

Essential oil and apple pomace affect fermentation and aerobic stability of alfalfa silage

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Abstract

This study assessed the effects of the additions of an essential oil (EO), composed of ricinoleic acid, cardol, cardanol, and apple pomace, on fermentation quality and aerobic stability of alfalfa silages. The experimental treatments consisted of T1) alfalfa (control), T2) alfalfa with EO, T3) alfalfa (75%) with apple pomace (25%), T4) alfalfa (75%) with apple pomace (25%) and EO, T5) alfalfa (50%) with apple pomace (50%), T6) alfalfa (50%) with apple pomace (50%) and EO, T7) alfalfa (25%) with apple pomace (75%), and T8) alfalfa (25%) with apple pomace (75%) and EO. The addition of apple pomace decreased the silage pH compared with the control ($P < 0.01$). Apple pomace at 25% level increased the total volatile fatty acid (tVFA) content ($P < 0.05$). Essential oil (EO) decreased tVFA and increased dry matter (DM) content 90 days after ensiling ($P < 0.01$). Apple pomace decreased ammonia (N-NH₃), crude protein (CP), and crude ash (CA) content and increased the amounts of neutral detergent fibre (NDF) and acid detergent fibre (ADF) ($P < 0.01$). Essential oil and apple pomace (level 75%) increased effective digestibility ($P < 0.05$). Apple pomace decreased aerobic stability and the addition of EO increased aerobic stability ($P < 0.05$). Thus, use of apple pomace as a source of fermentable carbohydrate and/or the addition of EO in the preparation of high-quality alfalfa silage is recommended to offset its high buffering capacity and low carbohydrate content.

Keywords: cardanol, cardol, ensiling, gas production, in vitro digestibility, ricinoleic acid, silage quality

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Introduction

Alfalfa is a valuable forage plant, which is fed to livestock as hay in Iran. However, significant amounts of nutrients are lost from alfalfa in the drying and storage process (Saruhan & Demirel, 2018). Production of alfalfa silage is attracting increasing attention (Jia *et al.*, 2021), but it is difficult to ensile owing to its high protein level and buffering capacity and low water-soluble carbohydrate content. Therefore, additives must be used during ensiling. Carbohydrate and bacterial additives have been studied for many years to improve the quality of alfalfa silage.

Citrus pulp, tomato pomace, apple pomace, and pistachio pulp are among the by-products from food processing that have been introduced for use in feeding livestock. These materials are produced during seasonal fruit production and are in large part discarded without contaminating the environment (Soycan-Önenç *et al.*, 2015; Besharati *et al.*, 2017). However, these by-products also can be incorporated into silages as a source of fermentable carbohydrate.

Exposure of silage to the air at the time of feeding causes degradation of its quality. Yeasts metabolize lactic acid, and raise the silage pH (Jalc *et al.*, 2010; Turan & Soycan-Önenç, 2018), and stimulate the growth of harmful microorganisms (Sadeghi *et al.*, 2012; Weiss *et al.*, 2016; Turan & Soycan-Önenç, 2018). Feeding degraded silage compromises livestock production. The use of additives with protective properties based on their organic acid content could prevent the growth of moulds and yeasts in silage and increase its aerobic stability (Muck *et al.*, 2018).

The antifungal, antibacterial, and antioxidant effects of aromatic plants and their products have prompted attention as dietary supplements. Plants that produce EO have an important place among

medicinal plants (Sameh *et al.*, 2019; Ali *et al.*, 2020), and have proved to be useful in animal nutrition (Abd El-Mola *et al.*, 2019).

However, there is little information about the effects of EOs on silage fermentation. Therefore, this study was conducted to investigate the effects of an EO on the chemical composition, fermentation quality, and aerobic stability of alfalfa silage. A second objective was to evaluate the addition of apple pomace to alfalfa silage as a source of fermentable carbohydrate.

Materials and Methods

Alfalfa was harvested at the flowering stage with a dry matter (DM) content of 200 g kg⁻¹, chopped to an approximate length of 2 to 2.5 cm, and wilted for 24 hours. An EO containing 30% ricinoleic acid, 30% cardol and 20% cardanol was applied to the forage to achieve final concentrations of 500 mg EO kg⁻¹ silage. The control silage was treated with an equivalent amount of ethanol.

The treatments consisted of T1) alfalfa silage (control), T2) alfalfa silage with 500 mg EO kg⁻¹, T3) alfalfa (75%) + apple pomace (25%) silage, T4) alfalfa (75%) + apple pomace (25%) silage + 500 mg EO kg⁻¹, T5) alfalfa (50%) + apple pomace (50%) silage, T6) alfalfa (50%) + apple pomace (50%) silage + 500 mg EO kg⁻¹, T7) alfalfa (25%) + apple pomace (75%) silage, and T8) alfalfa (25%) + apple pomace (75%) silage + 500 mg EO kg⁻¹. The silages were ensiled in polyvinyl chloride mini silos (10 cm in diameter × 90 cm high, with three replicates per treatment) with a capacity of 3 kg.

After 90 days, silos were opened, and the pH and dry matter contents of the silages were measured. Samples of each silage were stored at -20 °C until the ash, ADF, NDF, and CP contents had been determined (Van Soest *et al.*, 1991; AOAC, 2000) and the *in vitro* degradation of DM had been measured (Fedorah & Hruday, 1983). The fraction of water-soluble carbohydrates was assessed according to Dubois *et al.* (1956). The concentration of VFA was measured and aerobic stability was determined (Besharati *et al.*, 2020a; 2020b). When the temperature of silage mass reached 2 °C above ambient, it was considered corrupted (Xu *et al.*, 2019).

No animals were directly used in this research. Rumen fluid from a slaughterhouse was used to assess *in vitro* gas production. *In vitro* DM degradation of treatments was measured in three replicate bottles. The percentage of degradability (Y) of DM over time (t) was described by the exponential equation:

$$Y = a + b(1 - e^{-ct})$$

fitted to the data by iterative regression analysis (Palangi & Macit, 2019; Palangi *et al.*, 2020). The effective degradability (ED) of DM and CP for feedstuffs was estimated as:

$$ED (\%) = a + bc/(c + k)$$

In this equation, k refers to the fractional outflow rate of small particles from the rumen. The value of 0.02 fraction/h for k was assumed.

Analysis of variance was performed using SAS version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). The mean values were compared using Duncan's test.

Results and Discussion

The properties and chemical compositions of alfalfa and apple pomace before ensiling are listed in Table 1. The effects of treatments on chemical composition after ensiling for 90 days are shown in Table 2. The addition of apple pomace at a 25% level increased tVFA content ($P < 0.05$) compared with the control. Essential oil decreased tVFA ($P < 0.01$). Additions of EO and apple pomace produced a significant interaction on tVFA ($P < 0.01$). The addition of apple pomace reduced ammonia nitrogen compared with the control ($P < 0.01$).

Table 1 Chemical composition of alfalfa and apple pomace before ensiling

Feeds	Dry matter, %	pH	Neutral detergent fibre, %	Acid detergent fibre, %	Water soluble carbohydrate, %	Crude protein, %
Alfalfa	35.0	8.4	41.80	19.46	3.8	18.02
Apple pomace	36.0	3.5	40.42	36.64	5.2	6.12

Table 2 Effect of experimental treatments on chemical composition after ensiling for 90 days (% DM)

Treatments	Nutrients									
	DM	NDF, %DM	ADF, %DM	pH	tVFA, mm	CP, %DM	N-NH ₃ , %DM	EE, % DM	CA, %DM	
Apple pomace, %										
EO, mg										
0	0	29.29 ^a	38.09 ^b	20.95 ^c	5.1 ^a	11.33 ^{cde}	14.97 ^c	70.00 ^a	4.17 ^b	10.50 ^a
0	500	30.70 ^a	37.92 ^b	21.44 ^{bc}	5.1 ^a	10.33 ^{ef}	16.78 ^a	74.66 ^a	3.91 ^b	9.88 ^{ab}
25	0	29.70 ^{ab}	40.71 ^a	22.80 ^b	4.2 ^b	13.33 ^{ab}	13.36 ^d	49.00 ^b	2.38 ^b	8.33 ^b
25	500	30.70 ^a	40.26 ^a	21.97 ^{bc}	4.2 ^b	12.66 ^{abc}	15.27 ^b	43.00 ^{bc}	2.84 ^b	8.49 ^b
50	0	29.40 ^{ab}	41.05 ^a	25.24 ^a	4.1 ^c	13.66 ^a	10.88 ^e	39.60 ^{bcd}	4.49 ^b	5.56 ^c
50	500	32.10 ^a	41.72 ^a	26.01 ^a	4.1 ^c	9.66 ^f	11.47 ^f	25.66 ^e	7.46 ^a	5.98 ^c
75	0	27.10 ^b	40.87 ^a	25.63 ^a	3.8 ^d	10.66 ^{def}	9.36 ^g	28.00 ^{de}	4.41 ^b	3.88 ^d
75	500	31.90 ^a	40.60 ^a	26.69 ^a	3.7 ^d	12.00 ^{bc}	8.81 ^h	30.30 ^{cde}	4.88 ^b	5.37 ^{cd}
SE		0.47	0.63	0.51	0.03	0.51	0.25	3.87	0.94	0.54
EO, mg	0	29.03 ^b	40.18	23.66	4.28	12.25 ^a	12.39 ^b	46.66	3.86	7.31
	500	31.35 ^a	40.12	44.279	4.25	11.16 ^b	13.08 ^a	43.16	4.77	7.44
	SE	0.40	0.31	0.26	0.02	0.26	0.01	1.93	0.47	0.27
Apple pomace, %	0	30.32	38.00 ^b	21.19 ^c	5.04 ^a	10.83 ^b	15.87 ^a	72.33 ^a	4.04 ^{ab}	10.18 ^a
	25	30.19	40.41 ^a	22.39 ^b	4.25 ^b	13.00 ^a	14.31 ^b	45.50 ^b	3.91 ^b	8.41 ^b
	50	30.71	41.39 ^a	25.63 ^a	4.04 ^c	11.66 ^b	11.64 ^c	32.66 ^c	5.98 ^a	6.27 ^c
	75	29.55	40.74 ^a	26.16 ^a	3.72 ^d	11.33 ^b	9.08 ^d	29.16 ^c	4.64 ^a	4.62 ^d
	SE	0.57	0.44	0.36	0.02	0.36	0.02	2.74	0.66	0.38
Analysis of variance summary					<i>P</i> -values					
Essential oil		0.001	0.900	0.319	0.201	0.008	<0.001	0.219	0.188	0.754
Apple pomace		0.556	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	0.018	<0.001
Interaction		0.087	0.813	0.299	0.325	0.009	<0.001	0.097	0.362	0.225

DM: dry matter, CP: crude protein, EE: ether extract, CA: crude ash, NDF: neutral detergent fibre, ADF: acid detergent fibre, tVFA: total volatile fatty acids, AP: apple pomace effect

^{a,b,c} within a column, means with a common superscript did not differ with probability $P=0.05$

The pH of the silages with 25%, 50% and 75% apple pomace was lower than that of the control ($P < 0.01$). However, the EO treatment did not affect the pH of the silages ($P > 0.05$). Dry matter was higher in silages that were augmented with EO ($P < 0.05$). The addition of apple pomace increased NDF and ADF compared with the control ($P < 0.05$). Ammonia content was decreased as the level of apple pomace in the silage was increased ($P < 0.05$). The addition of apple pomace to alfalfa silage decreased aerobic stability compared with control and other experimental treatments (Figure 1). Aerobic stability was increased in treatments with EO additive compared with the control ($P < 0.05$).

The outcomes of the current experiment agree with those of others that investigated the effects of carbohydrate sources on silages (Hashemzadeh-Cigari *et al.*, 2011; Besharati *et al.*, 2020c). Guler *et al.* (2006) observed reduced pH and increased lactic acid concentration by adding apple pomace at 5% and 10% to alfalfa silage without affecting DM content. Kung *et al.* (2008) used Crina® (thymol, eugenol, vanillin, and limonene) to modify silage fermentation. They reported that the pH of the silage augmented with this additive did not differ from that of the control group, which was consistent with the present findings. The addition of peppermint and mint EOs at 120 and 240 mg decreased the pH of corn silage, but other EOs did not have an effect (Hodjatpanah-Montazeri *et al.*, 2016).

In the present experiment, tVFA was increased in the silages with 25% and 50% apple pomace compared with the control ($P < 0.05$). Abdollahzadeh & Abdulkarimi (2012) reported an increase in tVFA in silage containing either apple pomace or tomato pomace compared with the control. Other researchers who investigated the effects of carbohydrate sources on silages (Islam *et al.*, 2001; Aksu *et al.*, 2006; Khan *et al.*, 2006) reported increased amounts of tVFA. The addition of apple pomace reduced N-NH₃ compared with the

control ($P < 0.05$). Rodrigues *et al.* (2008) observed a significant decrease in gas production during in vitro digestion resulting from increased amounts of straw in apple pomace-straw mixtures. Yang *et al.* (2010) also reported that adding apple pomace to alfalfa silage reduced the amount of N-NH₃ significantly compared with control. By inhibiting proteolysis in silage, apple pomace probably decreased non-protein nitrogen and N-NH₃ compared with control. However, Guler *et al.* (2006) did not observe any changes in N-NH₃ production by adding apple pomace to alfalfa silage. The EO additive did not affect N-NH₃, which was consistent with Kung *et al.*'s (2008) findings. Hodjatpanah-Montazeri *et al.* (2016) reported that EO of cinnamon at 120 and 240 mg/kg had no effect on N-NH₃ in corn silage. However, in the same study, oregano and thyme EO and peppermint at the lower level decreased N-NH₃ content compared with control.

Treating alfalfa silage with EO produced no effect on the amounts of NDF and ADF compared with control in this experiment. In contrast, Hodjatpanah-Montazeri *et al.* (2016) found apple pomace and EO additives increased ADF significantly compared with control ($P < 0.05$). Rodrigues *et al.* (2008) showed that adding straw to apple pomace increased its ADF content significantly.

Hodjatpanah-Montazeri *et al.* (2016) observed that peppermint and oregano EOs at 120 and 240 mg/kg alfalfa silage increased the CP content of corn silage compared with control, which is consistent with the results of the current experiment. In contrast, Chavez *et al.* (2012) found no change in the level of CP by adding oregano EO to silage.

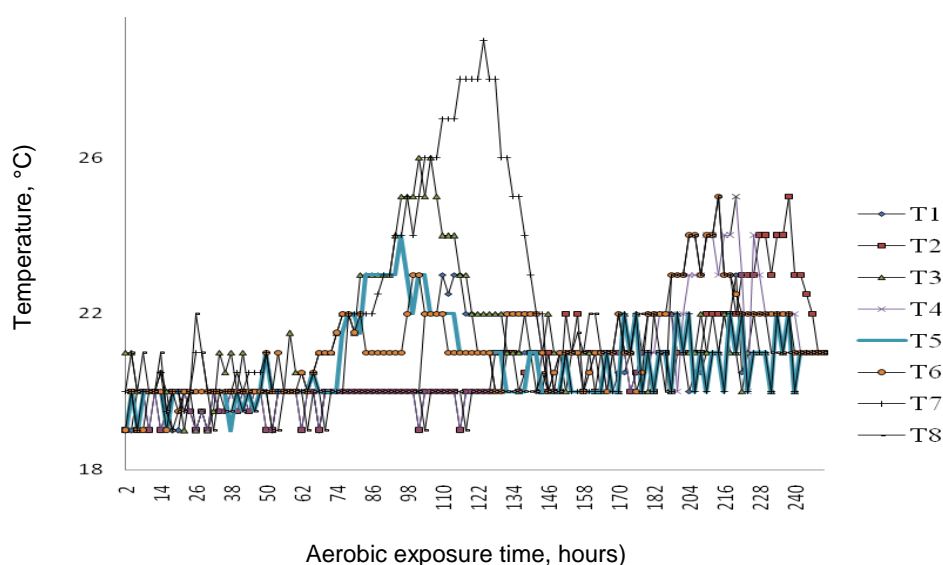


Figure 1 Silage temperature during hours of aerobic exposure

T1: alfalfa silage without additives, T2: alfalfa silage + 500 mg essential oil/kg, T3: alfalfa (75%) + apple pomace (25%) silage, T4: alfalfa (75%) + apple pomace (25%) silage + 500 mg essential oil/kg, T5: alfalfa (50%) + apple pomace (50%) silage, T6: alfalfa(50%) + apple pomace (50%) silage + 500 mg essential oil/kg, T7: alfalfa(25%) + apple pomace (75%) silage, T8: alfalfa(25%) + apple pomace (75%) silage + 500 mg essential oil/kg

The interaction of the level of apple pomace effects and the addition of EO consistently affected the parameter estimates of the exponential equation describing DM degradation of the silages ($P < 0.001$). The EO treatment decreased the soluble and very rapidly degradable fractions of the DM (a) in the silages with 25% or less apple pomace, had the opposite effect in the silage that contained 50% apple pomace and had no effect on (a) for the 75% apple pomace silage. Adding the EO to the silage without apple pomace greatly increased the insoluble but potentially degradable component (b) but had no effect on the silages that contained apple pomace. The fractional rate constant (c) was altered by the use of EO only in the silage containing 75% apple pomace.

Table 3 Effects of treatments on in vitro DM degradability of alfalfa silage augmented with apple pomace and essential oil

Silage	Additive	Parameter estimates			
		a, %	b, %	c, %/hour	ED, %
0% apple pomace	0 mg EO	16.53 ^b	24.98 ^d	0.097 ^a	37.25 ^{def}
0% apple pomace	500 mg EO	11.96 ^c	41.32 ^a	0.010 ^a	39.41 ^{bcd}
25% apple pomace	0 mg EO	12.49 ^c	32.96 ^b	0.061 ^{bc}	36.40 ^{ef}
25% apple pomace	500 mg EO	7.75 ^d	33.32 ^b	0.086 ^{ab}	34.75 ^f
50% apple pomace	0 mg EO	10.87 ^c	35.78 ^{ab}	0.062 ^{bc}	37.95 ^{cde}
50% apple pomace	500 mg EO	17.20 ^b	32.09 ^{bc}	0.067 ^{bc}	40.57 ^{bc}
75% apple pomace	0 mg EO	22.02 ^a	26.30 ^{cd}	0.054 ^c	41.18 ^{ab}
75% apple pomace	500 mg EO	23.36 ^a	24.42 ^d	0.093 ^a	43.44 ^a
SE		0.57	2.01	0.008	0.87
P-values	Essential oil	0.326	0.608	0.648	0.044
	Apple pomace	<.0001	0.001	0.647	<.0001
	Interaction	<0.001	<0.001	<0.001	0.082

a: soluble and rapidly degradable component, b: insoluble but potentially degradable component, c: constant fractional rate; ED: effective degradability
^{a,b,c,d,e,f} Within a column, means with a common superscript did not differ with probability $P=0.05$

The inclusion of apple pomace with alfalfa has been shown to diminish aerobic stability of silage possibly as a consequence of higher soluble carbohydrate and lactic acid contents (Ke *et al.*, 2015). Yang *et al.* (2010) demonstrated that the addition of apple pomace to alfalfa silage reduced aerobic stability and observed that it could be because of loss of nutrients and DM during storage. Aerobic stability was increased in the treatments with EO compared with control, which may be because of the antimicrobial activity and inhibitory effects of some EOs that prevent spoilage of silage by inhibiting deamination and proteolysis (Hodjatpanah-Montazeri *et al.*, 2016). Adding two EOs to barley silage increased aerobic stability compared with the control treatment, which agreed with data of the present study (Chaves *et al.*, 2012). Hodjatpanah-Montazeri *et al.* (2016) observed increased aerobic stability of silage with the addition of various EOs to corn silage. The addition of a combination of EOs to modify the fermentation of corn silage did not affect yeast, fungi, and *Enterobacteria* owing to aerobic stability compared with the control treatment (Da Silva *et al.*, 2018). In areas with high apple production, where most of the crop is used in processing factories, apple pomace is produced in large quantities and disposal of the by-products can result in environmental pollution. The use of apple pomace in the preparation of silage as feed for livestock in these areas could reduce environmental pollution and reduce feed costs.

Conclusion

The addition of EO improved the DM and CP content of silage and increased its effective degradability. Aerobic stability was enhanced in treatments with an EO. The use of a carbohydrate source as a fermentable dry matter for bacterial population and/or an EO additive is necessary to offset the deficiencies of alfalfa for making high quality silage.

Authors' Contributions

MB supervised the experiment, and ZN advised the project. MN conducted the laboratory analyses. TA and VP supervised the experiment and finalized the manuscript.

Conflict of Interest Declaration

The authors declare there are no conflicts of interest.

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