The Effect of Lavender Pomace on Chemical Composition, Microbiological and Some Fermentation Characteristics of Alfalfa Silages

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Published: 31 January 2020
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Abstract

In this study, the effects of lavender (*Lavandula angustifolia*) pomace added at different levels on chemical, microbiological and some fermentation properties of alfalfa silages were investigated. Lavender pomace was added to alfalfa silage at 0 % (control), 0.5, 1.0, 2.5 and 5.0 levels in laboratory conditions and the experiment silages were ensiled at room temperature for 75 days. At the end of the study, dry matter ($P<0.01$), NDF ($P<0.05$) and ADF ($P<0.01$) contents were increased and pH decreased with the treatment of lavender pomace to the alfalfa ($P<0.01$). 2.5 % and 5.0 % lavender reduced crude protein content of silages ($P <0.01$). Differences in lactic acid bacteria number, crude ash, lactic acid and propionic acid contents were not significant ($P>0.05$). Ammonia nitrogen, butyric acid and *Listeria*spp contents were not detected in silages. Sulphite reducing anaerobes, yeast and *Enterobacteriaceae* contents of experiment silages were below the detection limit. The highest mold content was observed in 5.0 % lavender pomace. Finally, it was concluded that the quality of silages could be improved by adding lavender pomace to the alfalfa.

Keywords: Lavender pomace, silage, quality, microbiology, volatile fatty acids.

Cite this article: DURU, A.A. (2020). The Effect of Lavender Pomace on Chemical Composition, Microbiological and Some Fermentation Characteristics of Alfalfa Silages. *European International Journal of Science and Technology*, 9(2), 1-8.

Introduction

Alfalfaas silage; it is in the class of feeds that are difficult to ensiled with high protein and mineral substance levels, low water-soluble carbohydrate content and high buffer capacity. Therefore, during the ensiling of forage crops, which are rich in protein and mineral substances, and which are poor in carbohydrates, it is necessary to use additives in order for fermentation to be of good quality. Lavender pomace is also thought to be one of these alternative additives.

Lavender, which is a perennial plant that grows in the Mediterranean region, is the generic name of the species that make up the genus *Lavandula* from the family of *Lamiaceae*. Spike-shaped purple flowers of lavender resemble shrubs.(Atalay, 2008; KesiciGüler et al., 2005). It is an important perfume, cosmetic and medicine plant that has antimicrobial properties and is cultivated in the world due to the high-quality essential oil it contains in its stems and flower (Arslancan and Sarıbaş, 2011).

In this study, it was aimed to investigate the effect of addition of different levels of lavender pomace the chemical composition, microbiological and some fermentation characteristics of alfalfa silage.

Materials and Methods

Silage Material and Treatments: Lavender pomace which is used as silage additive was obtained from Uşak University Faculty of Agriculture and Natural Science, Department of Crop Science. Alfalfa, which is used as main silage material, was harvested at the early bloom stage from a private farm in Uşak Province in Turkey. Alfalfa was chopped into lengths of 1.5-2 cm. The chopped alfalfa was weighed and lavender pomace were mixed by hand and then place into the 1 lt anaerobic plastic jars. Five different silage treatments were prepared from chopped alfalfa and lavender pomace (four replicates).The treatments were as follows: (i) no additives (control), (ii) silage treated 0.5%lavender pomace, (iii) silage treated 1.0% lavender pomace, (iv) silage treated 2.5 % lavender pomace, (v) silage treated 5.0 %lavender pomace. The experiment silages were stored at room temperature for 75 days.

The dry matter, crude ash and crude protein contents of experiment silages were asseyed following the procedure of Association of Official Analytical Chemists (AOAC, 1999). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using to methods described by Van Soest (1982).

The silage samples were opened after 75 day of ensiling. 25 g of silage were mixed with 100 mL of distilled water. then this extract was filtered through Whatman filter paper (No:1). The filtrate was immediately used for analyze of pH using a pH meter (Polan et al., 1998). 40 g of experiment samples were mixed with 360 mL of distilled water and then this extract was filtered throug Whatman filter paper (No:1). This filtrate was centrifuged at 14.000 rpm for 30 min. for analyses organic acids and ammonia nitrogen (Suzuki and Lund, 1980). Ammonia nitrogen contents were analyzed according to Broderick and Kang (1980) by the Kjeldahl distillation method. The contents of organic acids (lactic acid, propionic acid, acetic acid and butyric acid) were determined by HPLC (high-performance liquid chromatography). HPLC Conditions: Column: C18, 5 µm, 4,6 x 250-mm; Mobile Phase: Isocratic; 25-mM K-

phosphate buffer; pH 2,4; Flow Rate: 1.5 mL/min.; Column Temperature: 30 ºC; UV Detector: Wavelength: 210 nm; Injection Volume: 20 µL.

Silages were assayed according to the method reported by Stanley et al. (1971) and Harrigan, (1998) in terms of lactic acid bacteria, *Enterobacteriaceae*, sulphite-reducing anaerobes, yeast, mold and *Listeria* spp.

One-way Anova procedure was used to evaluate the data and Duncan Multiple Comparison Test was used for the differences of the experiment groups (SPSS, 2007).

Results and Discussion

Effects of lavender pomace on dry matter, crude ash, crude protein, NDF and ADF of alfalfa silages were given in Table 1.

Table 1. Effects of lavender pomace on chemical composition of alfalfa silages

Parameters	Lavender Pomace Levels, %				
	Control	0.5	1.0	2.5	5.0
DM, % **	34.82±0.58^{b}	35.63±0.20^{b}	35.14±0.62^{b}	35.56±0.54^{b}	37.82±0.37^{a}
CA, % DM	11.46±0.19	11.31±0.10	11.34±0.15	11.05±0.99	11.16±0.14
CP, % DM**	28.94±0.33^{a}	28.43±0.44^{a}	28.26±0.41^{a}	27.44±0.48^{b}	26.87±0.44^{b}
NDF, % DM*	25.14±1.92^{b}	24.30±2.19^{b}	26.52±2.04^{b}	27.57±2.66^{b}	31.16±1.90^{a}
ADF, % DM**	17.37±0.70^{c}	17.61±1.25^{c}	19.32±0.77^{b}	19.10±0.17^{b}	21.11±1.27^{a}

DM: Dry Matter; CA: Crude Ash; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber. CP: Crude Protein;

* $^{a-b:}$: The differences between the averages in the same line are significant (P < 0.05).

** $^{a-c:}$: The differences between the averages in the same line are significant (P < 0.01).

Dry matter content of silages the increased by 5.0 % lavender pomace treatment to alfalfa silage compared to other groups(P<0.01). When the necessary fermentation conditions for high quality silage cannot be achieved, microorganisms proliferate and consequently dry matter losses occur (Bolsen et al., 1996). As a result, it can be said that dry matter of the experiment silages treated with lavender pomace improved and due to the sufficient water-soluble carbohydrate content in the silages, lactic acid bacteria consuming this reduced the dry matter losses by stopping development of undesirable elements in the silage. Thus, the dry matter content of the silages increased as the dry matter content of lavender pomace was higher than that of alfalfa silage. Duru (2020) indicated that lavender (flower) supplementation increased the dry matter content of alfalfa silage.

Lavender pomace did not affect the crude ash contents of the experiment silages (P>0.05).Also, 2.5and 5.0 % lavender pomace had lower crude protein (P<0.01). It was determined that the highest crude protein content was in the control group. Lavender pomace

with low protein content can be said to reduce the crude protein content of alfalfa silage to which it is treated. The presence of high butyric acid and ammonia nitrogen, which are signs of deterioration in silages, was not observed in experiment silages. Guo et al. (2008)reported that alfalfa silage decreased crude protein content by adding different chemicals.

NDF contents of alfalfa silages were higher than the control and the other experiment groups ($P<0.05$).The ADF contents of 1.0 and 2.5 % lavender pomace were statistically lower than the 5.0 % lavender pomace and higher than the control and 0.5 % lavender pomace ($P<0.01$).ADF content is an important criterion that gives information about the degree of digestion of herbage.Also, NDF content reflects the amount of feed that the animal can consume. The possible mechanism of action on ADF and NDF contents in experiment silages can be attributed to the limited fermentation of lavender pomace caused by the active antimicrobial compound. Aksu et al. (2017) obtained similar results to this study.

Table 2. Effects of lavender pomace on some fermentation characteristics of alfalfa silages

Parameters	Lavender Pomace Levels, %				
	Control	0.5	1.0	2.5	5.0
pH*	4.86 ± 0.04^{a}	4.61 ± 0.05^{b}	4.60 ± 0.07^{b}	4.66 ± 0.02^{b}	4.54 ± 0.06^{c}
NH_3-N	ND	ND	ND	ND	ND
LA, %	2.95±0.81	2.64±0.74	2.77±0.70	1.97±0.09	2.32±0.93
AA, %	0.35±0.11	0.26±0.10	0.27±0.09	0.23±0.06	0.23±0.09
PA, %	0.42±0.12	0.24±0.11	0.31±0.09	0.27±0.10	0.31±0.12
BA, %	ND	ND	ND	ND	ND

*$^{a-c:}$ Differences between the means indicated by different letters on the same line are statistically significant ($P<0.01$).

LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid, NH_3-N: Ammonianitrogen. ND: Not Determined.

The pH value of alfalfa silage was significantly decreased with the addition of lavender pomace compared to the control group ($P<0.01$).In silages, pH indicates the acidity of the silage and thus the fermentation quality. Lower pH is preferred for quality silage. It was observed that the pH of the silages decreased when compared to the control group with the addition of lavender pomace to alfalfa. There was a statistically significant decrease compared to other groups, especially with 5% lavender pomace treatment. This may be due to the fact that silage is preserved due to the antimicrobial content of lavender pomace and that lactic acid bacteria are present in enough silage to produce good quality silage. The absence of undesirable microorganism in silages, butyric acid and ammonia nitrogen cannot be detected supports this phenomenon. Canbolat et al. (2013) concluded that the pH value of silage decreased with the addition of honey locus pods to alfalfa silage. These findings agreed with the results observed from the current study.

The differences in lactic acid, acetic acid and propionic acid were not statistically significant (P>0.05). The content of these organic acids in alfalfa silage was numerically high in the control group. Ammonia nitrogen and butyric acid could not be detected in experiment silages. What determines the quality of silages is pH and the amount and composition of organic acids formed during fermentation. In the early stage of fermentation, lactic acid contents and the rate of decrease in the pH in silages are of great importance to obtain good quality silage. Butyric acid bacteria are the most important competitor of acetic acid bacteria during the fermentation of silages. This is due to the presence of high butyric acid in silages and observed in a significant loss of nutrients. These bacteria consume the carbohydrates used by the acetic acid bacteria; thus, either reduce the nutrients they need to a great extent or completely consume them. Therefore, butyric acid is not desired in silages. In addition, since the desired level of lactic acid is provided in the silage, water-soluble carbohydrates prevent the formation of ammonia nitrogen by preventing proteolysis. The organic acid composition in experiment silages has been determined to be at the desired level for green feeds such as alfalfa in the current study. Hashemzadeh-Cigari et al. (2013) stated that lactic acid content did not change with the addition of molasses and inoculant to alfalfa silages. Tabacco et al. (2006) reported that no butyric acid content occurs as a result of adding chestnuttanninto alfalfa silages.

The effects of lavender pomace on lactic acid bacteria (LAB), sulphite reducing anaerobes (SRA), *Listeria* spp., mold and yeast and *Enterobacteriaceae* contents of alfalfa silages were shown in Table 3. No significant difference was found between the groups in terms of lactic acid bacteria (P> 0.05).The highest lactic acid bacteria were observed at 2.5% lavender pomace. SRA, mold and *Enterobacteriaceae*are below detection limit (<1.0x10¹). *Listeria* spp. couldn't be detected in all experiment silages. In the 1.0 % and 2.5% lavender pomace, yeast remained below the detection limit, while control and 1.5 % lavender pomace were detected at 2 Log (cfu/g) in one sample. In addition, 5.0 % lavender pomace was observed to have an average of 2.65 Log (cfu/g) yeast in both samples. In terms of the presence of microorganisms, while it is desired to have lactic acid bacteria, it is undesirable to have sulphite reducing anaerobes, *Listeria*, yeast, mold and *Enterobacteriaceae* in good quality silage. When research silage is evaluated in terms of microbiological properties, it is understood that good quality fermentation occurs. The presence of yeasts from undesirable microorganisms in a few examples and low levels supports this phenomenon. Arslan Duru (2019) reported that the number of lactic acid bacteria increased with the treatment of lavender (flower + stem) to alfalfa silage, there was no sulfide-reducing anaerobes, yeast and listeria, and mold was found in several samples.

Table 3. Effects of lavender pomace on microbiological characteristics of alfalfa silages

Parameters	Lavender Pomace Levels, %				
	Control	0.5	1.0	2.5	5.0
LAB (Log(cfu/g)	6.08±0.13	6.06±0.09	6.14±0.28	6.27±0.34	5.89±0.53
SRA (cfu/g)	$<1.0x10^1$	$<1.0x10^1$	$<1.0x10^1$	$<1.0x10^1$	$<1.0x10^1$
Listeria spp.	ND	ND	ND	ND	ND
Mold (cfu/g)	$<1.0x10^2$	$<1.0x10^2$	$<1.0x10^2$	$<1.0x10^2$	$<1.0x10^2$
Yeast	2 (1 sample) (Log(cfu/g)	2 (1 sample) (Log(cfu/g)	$<1.0x10^2$ cfu/g	$<1.0x10^2$ cfu/g	2.65 (2 sample mean) (Log(cfu/g)
Enterobacteriaceae (cfu/g)	$<1.0x10^2$	$<1.0x10^2$	$<1.0x10^2$	$<1.0x10^2$	$<1.0x10^2$

LAB: Lactic Acid Bacteria; SRA: Sulphite Reducing Anaerobes ND: Not Determined.

Conclusion

The alfalfa with lavender pomace supplement increased the dry matter content of the silages, the pH decreased, butyric acid and ammonia nitrogen were not found, and only a few yeasts was not found in the silages from unwanted microorganisms. Therefore, it was concluded that good quality silage can be obtained and the silage can be preserved for a long time by adding lavender pomace to alfalfa silages.

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