

Effect of almond (*Prunus dulcis*) hull addition to corn silage on silage quality, silage fermentation properties and *in vitro* digestibility

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Received 16.02.2023

Accepted 05.04.2023

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Summary

This study aimed to determine the effects of the addition of almond hull to corn silage on silage quality, fermentation properties and *in vitro* organic matter digestion (IVOMD). While corn silage without additives constituted the control group in the study, silages with almond hull added at the levels of 0.5%, 1%, 1.5%, 2% and 2.5% formed the experimental groups. The addition of almond hull caused an increase in the IVOMD and metabolizable energy (ME) values of the silages compared to the control group. For all the experimental groups, the methane (CH₄) values of the silages were lower than the values of the control group. The silage pH range of all silage groups was in the range of 3.62-3.70. The lactic acid (LA) and acetic acid (AA) values of the silages, on the other hand, were higher in all experimental groups compared with the values of the control group, and the highest values were observed in the group with the 1% almond hull addition. No propionic acid (PA), butyric acid (BA), or mold was detected in any of the silage groups. The yeast values, on the other hand, were lower in all silages that contained added almond hulls than the in the control group. The amount of CO₂ produced in the silage groups decreased in parallel with the increase in almond hull. This study will enable more use and evaluation of almond hulls in animal nutrition due to its positive effect on fermentation.

Keywords: Almond hull, IVOMD, maize silage, silage quality

It is generally accepted that animal products are essential for balanced and adequate nutrition of human beings. Among animal products, meat, milk, etc., obtained from cattle, sheep, and goats, called ruminants products, come to the fore. Feed expenses, which constitute approximately 70% of expenses accrued during the production phase of these products, have a significant impact on the profitability of the enterprise. In this regard, roughage that is relatively cheap and positively affects the digestive activities of ruminants comes to the fore (32). Developments in silage technology, innovations in additives and the cultivation of various plants, as forage crops, have been important in the spread of silage production (4). Since maize has higher dry matter content, low buffer capacity, and sufficient water-soluble carbohydrate (WSC) content, it can be easily ensiled. Due to the above-mentioned properties, corn is preferred more than other forage crops as a silage material because of its high fermentation efficiency. In recent years, there have been developments in silage technology,

especially in the use of additives (17). While the use of traditional feed products in animal nutrition constantly increases the cost, the use of food industry by-products in animal nutrition is both economical and environmentally friendly. In this study, it was thought that the use of almond hull (AH), which is one of the by-products of the food industry, in animal nutrition may provide added value to livestock as well as reduce the pollution and damage that this product may cause to the environment (10, 27). Since almond hull is a byproduct of the almond industry, its cost is low and it is important for the sustainability of animal production. Before 1948, almond hulls were not regarded as a valuable feed and were either burned for fuel or discarded (21). There are studies in which almond bark is used as a feed material in cows (38, 42), sheep (33, 43), pigs (22), broilers (40, 41) laying hens (39), and rabbits (1).

Almond hulls constitute approximately 54% of the total almonds (12). According to DePeters et al. (14) and Norollahi et al. (27), almond hulls contain 50-60% nitrogen-free extract, 10-30% moisture, 10-17% fiber,

4-7% ash, 2-5% protein and 1-4% oil. This nitrogen free extract in almond hull contains 25-37% sugar and other readily available carbohydrates (20). Almond hulls have a low protein content but are high in sugars and NDF (13). With lignin percentages ranging from only 6% to 11% based on DM, most of the NDF in almond hull can be degraded by ruminant microbes (2, 44). Almond hulls containing sucrose, fructose, glucose, inositol and sorbitol provide ruminants with a source of highly fermentable carbohydrates (28). Also almond hulls are particularly rich (up to 4.5%) in phenolic compounds, including phenolic acids and flavonol glycosides (5).

This study was carried out to determine the effects of the addition of almond hulls, which are considered waste, to corn silage on silage fermentation properties and *in vitro* organic matter digestion (IVOMD).

Material and methods

Study design and silage preparation. The corn plant was used in this study as the silage material. The method reported by Playne and McDonald (31) was used in the analysis of the buffering capacity of the fresh corn plant. In the study, an additive free corn plant constituted the control group, while the groups with 0.5% almond hull (0.5% AH), 1% almond hull (1% AH), 1.5% almond hull (1.5% AH), 2% almond hull (2% AH), and 2.5% almond hull (2.5% AH) added formed the experimental groups. Each silage group was prepared with six replications and was compressed and ensiled in 1.5 L glass jars equipped with a lid that enables gas release only by hand compressing to a final density of 600 g/L. Silages were stored at room temperature (22-24°C) for 60 days in a dark environment.

Fermentation profile analysis. The silages kept in a dark environment were opened at the end of the 60 day fermentation period. The upper 3-5 cm of the silages in the opened jars were discarded. One hundred millilitres of distilled water were added to 25 g of the silage sample taken homogeneously from each jar, which was then crushed for two minutes with the help of a blender. The pH value of the filtrate obtained after the silage was crushed was quickly measured with a pH meter (Hanna HI991002, pH/ORP meter, Romania) and recorded. In addition, the obtained filtrates were placed into 10 ml centrifuge tubes for ammonia nitrogen and volatile fatty acid analysis. While 0.1 ml of 1 M HCl was added to the tubes prepared for the determination of ammonia nitrogen, 0.25 ml of 25% metaphosphoric acid was added to the tubes prepared for the analysis of lactic and volatile fatty acids and kept in the deep freezer (-20°C) until the analysis was done. Ammonia nitrogen analyses of the silage samples were made according to the method reported by Broderick and Kang (9), and the lactic and volatile fatty acids (butyric, acetic and propionic acid) analyses were made according to the method reported by Suzuki and Lund (37). To analyze lactic acid (LA) and essential fatty acids, an HPLC device (with a Shimadzu L.C-20 AD HPLC pump, Isepe Coregel (87H3 column), a Shimadzu SIL-20 ADHT Autosampler, a Shimadzu cto-20ac Colum oven, and a Shimadzu SPD

M20A Detector (DAD)) were used. The opened silages were exposed to oxygen for five days to measure aerobic stability (determination of CO₂ production values) (7). Dry matter (DM) (method 950.01), crude ash (CA) (method 942.05) and crude protein (CP) (method 945.01) analyses of the silages obtained in the study were calculated according to AOAC (6). ADF and NDF analyses were performed by Van Soest et al. (34). For all analyses, fresh silage materials and the silages obtained at the end of the 60-day fermentation period were dried at room temperature, ground in a laboratory mill and passed through a 1 mm sieve to be prepared for analysis. The methane (CH₄) content of the silages was determined using the method described by Menke and Steingass (24), and the IVOMD and metabolizable energy (ME) values of the silages were determined using the method described by Menke et al. (23). The total amount of yeast and mold analyses of the silages followed the reports of Filya et al. (17).

Statistical method. In the study, One-Way Analysis of Variance (One-Way Anova) was used to determine whether the data obtained from the groups were widely different. Duncan's multiple comparison tests were used to control the significance of the difference between the groups, and for this purpose, the SPSS software program was used (35).

Results and discussion

The nutrient analysis results of the corn plant and almond hulls used in the study are presented in Table 1.

The nutrient content, IVOMD, ME and *in vitro* CH₄ values of the corn silages prepared by adding almond hulls at different rates (0.5%, 1%, 1.5%, 2% and 2.5%) are given in Table 2.

The differences between the groups in the DM, CA, ADF and NDF, IVOMD and ME values of the silages were statistically significant ($p < 0.05$). The differences between the CP values were not statistically significant ($p > 0.05$). In the silage groups, an increase in the DM values was observed in direct proportion with the addition of almond hull ($p < 0.05$). This increase in DM levels can be attributed to the high DM content of almond hulls. The chemical characteristics of the almond hulls in our experiment were generally in the range of published values (21, 38, 42-44). With the addition of almond hull, it was shown that the CP values of silages fell in an opposite manner. It is thought that this decrease is due to the increase in the ratio of added almond hull, which has a low protein content. This suggests that when almond hulls are ensiled with a low protein feed, protein supplementation may be necessary to meet animal nutrient needs and maintain yields. Aguilar et al. (2) found that giving almond hull

Tab. 1. Nutrient content of the corn plant used as silage material and the almond hull used as the additive in the study

	DM	CA	CP	ADF	NDF	IVOMD	ME	CH ₄
Corn	27.40	7.00	6.85	37.90	69.50	54.84	8.31	10.36
Almond hull	92.50	10.60	3.80	32.92	46.96	34.39	4.98	1.30

Explanations: DM – dry matter, %; CA – crude ash, DM%; CP – crude protein, DM%; ADF – acid detergent insoluble fiber, DM%; NDF – neutral detergent insoluble fiber, DM%; IVOMD – *in vitro* organic matter digestion; ME – metabolic energy; CH₄ – *in vitro* methane, %

Tab. 2. Nutrient content and IVOMD, ME and *in vitro* CH₄ values of the corn silages prepared by adding almond hulls at different rates

Groups	DM	CA	CP	ADF	NDF	IVOMD	ME	CH ₄
Control	26.01 ^b	7.05 ^a	7.23 ^a	33.99 ^c	60.56 ^b	53.24 ^d	8.13 ^b	8.92 ^a
0.5% AH	26.93 ^a	6.07 ^c	6.36 ^{ab}	34.34 ^{bc}	60.64 ^b	54.32 ^{cd}	8.28 ^b	7.59 ^c
1% AH	27.03 ^a	6.21 ^{bc}	6.27 ^b	35.36 ^{ab}	62.88 ^a	56.09 ^b	8.40 ^b	8.26 ^{abc}
1.5% AH	26.94 ^a	6.28 ^{bc}	6.22 ^b	35.47 ^{ab}	63.12 ^a	55.08 ^{bc}	8.16 ^b	8.41 ^{ab}
2% AH	27.02 ^a	6.35 ^{bc}	6.18 ^b	35.82 ^a	63.54 ^a	54.47 ^{cd}	8.16 ^b	7.80 ^{bc}
2.5% AH	27.52 ^a	6.65 ^{ab}	6.15 ^b	36.13 ^a	64.17 ^a	58.58 ^a	8.81 ^a	8.07 ^{bc}
SEM	0.128	0.094	0.129	0.219	0.346	0.397	0.069	0.122
P	0.012	0.012	0.136	0.011	0.000	0.000	0.017	0.010

Explanations: a-d – values with different letters in the same column were different ($p < 0.05$); DM – dry matter, %; CA – crude ash, DM%; CP – crude protein, DM%; ADF – acid detergent insoluble fiber, DM%; NDF – neutral detergent insoluble fiber, DM%; IVOMD – *in vitro* organic matter digestion; ME – metabolizable energy MJ/kg DM; CH₄ – *in vitro* methane, %

at a TMR of up to 25% to lactating dairy cows had no negative effects on the cows ability to produce milk or consume the feed. Williams et al. (42) discovered the opposite, finding that feeding almond hull to lactating dairy cows at 17.6% of their diet decreased their intake of CP and NDF as well as the yields of milk and milk protein. Both of these previous studies used almond hull instead of alfalfa hay, and Aguilar et al. (2) study also used urea supplementation. As was previously mentioned, almond hulls are high in fermentable carbohydrates but low in CP. Due to the highly fermentable sugars such as sucrose and glucose in almond hulls, it can be used as an alternative to concentrates instead of forage in the diets of lactating cows.

The ADF and NDF values of the silages increased in parallel with the increase in almond hull, with the highest value observed the almond hull group of 2.5% ($p < 0.05$). In this study, it is thought that the reason for the increase in ADF and NDF values is due to the high ADF and NDF content in the almond hull.

When the IVOMD and ME values of the silages were examined, increases were observed in all the experimental group silages compared to the control group silage ($p < 0.05$). The highest IVOMD (58.58) and ME (8.81) values were observed in the silage group with the 2.5% almond hull addition. In Table 4, a positive correlation was observed between IVOMD and ME ($R: 0.700$). The reason for the high ME value of almond hulls is thought to be due to the high amount of *in vitro* gas released as a result of the fermentation of this feed. Because the metabolizable energies of the silages were calculated by considering the 24-hour gas production values. A decrease in CH₄ values was observed in all the experimental groups compared to

the control group ($p < 0.05$). Components of almond hulls, including phenolics, triterpenoids, and antibacterial agents, have been suggested as potential factors inhibiting methane formation in *in vitro* studies (15). It is thought that the addition of almond hull causes a decrease in the total bacteria and cellulotic bacteria population in the rumen fluid, suppresses the rumen protozoa and therefore reduces the formation of methane (18). As seen in Table 4, there was a negative correlation between the IVOMD and CH₄

values of the silages ($R: 0.243$).

In the study, the fermentation characteristics of the silages prepared by adding different amounts of almond hull to the corn plant and the correlation in the analyses are presented in Tables 3 and 4.

When the fermentation properties (pH, NH₃-N, LA, AA, yeast, mold and CO₂) of the silages were examined, the differences between the groups were found to be statistically significant ($p < 0.05$), while propionic acid (PA), butyric acid (BA) and mold were not detected. When the pH values of the silages were examined, it was determined the highest pH value (3.70) was found in the silage group with 2.5% AH, while the lowest pH value (3.62) was found in the group with 0.5% AH ($p < 0.05$). Ergün et al. (16) reported that silage pH value is one of the most important factors affecting silage fermentation, the most suitable silage pH range for the development of LABs grown in acid environment is 3.8-4.2, and bacteria that cause deterioration in silages in this pH range cannot survive. The low pH values in the study can be attributed to the high amount of LA present in all the silage groups. In the study, when the silage NH₃-N/TN values were compared with the control group, although a decrease was observed in the silages due to the almond hull addition, the highest NH₃-N/TN value (7.97) was found in the control group, and the lowest NH₃-N/TN value (6.61) was observed in the group with

Tab. 3. Fermentation properties of the silages prepared by adding almond hulls at different rates

Groups	pH	NH ₃ -N/TN	LA	AA	PA	BA	Yeast	Mold	CO ₂
Control	3.63 ^{bc}	7.97 ^a	7.55 ^d	2.33 ^f	–	–	8.36 ^a	–	1.78 ^a
0.5% AH	3.62 ^c	7.78 ^{ab}	8.88 ^c	2.96 ^b	–	–	5.62 ^c	–	0.40 ^b
1% AH	3.65 ^{ab}	7.17 ^{bc}	13.36 ^a	4.23 ^a	–	–	5.65 ^b	–	0.35 ^c
1.5% AH	3.66 ^b	7.48 ^{ab}	7.55 ^d	2.72 ^e	–	–	3.57 ^e	–	0.19 ^e
2% AH	3.65 ^{ab}	7.46 ^{ab}	11.29 ^b	2.91 ^c	–	–	2.17 ^f	–	0.15 ^f
2.5% AH	3.70 ^a	6.61 ^c	7.61 ^d	2.84 ^d	–	–	4.03 ^d	–	0.25 ^d
SEM	0.006	0.121	0.462	0.123	–	–	0.409	–	0.119
P	0.000	0.006	0.000	0.000	–	–	0.000	–	0.000

Explanations: a-f – values with different letters in the same column were found to be different ($p < 0.05$); NH₃-N/TN – ammonia nitrogen; LA – lactic acid, g/kg DM; AA – acetic acid, g/kg DM; PA – propionic acid, g/kg DM; BA – butyric acid, g/kg DM; CO₂ – carbon dioxide, g/kg DM

Tab. 4. Correlation relationship between fermentation characteristics and yeast values of the corn silages prepared by adding almond hulls at different rates

		pH	NH ₃ -N	LA	AA	Yeast	IVOMD	ME	CH ₄	CO ₂
pH	PC	1	-0.617**	-0.387	-0.040	-0.432*	0.684**	0.530**	0.001	-0.387
	P		0.001	0.061	0.854	0.035	0.000	0.008	0.998	0.061
NH ₃ -N	PC		1	-0.103	-0.198	0.363	-0.700**	-0.284	-0.045	0.426*
	P			0.631	0.354	0.081	0.000	0.178	0.833	0.038
LA	PC			1	0.864**	-0.185	0.021	-0.041	-0.214	-0.300
	P				0.000	0.387	0.921	0.849	0.315	0.154
AA	PC				1	0.170	0.196	0.164	-0.091	-0.103
	P					0.427	0.359	0.444	0.672	0.631
Yeast	PC					1	-0.337**	-0.107	0.441*	0.868**
	P						0.107	0.618	0.031	0.000
IVOMD	PC						1*	0.700**	-0.243**	-0.462*
	P							0.000	0.253	0.023
ME	PC							1	-0.329	-0.234
	P								0.116	0.272
CH ₄	PC								1	0.561**
	P									0.004
CO ₂	PC									1

Explanations: PC – Pearson correlation; * – correlation is significant at the 0.05 level; ** – correlation is significant at the 0.01 level; P – significance degree; NH₃-N/TN – ammonia nitrogen; CO₂ – carbon dioxide, g/kg DM; LA – lactic acid, g/kg DM; AA – acetic acid, g/kg DM; PA – propionic acid, g/kg DM; BA – butyric acid, g/kg DM; IVOMD – *in vitro* organic matter digestibility, %; ME – metabolizable energy, MJ/kg DM; CH₄ – *in vitro* methane, %

the 2.5% AH addition ($p < 0.05$). It is thought that the easily soluble carbohydrate sources in the AHs provide good fermentation for LAB, and the rapid decrease in silage pH is thought to occur by suppressing the activities of microorganisms and plant proteases that ferment amino acids, thereby protecting some of the real proteins (8, 19, 25). The report of Paydaş (30) that the addition of pistachio hull to corn silage reduced the amount of silage NH₃-N/TN supports the results of the current study. The LA and acetic acid (AA) values of the silages, on the other hand, increased in all the additive experimental groups compared to the control group, but were observed in the group with the highest 1% almond hull additive ($p < 0.05$). The sugar content in the silage during fermentation is converted to LA and AA, reducing the pH of the environment (36). When Table 4 was examined, a positive correlation ($R: 0.864$) between LA and AA was observed, which supports this report. Contrary to the current study, Paydaş et al. reported that the addition of pistachio hulls at different rates to corn silage reduced LA and AA levels. PA and BA were not detected in any of the study groups (30). The fact that BA was not detected in the silage groups indicates that the quality of the obtained silages was good.

As seen in Table 3, mold could not be detected in the silages, and the yeast values were lower in the experimental groups compared to the control group ($p < 0.05$). When the AA content of the silages were examined, an increase in the AA content of the experimental groups was observed compared to the control

silage, and a decrease was observed in the aerobic stability and yeast values compared to the control group. The reason for this observation is that the easily soluble carbohydrate sources in almond hulls decrease the pH values of silage due to the conversion of LA and AA, and it can be postulated that it prevents yeast and mold growth due to the antimicrobial effect of AA (29). The positive correlation between LA and AA ($R: 0.864$) shown in Table 4 supports this report. It was reported that silages obtained from plants with high WSC content were more susceptible to aerobic

degradation (11). In the study, it was observed that on the fifth day of aerobic stability, the CO₂ production of the silage groups varied between 0.15 g/kg and 1.78 g/kg DM and that there was a decrease in all experimental groups due to the addition of almond hull compared to the control group ($p < 0.05$). This decrease in the experimental groups supports the report that the amount of AA produced as a result of the fermentation of LA bacteria in silages has an inhibitory effect against the microorganisms that cause the spoilage of the silage and has a reducing effect on CO₂ production by preventing the growth of yeasts (3). From Table 4, a positive correlation was observed between yeast and CO₂ ($R: 0.868$).

This study was conducted to evaluate the effect of adding almond hulls to corn silage at different rates on silage quality, silage fermentation, IVOMD and *in vitro* gas production. When all parameters were examined, the 2% AH and 2.5% AH silages had positive effects on silage fermentation properties, silage quality and IVOMD. Due to its low protein value, it is thought that additional protein supplementation may be required when almond hulls are used in ruminant feeding. As a result, based on *in vitro* measurements and fermentation properties, almond hulls can be used as silage additives as they increase IVOMD and ME, reduce methane and have a positive effect on fermentation properties. It is recommended to conduct more detailed (*in vivo*, *in vitro*) and different studies to support the results of this study and other studies.

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