrition, consists of the bark and stem parts of the mulberry and constitutes approximately 8 % of its weight [6]. It has been reported that ensiling of mulberry pomace is a practical and effective way for long-term storage and industrial use [7]. The water-soluble carbohydrate content of mulberry pomace silage was 15.64 % and the protein content was 19.79 % [7].

The use of additives that can prevent or reduce proteolysis, such as tannins, as silage additives for silage feeds with high protein content but high water-soluble carbohydrate content suitable for silage production has increased in recent years [8]. Tannins are considered to be effective substances in silage production because they prevent excessive breakdown of proteins during ensiling and in the digestive system of ruminants. However, in order to obtain optimum silage in the pH range of 3.8 - 4.2, it is recommended to add materials rich in water-soluble carbohydrates (WSC) and tannins to the silage [9, 10]. Studies reported that additives with high tannin content increased dry matter (DM) and decreased crude ash (CA), ammonia and pH parameters in alfalfa silage [11].

Sumac (*Rhus coriaria*), belonging to the family *Anacardiaceae*, includes about 150 species worldwide: only two species, *Rhus coriaria* L. (Tanner's sumac) and *Cotinus coggyria* Scop. (Syn: *Rhus cotinus* L.) (dyer's sumac), are cultivated and economically valuable. Tanner sumac leaves contain about 15-20 % tannins and myricetin, 7 % water, 11 % CA, sugars (glucose, rhamnose, sucrose, galactose, etc.) and waxy substances [8]. Analysis of the content of sumac plant showed richness in tannin [8]. In silages obtained from high protein plants, a large amount of non-protein-N is produced with the effect of plant proteases and microbial activities. This causes economic loss and environmental pollution [12]. It is known that tannins reduce non-protein-N production by decreasing proteolysis in silage fermentation [13, 14]. From this point of view, it was predicted that tannin-added

silages would contribute to the reduction of economic loss and environmental pollution. This study was planned based on the hypothesis that the addition of sumac to mulberry pomace would prevent the formation of butyric acid by inhibiting the protein digestion of mulberry and the organic acids in sumac would improve the fermentation of mulberry pomace silage. In this study, the contribution of sumac to mulberry pomace silage and its effects on nutrient levels, nutrient degradability and some silage fermentation parameters were investigated.

## MATERIALS AND METHODS

### Silage preparation

The fresh white mulberry pomace, the silage material for the study, was obtained from Yenice village in the Karakoçan district of Elazığ province. At the same time, sumac, used as a silage additive, was procured from a commercial company (Arifoğlu). The dry matter (DM) levels of mulberry pomace and sumac were determined through a preliminary study by drying (Binder, Tuttlingen, Germany) at 60 °C for 72 hours (h). The amounts of sumac to be added to mulberry pomace, at 5 % and 10 % levels based on DM [15], were calculated. Subsequently, these calculated amounts were thoroughly mixed to achieve homogeneity, and three different silage groups were formed. The experimental groups were as follows: mulberry pomace 0 % (control), mulberry pomace+sumac 5 % (MS-5), and mulberry pomace+sumac 10 % (MS-10). Mulberry pomace was ensiled in six replicate per treatment in vacuum jars. The prepared silages were compacted into 18 1.8 L, vacuum jars sealed airtight, and stored at room temperature (20-24 °C). Silos were stored for 45 days (d).

### Organoleptic evaluations

Organoleptic evaluations of silage samples were carried out according to the methods described by Başaran *et al.* [16]. In addition, Flieg scores of silages were determined using the following equation [16, 17].

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

### Chemical analysis

After silaging, fresh mulberry pomace silage samples taken from glass jars were dried at 60 °C for 72 h, ground to a 1 mm particle size. DM, CA (Protherm PLF 120/7, Türkiye), organic matter (OM), ether extract (EE) (Gerhardt, Germany) and crude protein (CP) (Vapodest, Gerhardt, Germany) were analysed using AOAC methods [17]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), crude fiber (CF) and hemicellulose (HC) contents were determined using Ankom 220 Fibre Analyzer (Ankom Technology, Macedon, USA) according to Van Soest *et al.* [18]. Cellulose content is determined by the difference between ADF and ADL, while hemicellulose content is calculated as the difference between NDF and ADF. To analyze ammonia content, 20 grams of ensiled mulberry pomace material were placed in filter stomacher bags, 180 mL of distilled water was added, thoroughly mixed, homogenized for 5 minutes

using a stomacher device (Colworth stomacher 400, England), and then filtered. The pH of the obtained filtrate was measured with a calibrated pH meter (Hanna pH 211, Hanna Instruments Italia Srl, Padova, Italy). Subsequently, 100 mL of the filtrate was taken, and ammonia content was determined by distillation and titration according to the Kjeldahl method [8]. The metabolizable energy (ME) content of feeds is calculated using the following formula developed by Kirchgeßner et al. [19]:  $ME (Kcal/kg DM) = 3309.5 - 35.64 \times CF$

The tannin content of sumac was analyzed and determined to be 311 µg/g [8]. In a previous study [8], the nutrient content of sumac used as silage additive was determined as 89.04, 7.45, 10.23, 10.31, 20.02, 22.90, 18.22, 2.2% in DM, CA, CP, EE, CF, NDF, ADF, ADL levels, respectively.

The choice of farms was guided by the free manager's acceptance to cooperate or not with the study. The epidemiological questionnaire of farms contained 76 closed-type questions. These were related to the infrastructure, food, hygiene, care, and supervision of the lambs on each farm. The information thus gathered was based both on personal observations and on data collected from the owners.

### ***In-vitro* true degradability (IVTD)**

*In-vitro* true degradability analyses of silage samples on the 45th d were conducted using an Ankom Daisy incubator (Ankom Technology, Macedon, USA) according to Tilley and Terry's method [20]. Rumen fluid used for *in vitro* true degradability analyses was obtained from two male Simmental breed cattle of the same age and weight, carefully selected from the ELKAS Meat Integrated Facility in Elaziğ. Calculations of *in-vitro* true DM degradability and *in-vitro* true OM degradability of feed samples were performed using the equations provided by Ankom [21].

### **Statistical analysis**

SPSS software package was used for statistical analyses. One-way analysis of variance (ANOVA) was applied. Differences between groups were determined using Duncan's multiple range test. The significance level was accepted as  $P < 0.05$  and the data were presented as mean  $\pm$  standard error [22].

## **RESULTS AND DISCUSSION**

### **Chemical composition of fresh and mulberry pomace silages**

The nutrient composition values of mulberry pulp used in the study are presented in TABLE I. The nutrient composition,  $NH_3$ -N concentrations and pH values of M, MS-5 and MS-10 silage groups are presented in TABLE II. The DM contents of fresh mulberry pomace and sumac were determined as 34.05% and 89.04%, respectively. The effect of sumac addition on the DM level of mulberry pomace silage was found to be statistically significant ( $P < 0.01$ ), and the highest DM content was observed in the silage group to which 10 % sumac was added.

**TABLE I**  
**Nutrient composition of fresh mulberry pomace used in silage production**

Parameters	Mulberry Pomace
DM, %	34.05
CA, %	4.52
CP, %	14.31
EE, %	15.93
CF, %	12.51
NDF, %	24.35
ADF, %	15.90
ADL, %	7.51
ME, kcal/kg	2863

DM: Dry matter; CA: Crude ash; CP: Crude protein; EE: Ether extract; CF: Crude fiber; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; ME: Metabolizable energy; \*Calculated.

The effect of sumac addition on the CA content, OM, EE, HC, and cellulose of the silages was statistically insignificant ( $P > 0.05$ ), indicating that there was no significant difference between the groups. When the CP contents of the experimental groups were examined, the addition of 10 % sumac to mulberry pomace significantly increased the CP content ( $P < 0.05$ ). It is seen from TABLE II that the pH values of the silages varied between 3.71 and 3.69. There was a statistically significant difference in pH values between the M group and MS-10 group silages ( $P < 0.05$ ).  $NH_3$ -N values for each group are presented in TABLE I. A statistically significant difference was observed in  $NH_3$ -N values between the M group silage and the MS-10 group silage ( $P < 0.05$ ).

In this study, the effects of sumac addition to silages obtained from mulberry pulp, a by-product of the fruit industry, on nutrient levels, *in vitro* nutrient degradability and silage quality were investigated. When the DM contents of the groups were examined, the highest DM content was determined in the silage group with 10 % sumac addition ( $P < 0.001$ ). The increase in DM content in MS-5 and MS-10 groups was attributed to the higher DM content of sumac (89.04 %) used for silage compared to mulberry pulp (34.05 %, TABLE I) (TABLE II). Similar trends have been shown in previous studies; for example, in a study on sugar beet pulp silage with gladicia fruit addition (*Gleditsia triacanthos*) [23], the addition of gladicia fruit increased the DM content of the silage according to the added amounts (3, 6, 9 %), and the DM content of gladicia fruit (93.94 %) was significantly higher than the DM content of sugar beet pulp (17.79 %).

In another study on alfalfa silage with oak tannin extract added (DM 91.95 %), it was reported that the increase in oak tannin addition led to a significant increase in the DM content of the silage [24]. When the CA content was examined, the addition of sumac to mulberry pulp silage did not have a statistically significant effect ( $P > 0.05$ ; TABLE II). Similarly, in a study on alfalfa silage with sumac and molasses added, it was reported that there was no significant difference in CA values compared to the control group on the 45th d [8]. In addition, when this research findings on organic matter were evaluated, no statistically significant difference was found between the OM levels of the groups on the 45th d ( $P > 0.05$ ; TABLE II).



**TABLE II**  
Nutrient content, NH<sub>3</sub>-N concentrations, pH, NDF, ADF, ADL, cellulose, and hemicellulose values of mulberry pomace silages

	Groups			P<0.05
	M	MS-5	MS-10	
DM, %	31.46 ± 0.47 <sup>c</sup>	33.72 ± 0.14 <sup>b</sup>	35.29 ± 0.10 <sup>a</sup>	0.001
CA, %	5.01 ± 0.10	4.76 ± 0.03	4.79 ± 0.04	0.199
OM, %	88.75 ± 0.31	89.12 ± 0.33	89.23 ± 0.23	0.484
EE, %	16.80 ± 0.41	16.56 ± 0.39	16.09 ± 0.25	0.406
CP, %	12.49 ± 0.10 <sup>b</sup>	12.84 ± 0.08 <sup>AB</sup>	12.96 ± 0.13 <sup>A</sup>	0.016
pH	3.71 ± 0.01 <sup>A</sup>	3.70 ± 0.01 <sup>AB</sup>	3.69 ± 0.01 <sup>B</sup>	0.034
NH <sub>3</sub> -N, %	4.46 ± 0.16 <sup>A</sup>	3.93 ± 0.14 <sup>AB</sup>	3.85 ± 0.13 <sup>B</sup>	0.028
NDF, %	28.19 ± 0.89 <sup>A</sup>	26.19 ± 0.66 <sup>A</sup>	23.92 ± 0.41 <sup>B</sup>	0.005
ADF, %	18.72 ± 1.04 <sup>A</sup>	17.67 ± 0.41 <sup>A</sup>	15.41 ± 0.46 <sup>B</sup>	0.024
ADL, %	9.55 ± 0.52	8.30 ± 0.40 <sup>AB</sup>	7.24 ± 0.38 <sup>B</sup>	0.015
Cellulose, %	9.47 ± 0.41	8.52 ± 0.56	8.52 ± 0.05	0.209
Hemicellulose, %	9.18 ± 0.62	9.37 ± 0.25	8.17 ± 0.42	0.186

DM: Dry matter; CA: Crude ash; OM: Organic matter; EE: Ether extract; CP: Crude protein; NH<sub>3</sub>-N: Ammonia nitrogen; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; M: Mulberry pomace silage; MS5: Mulberry pomace+5 % Sumac; MS10: Mulberry pomace+10 % Sumac. Data are presented as mean ± standard error. <sup>A</sup>, <sup>B</sup>, <sup>C</sup>Values with different letters in the same columns are significantly different.

In a study [25], an increase in the OM content of silages with alfalfa, bran and pudding was found and this was attributed to the low CA content. However, in this study, since the CA levels of the silage groups with and without sumac were statistically insignificant (P>0.05), no statistically significant difference was found in the OM levels. In a similar study [26], 0, 20, 40 and 60 g.kg<sup>-1</sup> gladicia fruit (CA, 3.28 %) were added to alfalfa silage, respectively (fresh alfalfa CA, 5.97 %). It was reported that the CA levels of fresh alfalfa and gladicia added silages (0, 20, 40 and 60 g.kg<sup>-1</sup>) were determined as 5.92, 5.84, 5.81 and 5.76 %, respectively (P>0.05).

Ether extract (EE) content of silages is presented in TABLE II. EE content of mulberry pulp and sumac was determined as 15.93 % (TABLE I) and 10.31 %, respectively. As sumac addition increased, EE content in silage groups decreased numerically. However, no statistically significant difference was observed between the groups (P>0.05). In a similar study on alfalfa silage with sumac addition, no significant difference was reported in EE content compared to other silages without sumac [8]. However, in a different study [24], when the EE of silages with 1.5, 3 and 6 % tannin added to alfalfa silage were examined, it was found that there was no statistically significant difference between the 1.5 % and 3 % tannin groups, but the EE of the 6 % tannin silage group was statistically significantly lower than the other two groups. It was concluded that there may be differences in the EE of the silages depending on the oil content of the silage material and the silage additives used.

Crude protein (CP) content of the silages is presented in TABLE II. Addition of 10 % sumac to mulberry pulp significantly increased the CP content (P<0.05). The level of ammonia nitrogen (NH<sub>3</sub>-N) in silages [13], an indicator of protein degradation, was lower in MS-5 and MS-10 groups compared to M group (P<0.05), indicating a decrease in proteolysis. This suggests that tannins in sumac may reduce proteolysis during fermentation. Consistent with this study, a study conducted on sugar beet pulp silage with the addition of gladicia fruit reported that the addition of gladicia

fruit, rich in tannin content, increased the protein content and this reduced proteolysis by the tannin in its structure [23].

The pH and NH<sub>3</sub>-N values of mulberry pulp silages are given in TABLE II. The pH values of the silages were found to be between 3.71 and 3.69. The pH of the MS-10 group was significantly lower than the pH of the M group (P<0.05). The proteolysis rate is considered an important parameter during silage fermentation. Proteins are hydrolyzed to peptides, amino acids, and ammonia by plant proteases and microbial activity. Proteolytic activity and protein degradation have been associated with acidic environmental conditions, especially after silage capping. Therefore, a rapid decrease in pH during the critical period at the beginning of ensiling has been reported to be an important factor [27, 28, 29]. In a study where oak grain extract was selected as an additive due to its tannin content like sumac and used in silage production with alfalfa (*Medicago sativa*) [24], a decrease of 0.0813 units in silage pH was observed with a one-unit increase in oak grain additive ratio. In a similar study [9], commercial hydrolyzable tannin obtained from chestnut was added to alfalfa silage. It was reported that the group in which 2 % chestnut grain was added to alfalfa silage was found to be statistically lower in terms of pH compared to the control group (P<0.05).

When the NH<sub>3</sub>-N values of mulberry pomace silages were examined, a statistically significant difference was found in silages constituting the M group and MS-10 group (P<0.05). It was found that the NH<sub>3</sub>-N value was higher in the unmodified mulberry pulp silage. This ratio decreased with the addition of sumac. This situation can be explained by the decrease in proteolysis during silage [30]. The results of CP and NH<sub>3</sub>-N of the silages are consistent (TABLE II). The NH<sub>3</sub>-N content decreased significantly in the MS-10 group compared to the other groups. This is probably due to the low pH and antimicrobial activity resulting from the restriction of deamination of peptides by the addition of sumac and thus the decrease in NH<sub>3</sub>-N production [31].

In the study where oak grain was also used [24], the ammonia content of the silages varied between 4.48 and 29.34 %, while the highest NH<sub>3</sub>-N level was detected in the alfalfa silage produced without the use of additives. It was determined that the ammonia content of the silage decreased by 4.169 units with an increase of one unit of oak grain.

## Cell wall components of mulberry pomace silages

The NDF, ADF, ADL, cellulose, and hemicellulose values of mulberry pomace silages are presented in TABLE II. When looking at the study groups, the NDF values were found to be 28.19 % in group M silage, 26.19 % in group MS-5 silage, and 23.92 % in group MS-10 silage. A statistically significant difference in NDF values was observed in group MS-10 silage compared to the other groups (P<0.01). Examining the ADF values of the study groups (TABLE II), values of 18.72 % for group M, 17.67 % for group MS-5, and 15.41 % for group MS-10 were found. A statistically significant difference in ADF values was detected in group MS-10 silage compared to the other groups (P<0.05). When the ADL values of the groups were examined (TABLE II), values of 9.55 % for group M, 8.30 % for group MS-5, and 7.24 %

for group MS-10 were found. A statistically significant difference in ADL values was observed between group M and MS-10 silage ( $P < 0.05$ ).

The cellulose values in TABLE II for the study groups ranged from 9.47 to 8.52 %, and hemicellulose values ranged from 9.37 to 8.17 %. No statistically significant difference ( $P > 0.05$ ) was determined among the groups regarding cellulose and hemicellulose. The NDF values of the groups showed a significant difference, with the MS-10 group having lower NDF content than other groups ( $P < 0.01$ ) (TABLE II). Adding sumac was associated with a lower NDF content (22.90 %) than mulberry pomace NDF (24.35 %). A similar study on sugar beet pulp silage (46.88 %) with the addition of low-NDF apple fruit (6.45 %) showed a significant decrease in NDF levels in silages with increasing apple fruit addition rates (5 and 10 %) [32]. The study indicates that the addition of 10 % sumac significantly reduced the ADF content in mulberry pomace silage ( $P < 0.05$ ) (TABLE II). This reduction is attributed to the positive impact of sumac on silage fermentation, possibly increasing the number of anaerobic bacteria like lactic acid bacteria, leading to enhanced hydrolysis of cell wall carbohydrates such as NDF, ADF, and HC [8, 33, 34]. Similar effects were observed in a study [33] where molasses and formic acid were added to silage, resulting in significantly lower NDF and ADF levels compared to the control group ( $P < 0.01$ ). The ADL values of mulberry pomace silages showed a decrease, especially in the MS-10 group compared to the M group ( $P < 0.05$ ), attributed to the positive effects of sumac on silage fermentation (reduced pH and  $\text{NH}_3\text{N}$ ), leading to increased anaerobic bacteria and subsequent hydrolysis of cell wall carbohydrates [34, 35]. However, this effect was not observed in cellulose and hemicellulose levels, with numerical decreases in the MS-10 group but no statistical significance ( $P > 0.05$ ; TABLE II).

## Evaluation of silage based on organoleptic characteristics and Flieg score

The silage groups M, MS-5, and MS-10 created for the research were evaluated based on their organoleptic characteristics for quality classification, and the Flieg score is presented in TABLE III. Upon the evaluation of silage based on organoleptic characteristics (odor, external appearance, and color), group M was rated as "good," while groups MS-5 and MS-10 were rated as "very good." As a result of the study, the Flieg silage score was above 100 points based on DM content and pH values. The calculated scores were 119.42 for group M, 123.85 for group MS-5, and 127.68 for group MS-10, with a statistically significant difference detected among the groups ( $P < 0.01$ ).

The organoleptic evaluation results and Flieg score of the silage groups are given in TABLE III. In the organoleptic evaluation of the silages in terms of odor, appearance, and color, the M group obtained the lowest score, and the MS-10 group obtained the highest score. The Flieg scores of the groups in which sumac was added were statistically significantly higher than the silage group without additive ( $P < 0.01$ ). When appropriate pH and DM ratio are provided in silage feed, the Flieg score is also high [36]. The increase in the Flieg score of the sumac-added groups was attributed to the positive effect of

the tannin content of sumac on silage fermentation by reducing proteolysis and lowering pH during fermentation [13]. Başar and Atalay [37] reported that the Flieg score values of various citrus pomace silages varied between 80.50 and 111.78. In another study in which the ensiling of waste red and white grape pomace was examined, Flieg score was 160.20 in the white grape pomace group, while Flieg score was 157.65 in the red grape pomace group [36]. When the Flieg score of the two pulp studies and this study were compared, it was observed that the values of mulberry pomace silage were higher than the Flieg score of citrus pomace silage and lower than that of grape pomace. This was attributed to the differences in the nutrient levels and silage fermentation of the pulps.

Groups	Odor	External Appearance	Color	Total Score	Flieg Score
M	12	3	2	17	119.42 ± 1.60 <sup>c</sup>
MS-5	13	4	2	19	123.85 ± 0.50 <sup>b</sup>
MS-10	14	4	2	20	127.68 ± 0.26 <sup>a</sup>
$P < 0.01$					

M: Mulberry pomace silage; MS5: Mulberry pomace+5 % Sumac; MS10: Mulberry pomace+10 % Sumac. Quality grades are classified as excellent-good (16-20 points); satisfactory (10-15 points); moderate (5-9 points); and very poor (0-4 points). Values with different letters in the same columns (A-C) are significantly different ( $P < 0.01$ ).

## In vitro degradability

The *in vitro* true degradability values of the silage groups are presented in TABLE IV. The *in vitro* DM degradability values for groups M, MS-5, and MS-10 were 68.96, 69.31 and 71.19 %, respectively. The *in vitro* OM degradability values were 67.19, 67.68 and 69.63 %, respectively. No statistically significant difference was found among the groups regarding *in vitro* DM and OM degradability ( $P > 0.05$ ).

Sumac addition did not significantly affect the *in vitro* degradability (DM, OM) of silage. Similarly, a study on alfalfa silage supplemented with sumac and molasses [8] reported that the addition of sumac alone did not significantly affect the *in vitro* degradability (DM, OM).

	IVDMD, %	IVOMD, %
M	68.96 ± 1.27	67.19 ± 1.33
MS-5	69.31 ± 0.65	67.68 ± 0.69
MS-10	71.19 ± 1.98	69.63 ± 2.09
<i>P</i>	0.484	0.467

M: Mulberry pomace silage; MS-5: Mulberry pomace+5 % Sumac; MS-10: Mulberry pomace+10 % Sumac; IVDMD: *In vitro* DM degradability; IVOMD: *In vitro* OM degradability.

## CONCLUSION

The results of this research show a promising perspective for the use of sumac as a silage additive. The MS-10 group demonstrated significant increases in DM and CP, while CA and OM contents were not significantly affected. The rise in silage CP levels, attributed to the tannin content in sumac, is a critical factor in reducing proteolysis during fermentation. The high Flieg score and significant decreases in pH and NH<sub>3</sub>-N values for silages with sumac addition indicate a favorable fermentation process. The positive effect of sumac on silage fermentation in mulberry pomace silage, leading to a decrease in pH and NH<sub>3</sub>-N, suggests an increase in the hydrolysis of cell wall components. While there was no significant effect of sumac addition on *in-vitro* DM and OM degradability, these findings pave the way for further exploration and potential advancements in silage production.

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## Conflict of Interest

The authors declared that they have no conflict of interest.

## BIBLIOGRAPHICS REFERENCES

- [1] Shuo W, Peishan H, Chao Z, Wei Z, Xiaoyang C, Qing Z. Novel strategy to understand the aerobic deterioration of corn silage and the influence of *Neolamarckia cadamba* essential oil by multi-omics analysis. *Chem. Eng. J.* [Internet]. 2024; 482:148715. doi: <https://doi.org/ph9q>
- [2] Büyükkılıç-Beyzi S, Ülger İ, Kaliber M, Konca Y. Determination of chemical, nutritional, and fermentation properties of citrus pulp silages. *TURJAF.* [Internet]. 2018; 6(12):1833. doi: <https://doi.org/ph9r>
- [3] Ayaşan T, Sucu E, Ülger I, Hızlı H, Çubukcu P, Özcan BD. Determination of in vitro rumen digestibility and potential feed value of tiger nut varieties. *S. Afr. J. Anim. Sci.* [Internet]. 2020; 50(5):738-744. doi: <https://doi.org/ph9s>
- [4] Büyükkılıç Beyzi S, Ülger İ, Konca Y. Chemical, fermentative, nutritive, and anti-nutritive composition of common reed (*Phragmites australis*) plant and silage. *WBV.* [Internet]. 2022; 14(4):1-10. doi: <https://doi.org/gvvpvk>
- [5] Kaya İB, Selçuk Z. Investigation of availability of dried mulberry pomace instead of barley for ruminants. *Kocatepe Vet. J.* [Internet]. 2021; 14(2):177-186. doi: <https://doi.org/ph9t>
- [6] Köksal Y, Bölükbaş B, Selçuk Z. An In Vitro Evaluation of the Silage Mixtures Containing Different Levels of White Mulberry Pomace and Meadow Grass. *KVJ.* [Internet]. 2021;14(3):309-15. doi: <https://doi.org/ph9v>
- [7] Zhou B, Meng QX, Ren LP, Shi FH, Wie Z, Zhou ZM. Evaluation of chemical composition, *in situ* degradability, and *in vitro* gas production of ensiled and sun-dried mulberry pomace. *J. Anim. Feed Sci.* [Internet]. 2012; 21(1):188-197. doi: <https://doi.org/f3wttb>
- [8] Tatlı-Seven P, Yıldırım EN, Seven İ, Kaya CA, İflazoğlu-Mutlu S. An evaluation of the effectiveness of sumac and molasses as additives for alfalfa silage: Influence on nutrient composition, *in vitro* degradability and fermentation quality. *J. Anim. Physiol. Anim. Nutr.* [Internet]. 2024; 108(4):1096-1106. doi: <https://doi.org/ph9w>
- [9] Tabacco E, Borreani G, Crovetto GM, Galassi G, Colombo D, Cavallarin L. Effect of chestnut tannin on fermentation quality, proteolysis, and protein rumen degradability of alfalfa silage. *J. Dairy Sci.* [Internet]. 2006; 89(12):4736-4746. doi: <https://doi.org/dkbzt9>
- [10] Besharati M, Maggiolino A, Palangi V, Kaya A, Jabbar M, Eseceli H, De Palo P, Lorenzo JM. Tannin in ruminant nutrition: Review. *Mol.* [Internet]. 2022; 27(23):8273. doi: <https://doi.org/gtkgxt>
- [11] Şengül AY, Aydın R. Use of farmatan as an additive to make alfalfa silage. *TURKJANS.* [Internet]. 2019; 6(3):579-587. doi: <https://doi.org/ph9x>
- [12] He L, Lv H, Xing Y, Chen X, Zhang Q. Intrinsic tannins affect ensiling characteristics and proteolysis of *Neolamarckia cadamba* leaf silage by largely altering bacterial community. *Bioresour Technol.* [Internet]. 2020; 311:123496. doi: <https://doi.org/gzkv7w>
- [13] He L, Lv H, Chen N, Wang C, Zhou W, Chen X, Zhang Q. Improving fermentation, protein preservation and antioxidant activity of *Moringa oleifera* leaves silage with gallic acid and tannin acid. *Bioresour. Technol.* [Internet]. 2020; 297:122390. doi: <https://doi.org/ghj27m>
- [14] Jayanegara A, Sujarnoko TUP, Ridla M, Kondo M, Kreuzer M. Silage quality as influenced by concentration and type of tannins present in the material ensiled: a meta-analysis. *J. Anim. Physiol. Anim. Nutr.* [Internet]. 2019; 103(2):456-465. doi: <https://doi.org/ph96>
- [15] Wischer G, Boguhn J, Steingaß H, Schollenberger M, Rodehutschord M. Effects of different tannin-rich extracts and rapeseed tannin monomers on methane formation and microbial protein synthesis in vitro. *Anim.* [Internet]. 2013; 7(11):1796-805. doi: <https://doi.org/f5cftt>

- [16] Başaran U, Gulumser E, Mut H, Çopur-Doğrusöz M. Determination of Silage Yield and Quality of Grasspea+ Cereal Intercrops. Turkish JAF Sci.Tech. [Internet]. 2018[cited Nov 23 2024]; 6(9):1237-1242. Available in: <https://goo.su/zr17H>
- [17] AOAC. Official Methods of Analysis. 18th.Ed. Association of Official Analytical Chemists, Gaithersburg: MD; 2006.
- [18] Van-Soest PJ, Robertson JD, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. JDS. [Internet]. 1991; 74(10):3583-3597. doi: <https://doi.org/b6c78f>
- [19] Kirchgessner M, Kellner RJ, Roth FX .Zur schätzung des futterwertes mittels rohfaser und der zellwandfraktionen der detergentien-analyse. Landwirtsch. Forsch. [Internet]. 1977[cited Nov 18 2024]; 30:245-250. Available in: <https://goo.su/sznMR1>
- [20] Tilley JMA, Terry RA. A two-stage technique for the in vitro digestion of forage crops. Grass Forage Sci. [Internet]. 1963; 18(2):104-111. doi: <https://doi.org/c4kw3p>
- [21] ANKOM. Ankom Technology Method 3. *In-vitro* true digestibility using the DAISYII Incubator; 2020. [Internet]. Available in: <https://goo.su/9jZ9kz>
- [22] SPSS. Statistics for Windows, Version 22.0. IBM Copyright SPSS Inc, Armonk, NY; 2013.
- [23] Özkan ÇÖ. Utilization of *Gleditsia* (*Gleditsia triacanthos*) fruit in sugar beet pulp silage. [Ph.D. thesis]. Kahramanmaraş (Turkey): Kahramanmaraş Sütçü İmam University, Institute of Natural Sciences; 2012: 81 p. Available in: <https://goo.su/kFXs>
- [24] Kamalak A, Şahin M, Canbolat Ö, Kurt Ö. The effect of tannin extract (Artutan) as a silage additive on the quality characteristics of clover silage and its effects on feed consumption, digestion, and rumen fermentation in sheep. VIth International Balkan Animal Conference BALNIMALCON 2013. 3-5 October 2013 Tekirdag / Turkey. Project No: 111 O 821. TUBITAK. [Internet]. 2014. Available in: <https://goo.su/uYau>
- [25] Malhatun Çotuk G, Soycan Önenç S. Yonca silajına kepek ve puding ilavesinin silaj fermentasyonu, aerobik stabilite ve *in vitro* sindirilebilirlik üzerine etkileri. Hayvansal Üret. [Internet]. 2016[cited Nov 23 2024]; 58:13-19. Available in: <https://goo.su/om1N>
- [26] Canbolat Ö, Kalkan H, Filya İ. Yonca silajlarında katkı maddesi olarak gladiçya meyvelerinin (*Gleditsia triacanthos*) kullanıma olanakları. Kafkas Univ. Vet. Fak. Derg. [Internet]. 2013; 19:291-297. doi: <https://doi.org/ph98>
- [27] Filya I. Silage fermentation. Atatürk Univ. J. Agric. Fac. [Internet]. 2001[cited Nov 13 2024]; 32:87–93. Available in: <https://goo.su/H0Qtck>
- [28] Bai J, Dongmei X, Dongmei X, Musen W, Ziqian L, Xusheng G. Effects of antibacterial peptide-producing *Bacillus subtilis* and *Lactobacillus buchneri* on fermentation, aerobic stability, and microbial community of alfalfa silage. Bioresour. Technol. [Internet]. 2020; 315:123881. doi: <https://doi.org/gwj8wk>
- [29] Koc F, Karapinar B, Okuyucu B, Korucu-Erdem D. The effects of kefir addition on the fermentation characteristics and aerobic stability of alfalfa silages. KSU J. Agric Nat. [Internet]. 2020;23(2):535–542. doi: <https://doi.org/pjbb>
- [30] Li X, Tian J, Zhang Q, Jiang Y, Wu Z, Yu Z. Effects of mixing red clover with alfalfa at different ratios on dynamics of proteolysis and protease activities during ensiling. J. Dairy Sci. [Internet]. 2018; 101(10):8954-8964. doi: <https://doi.org/gfcbxt>
- [31] Song C, Li J, Xing J, Wang C, Li J, Shan A. Effects of molasses interacting with formic acid on the fermentation characteristics, proteolysis and microbial community of seed-used pumpkin leaves silage. J. Clean. Prod. [Internet]. 2022; 380(2):135186. doi: <https://doi.org/gtbdgb>
- [32] Bağuç Y, Aksu T. Elma (*Malus pumila*) katkısının yaş şeker pancarı posası silajı kalitesine etkisi. Atatürk Univ. Vet. Bilim. Derg. [Internet]. 2021; 16(1):49–56. Available in: <https://goo.su/BsStnoU>
- [33] Bingöl NT, Karslı MA, Bolat D, Akça İ. Vejetasyonun farklı dönemlerinde hasat edilen korungaya ilave edilen melas ve formik asit'in silaj kalitesi ve in vitro kuru madde sindirilebilirliği üzerine etkileri. Van. Vet. J. [Internet]. 2008[cited Dec 3 2024]; 19(2):61-66. Available in: <https://goo.su/kJGv>
- [34] Uğurlu S, Okuyucu B, Özdüven ML. Bakteriyel inokulantların çavdar (*Secale cereale* L.) hasılı silajlarında fermentasyon, aerobik stabilite ve yem değeri üzerine etkileri. TURJAF. [Internet]. 2022; 10(3):426–433. doi: <https://doi.org/pjbc>



- [35] Koç F, Coşkuntuna L, Ozduven ML. The effect of bacteria+enzyme mixture silage inoculant on the fermentation characteristic, cell wall contents and aerobic stabilities of maize silage. Pak. J. Nutr. [Internet]. 2008; 7(2):222-226. doi: <https://doi.org/bv98rb>
- [36] Ulger İ, Özdemir M. Atık kırmızı ve beyaz üzüm posalarının alternatif kaba yem kaynağı olarak silolanmasının besin madde kompozisyonu ve silaj kalite özellikleri üzerine etkileri. OKÜ Fen Bil. Ens. Dergisi. [Internet]. 2023; 6(1):792-805. doi: <https://doi.org/pjbd>
- [37] Başar Y, Atalay Aİ. The use of citrus pulps as an alternative feed source in ruminant feeding and ist methane production capacities. JIST. [Internet]. 2020;10(2):1449–1455. doi: <https://doi.org/pjbf>