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Effects of Harvesting Stages and Additives on the Chemical Composition, Fermentation Quality and Relative Feed Value of Soybean Silages Varieties

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ABSTRACT

In this study, the chemical composition, silage fermentation quality and relative feed value of soybean silages varieties, namely Adasoy (A), Derry (D) and Yeşilsoy (Y), with Pioneer11C33 (I), molasses (M) and cracked wheat (W) additives at the three harvesting stages, i.e., full flowering (R2), full pod (R4) and full seed (R6) stages, were determined. It was observed that the A and D varieties were the best silage materials for the production of good quality silage in terms of both nutrient contents such as DM and CP and fermentation criteria such as LA and BA concentrations during the R4 and R6 harvest periods. Molasses and cracked wheat additives significantly improved the fermentation qualities of all soybean varieties ($P<0.05$), but the fermentation effect of the inoculant on silages was less than that of molasses and cracked wheat. The harvesting of soybean varieties in the full seed and applying molasses or cracked wheat as an additive optimally improves silage characteristics and results in well-preserved silage.

Keywords: Soybean, Lactic acid bacteria, Molasses, Fermentation quality, Interactions

1. Introduction

The soybean, as one of the most important agricultural products of Asian countries, has been the staple food of the people of that region for centuries. It was originally cultivated in northern Asia and, in recent decades, in North America and the countries of the Southern Cone of Latin America (Pratapet al. 2016). Brazil is the largest producer of soybeans, accounting for about 37.6% of world production, followed by the United States (30.7%), Argentina (12.9%), China (5%) and India (3%) (Soystats 2022). Turkish soybean production in 2021 totaled 182 thousand tons, with a 17.2% increase in production over 2020. The major provincial producers of soybeans in Turkey include Adana province constituting around 67.2%, followed by Mersin province (16.8%) (Tüik 2019). In Turkey, it can be grown as a main crop and a secondary crop since there are a variety of genotypes in terms of maturation times. With the rapid development of animal husbandry, high quality green feed demand throughout the year has been increasing (Hisham et al. 2022). Increasing the productivity of pasture and production of forage crops are of great importance in closing the current forage deficit. Silage preserves fresh grass for longer, reduces nutrient loss and is more digestible by ruminants in the absence of green forage (Kung et al. 2018). The soybean is well-adapted to dry conditions, has high grain productivity, with high protein content, and low fiber to protein ratio (Garcia 2020). Forage soybeans have an important place among legumes due to their high nutritional value and low cost (Chang et al. 2012; Zambom et al. 2012; Asekowa et al. 2014). The most important conservation methods in feed legumes are ensiling and drying. As they possess higher CP concentrations and relatively high concentrations of organic acids and cations, legumes such as alfalfa, vetch and soybean have a high buffering capacity. Moreover, they contain low levels of water-soluble carbohydrates and, therefore, limited substrate is available for fermentation by lactic acid bacteria (McAllister et al. 1998; Hartinger at al. 2019). Moisture content is vital for fermentation in the silo. For soybeans, this ideal moisture occurs just before the pods are full. Waiting until the soybean has achieved fully maturity results in higher dry matter and lower digestible feed that can lead to fermentation problems due to the high oil content of the seeds (Garcia 2020). Due to the above-mentioned factors, it resists the rapid pH decrease and storage stability of forage legumes from being ensiled and classifies them as difficult forages to ensilage. Forage soybean quality varies according to variety, growing stage and harvest losses. When legumes are harvested at the appropriate dry matter content and the appropriate additives are used, high quality silages can be produced. Therefore, the questions addressed or examined in this study include (1) what is the best period to harvest in order to produce good quality soybean silage, (2) will the inoculant, molasses and cracked wheat used as additives improve the silage fermentation quality (3) which is the best silage material to produce high quality silages among the three of soybean varieties, namely Adasoy, Derry and Yeşilsoy, grown as a second crop in the Eastern Mediterranean conditions.

2. Material and Methods

2.1. Field trial

The field experiment was carried out at the East Mediterranean Agricultural Research Institute (EMARI), ($36^0$50' N, $35^0$34' E, 12 m above sea level) in Dogankent town, trial area/location, Adana province, Turkey. The mean temperatures of the months of June, July, August and September, when the research was carried out, was determined to be between 26.5 and 27.4 ºC, and the total precipitation values were determined as 83.9- and 49.5-mm. The average relative humidity values were found to be between 66.6% and 69.0%, and the soil organic matter content in this area is at a low level of 1.07% and has a clay loam structure.

2.2. Experimental materials

In this study, carried out in the experimental field of the Eastern Mediterranean Agricultural Research Institute, Derry and Yeşilsoy were used as the soybean varieties for feed, and Adasoy was used for the grain varieties.

Adasoy (A) variety: It is a medium late soybean variety registered as grain by the Eastern Mediterranean Agricultural Research Institute. This variety is in the IV maturation group.

Derry (D) variety: It is a forage soybean variety developed in 1997 with the USDA-ARS breeding program. It is in the VI maturation group.

Yeşilsoy (Y) variety: It has been registered as silage by the Eastern Mediterranean Agricultural Research Institute. It is in the V maturation group.

Chopped forages were treated with 1) control-additive free (C); 2) Pioneer 11C33 produced by Pioneer Hi-Bred International, Des Moines, IA containing lactic acid bacteria, namely *Lactobacillus buchneri*, *Lactobacillus plantarum* and, *Enterococcus faecium*. Pioneer 11C33 inoculant was applied at the recommended rates; that is, 5 mg/kg of fresh forage; 3) Sugar beet molasses (M) containing 75% DM and 65% sucrose on a DM basis, was applied at 4% of fresh material; 4) The cracked wheat (W) which was added on the fresh material at a rate of 4%.

2.3. Experimental design

The plantings were arranged in a four-replication split plot design. The soybean varieties were the main plots and included three varieties, i.e., Adasoy (A), Derry (D) and Yeşilsoy (Y). Harvesting of soybean varieties was done in 3 different stages, i.e., the full flowering (R2), full pod (R4) and full seed (R6). Soybean varieties were ensiled with additive-free (C), Pioneer 11C33 (I), molasses (M) and cracked wheat (W) during all harvesting stages.

The soybean forage in each plot was cut at a height of 5 cm from the ground to determine their fresh weight and samples of approximately 500 g were collected and then dried in an oven at 65 °C for 48 hours to determine the DM content (Martin et al. 1990). The soybean herbage was chopped into 2 cm lengths using a forage chopper and filled into a 5 kg polyethylene bottle with a screw cap. The preparation of silages was as mentioned below: Silage treatments included control (no additives), 0.005 g/kg inoculant, 4% molasses, and 4% cracked wheat of fresh forage according to the instructions and, the same level of distilled water used during the preparation of the other groups was added to the group without additives. A total of 144 bottles of soybean silage were prepared and fermented for 60 days at a temperature of 25±2 ºC.

2.4. Determination of silage fermentation quality and chemical composition

The pH of the silage was determined with a pH meter after homogenization of 10 g of silage with 100 ml of distilled water for 1 min in a blender (Chen et al. 1994). Then, liquid was filtered using Whatman paper and the liquid was stored at -20 ºC. After the liquid of the silage was filtered through 0.22 mm membrane filter, content lactic acid and volatile fatty acids were determined with HPLC (Agilent 1100, Agilent Technologies Inc.). The chromatographic conditions were as follows: The Hi-Plex H column (7.7x300 mm, 8µm) was selected, column temperature was 50 ºC, the mobile phase was 0.004 M H_2SO_4, the flow rate was 0.6 mL/min, the detective wavelength was 210 nm, and the injection volume was 20 µl (Muck & Dickerson 1988).

Crude protein was calculated by multiplying N measurements obtained from a Kjeldahl N analyzer by 6.25 (AOAC 1990). The methods of Van Soest et al. (1991) were used for the NDF and ADF analysis. Amylase and sodium sulphite were used in the analysis of NDF, and the results were expressed on a dry matter basis, including the ash content.

The digestible dry matter (DMD), dry matter intake (DMI) and relative feed value (RFV) were calculated using the following equations (Rohweder 1978):

Digestible dry matter (DDM%) = 88.9 - (0.779 x ADF%)

Dry matter intake (DMI%) = 120 / NDF%

Relative feed value (RFV) = (DDM% x DMI%) / 1.29

2.5. Statistical analysis

SAS version 9.4 (2020) was used as a program for statistical analysis. The data analyzed included the chemical composition, fermentation quality and relative feed value of the silage. Duncan's multiple comparison test and LS-Means were applied to compare differences between means.

3. Results

The variance analysis results on the differences in chemical composition, fermentation quality and relative feed value of silage are presented in Table 1. The analysis of variance indicated that there were significant differences on the parameters in each variable within the soybean varieties, harvesting stage and additives. Therefore, in addition to the effects of the factors, the effects of the interactions are also important and should be considered together.

Table 1- Variance analysis of the differences in chemical composition, fermentation quality and relative feed value of the silage

	Chemical composition				Fermentation quality					Relative feed value		
Variable	DM	CP	ADF	NDF	pH	LA	PA	BA	AA	DMD	DMI	RFV
Within the variety	128.96**	29.83**	18.89**	28.02**	8.34**	80.49**	17.23**	12.18**	12.09**	18.78**	22.15**	21.55**
Within the stages	459.28**	17.33**	10.02**	19.74**	103.20**	9.73**	52.93**	72.31**	132.02**	9.93**	15.37**	11.56**
Within the additive	129.87**	13.28**	44.46**	50.41**	70.78**	138.29**	44.49**	156.72**	74.64**	44.45**	46.12**	51.31**
Variety x stage	10.90**	8.49**	3.73*	-	-	7.90**	-	4.73**	-	3.70*	-	-
Variety x additive	4.39**	-	-	2.92*	2.56*	6.48**	4.46**	-	4.71**	-	-	-
Stage x additive	3.04**	5.13**	-	-	-	5.88**	10.24**	8.40**	4.12**	-	-	-
Variety x stage x additive	-	-	-	-	2.12*	-	-	-	3.17**	-	-	-

*: indicates significant differences at the 0.05 level; **: indicates significant differences at the 0.01 level and "-" indicates no interaction. DM: dry matter; CP: crude protein; ADF:acid detergent fiber; NDF:neutral detergent fiber; LA: lactic acid; PA: propionic acid; BA: butyric acid; AA: acetic acid; DMD: digestible dry matter; DMI: dry matter intake; RFV: relative feed value.

Table 2- Differences in the chemical composition, silage fermentation quality and relative feed quality within the soybean varieties, harvesting stage and additives

		Chemical composition					Fermentation quality				Relative feed quality		
Factor	Ex	DM, %	CP, %	ADF, %	NDF, %	pH value	LA	PA	BA	AA	DMD,%	DMI	RFV
Variety	A	27.38±0.18b	14.33±0.22a	40.89±0.48c	47.32±0.47c	4.66±0.03a	3.70±0.05a	0.44±0.01b	0.39±0.01b	0.51±0.02a	57.04±0.38a	2.56±0.02a	113.88±1.68a
	D	28.30±0.18a	12.11±0.22b	45.03±0.48a	52.22±0.47a	4.47±0.03b	3.42±0.05b	0.44±0.01b	0.38±0.01b	0.44±0.02b	53.83±0.38c	2.34±0.02c	95.54±1.68c
	Y	24.38±0.18c	13.95±0.22a	42.31±0.48b	49.09±0.47b	4.68±0.03a	2.89±0.05c	0.52±0.01a	0.45±0.01a	0.55±0.02a	55.94±0.38b	2.49±0.02b	108.62±1.68b
Stage	R2	22.59±0.18c	12.93±0.22b	44.35±0.48a	50.31±0.47a	5.01±0.04a	3.23±0.05b	0.56±0.01a	0.51±0.01a	0.70±0.02a	54.36±0.38b	2.43±0.02b	103.22±1.68b
	R4	27.19±0.18b	14.51±0.22a	42.59±0.48b	51.12±0.47a	4.58±0.04b	3.28±0.05b	0.42±0.01b	0.38±0.01b	0.43±0.02b	55.72±0.38a	2.39±0.02b	104.24±1.68b
	R6	30.29±0.18a	12.96±0.22b	41.29±0.48c	47.18±0.47b	4.22±0.04c	3.50±0.05a	0.42±0.01b	0.34±0.01c	0.37±0.02c	56.73±0.38a	2.57±0.02a	113.57±1.68a
Additive	C	24.75±0.21b	12.74±0.25b	44.95±0.56b	52.02±0.54a	4.84±0.04a	2.52±0.05d	0.58±0.01a	0.60±0.01a	0.69±0.02a	53.89±0.44b	2.33±0.03c	97.97±1.94c
	M	29.01±0.21a	14.30±0.25a	39.07±0.56c	45.35±0.54c	4.09±0.04c	3.91±0.05a	0.40±0.01c	0.28±0.01c	0.33±0.02d	58.47±0.44a	2.68±0.03a	122.22±1.94a
	W	28.47±0.21a	14.21±0.25a	40.15±0.56c	47.30±0.54b	4.56±0.04b	3.74±0.05b	0.40±0.01c	0.30±0.01c	0.43±0.02c	57.62±0.44a	2.57±0.03b	115.01±1.94b
	I	24.53±0.21b	12.60±0.25b	46.81±0.56a	53.48±0.54a	4.93±0.04a	3.18±0.05c	0.50±0.01b	0.46±0.01b	0.55±0.02b	52.44±0.44c	2.28±0.03c	92.83±1.94c

Different letters in the same column mean significantly differences at P<0.05. DM: dry matter; CP crude protein: ADF:Acid detergent fiber, NDF: Neutral detergent fiber, LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; DMD: Digestible dry matter; DMI: Dry matter intake; RFV: Relative feed value, A:Adasoy; D: Derry; Y:Yeşilsoy; R2:Full flowering; R4:Full pod; R6:Full seed; C: Additive free (Control); M:Molasses, W:Cracked wheat; I:Pioneer11C33(*Lactobacillus buchneri, Lactobacillus plantarum, Enterococcus faecium*)

Table 3- Differences in the chemical composition, silage fermentation quality and relative feed quality for the interaction of soybean variety and additives

		Chemical composition				Fermentation quality					Relative feed quality		
V	A	DM, %	CP, %	ADF, %	NDF, %	pH value	LA	PA	BA	AA	DMD,%	DMI	RFV
A	C	25.79±0.36d	13.66±0.44	42.45±0.97	49.23±0.94de	4.94±0.08a	3.12±0.09f	0.50±0.02c	0.60±0.02	0.69±0.03b	55.83±0.75	2.45±0.05	106.88±3.36
	M	29.79±0.36b	15.18±0.44	36.85±0.97	43.77±0.94g	4.04±0.08e	4.28±0.09a	0.39±0.02d	0.27±0.02	0.32±0.03g	60.18±0.75	2.77±0.05	129.33±3.36
	W	28.85±0.36b	14.58±0.44	40.04±0.97	46.66±0.94ef	4.68±0.08bc	3.77±0.09c	0.38±0.02d	0.30±0.02	0.51±0.03cd	57.70±0.75	2.59±0.05	115.96±3.36
	I	25.10±0.36e	13.92±0.44	44.22±0.97	49.60±0.94de	4.98±0.08a	3.64±0.09cd	0.52±0.02bc	0.43±0.02	0.52±0.03cd	54.45±0.75	2.45±0.05	103.33±3.36
D	C	26.39±0.36d	11.70±0.44	48.10±0.97	55.40±0.94ab	4.60±0.08c	2.53±0.09g	0.57±0.02b	0.58±0.02	0.57±0.03c	51.45±0.75	2.20±0.05	87.67±3.36
	M	29.65±0.36b	12.65±0.44	41.40±0.97	47.93±0.94e	4.14±0.08de	3.83±0.09bc	0.38±0.02d	0.27±0.02	0.34±0.03fg	56.65±0.75	2.53±0.05	111.96±3.36
	W	31.05±0.36a	13.12±0.44	40.47±0.97	47.69±0.94e	4.31±0.08d	4.05±0.09ab	0.40±0.02d	0.24±0.02	0.38±0.03fg	57.38±0.75	2.55±0.05	113.83±3.36
	I	26.15±0.36d	10.97±0.44	50.15±0.97	57.84±0.94a	4.85±0.08ab	3.27±0.09ef	0.40±0.02d	0.43±0.02	0.49±0.03de	49.84±0.75	2.09±0.05	80.71±3.36
Y	C	22.06±0.36f	12.87±0.44	44.30±0.97	51.44±0.94cd	4.96±0.08a	1.92±0.09h	0.67±0.02a	0.64±0.02	0.80±0.03a	54.39±0.75	2.35±0.05	99.38±3.36
	M	27.58±0.36c	15.06±0.44	38.94±0.97	44.34±0.94fg	4.09±0.08e	3.60±0.09cd	0.43±0.02d	0.30±0.02	0.35±0.03fg	58.56±0.75	2.73±0.05	125.38±3.36
	W	25.53±0.36de	14.94±0.44	39.93±0.97	47.55±0.94e	4.70±0.08bc	3.41±0.09de	0.41±0.02d	0.34±0.02	0.41±0.03ef	57.79±0.75	2.56±0.05	115.25±3.36
	I	22.34±0.36f	12.92±0.44	46.06±0.97	53.01±0.94bc	4.95±0.08a	2.63±0.09g	0.57±0.02b	0.52±0.02	0.64±0.03b	53.02±0.75	2.29±0.05	94.46±3.36

Different letters in the same column mean significantly differences at P<0.05. DM: dry matter; CP crude protein: ADF:Acid detergent fiber, NDF: Neutral detergent fiber, LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; DMD: Digestible dry matter; DMI: Dry matter intake; RFV: Relative feed value; A:Adasoy; D: Derry; Y:Yeşilsoy; R2:Full flowering; R4:Full pod; R6:Full seed; C: Additve free (Control); M:Molasses, W:Cracked wheat; I:Pioneer11C33 (*Lactobacillus buchneri, Lactobacillus plantarum, Enterococcus faecium*)

3.1. Differences in the chemical composition, fermentation quality and relative feed value of silages for the single factor

3.1.1. Soybean varieties (V)

The mean DM, ADF and NDF of the D variety at different harvesting stages were significantly higher than that of the A and Y varieties. Compared with the D variety, the average content the CP of the A and Y varieties was significantly greater. Both the low DM and high HP content of the Y variety negatively affected the fermentation quality compared to the other two varieties. The mean pH of the D variety was significantly lower than the A and Y varieties at different harvesting stages and additives, but the average LA levels of the A varieties were significantly higher than that of the D and Y variety. Compared with the Y variety, the A variety had higher LA content and lower BA and PA content, indicating that the fermentation quality of A was better than that of the Y variety. The mean DMD, DMI and RFV of the A variety at different harvesting stages was significantly higher than that of the D and Y varieties (P<0.05; Table 2).

3.1.2. Harvesting stage (S)

With the advancing maturity, the mean dry matter content of the soybean varieties gradually increased and reached the optimum dry matter content in order to produce quality silage at the R6 stage. The highest average CP content was observed in the R4 stage during the harvesting stages (P<0.05). The mean pH of soybean varieties ensiled with different additives had the lowest value at the R6 stage, followed by R4, both significantly lower than pH values at the R2 harvest stage (P<0.05). With the delay of the harvesting period and with the increase of maturing, the average LA content increased, while the concentration of PA, BA and AA significantly decreased. In terms of DMD and other relative feed value parameters, the best harvesting stage for the three silages was the R6 stage (Table 2).

3.1.3. Additives (A)

In relation to the different additive treatments, significant differences were observed for the mean nutrient composition, fermentation quality and relative feed value parameters among the different varieties harvested at different stages. While the M and W additives increased the DM content of the silages, they decreased the ADF and NDF content. The mean pH value, PA, BA and AA concentrations of the M and W treatments were significantly lower than the ones in C and I treatments. Compared with C treatment, the mean concentration for LA of I treatment was significantly higher. The opposite was observed for PA, BA, and AA; this indicated that the ensiling with additive I had a better fermentation quality than ensiling without additives (P<0.05). Table 2 showcases that the mean silage fermentation quality of the M treatment was the best, followed by W, and the fermentation quality of W treatment was significantly higher than that of I and C (P<0.05). The addition of M and W additives to the silages increased the DMD, DMI and RFV values (P<0.05) but these values of the silages were not affected by the addition of I additive in comparison to the control silage.

3.2. The interactions

3.2.1. Soybean varieties (V) x harvesting stages (S)

The V x S interaction was not significant (P>0.05) for most of the measured components, with the exception of the DM, CP, ADF, LA, BA concentrations and DMD% (Table 1 and Figure 1). With the advancing maturity, the DM of the soybean varieties increased gradually, and at the R6 stage the DM contents ranged from 27.71% to 32.53%, the most suitable DM to produce a quality silage. The CP content of silage harvested at the R4 stage was significantly higher than that of R2, because the R4 stage soybeans possessed well-developed leaves and pods. Regarding the Vx S interaction, the ADF concentration decreased from the early to the late stage of the harvest in all soybean varieties.

For the three silages with different additives at stages R2, R4 and R6, no differences were found for the average pH, AA and PA concentrations. Silage LA and BA concentrations were affected by the V x S interaction. The LA of the all-soybean varieties increased with the stage of harvest, although the increase was higher with the D and Y varieties in R6 harvesting stage than with the D and Y varieties in R4 harvesting stage (P<0.05). The mean BA concentration of the Y variety with different additives at the R2 and R4 stages was significantly higher than that of the A and D varieties.

Silages digestible dry matter ranged from 51.92% to 58.38%. The highest value was calculated for the silage from the A variety at the R4 and R6 harvesting stage. The lowest value was calculated for the silage from the D variety for all the harvesting stages. No significant differences were found in the dry matter intakes and relative nutritive values of the silages of the three soybean varieties at stages R2, R4 and R6.

3.2.2. Soybean varieties (V) x additives (A)

The differences in the chemical composition, silage fermentation quality and relative feed quality for the interaction of soybean variety and additives are shown in Table 3. Interactions were detected between variety and additives for DM (P<0.01), NDF (P<0.05), pH (P<0.05), LA (P<0.01), PA (P<0.01) and AA (P<0.01). The DM was the highest in the D variety cracked wheat additives (31.05%) followed by the A variety with cracked wheat (28.85%) and molasses (29.79%) additives in silages. Furthermore, the effect of cracked wheat was greater in the D variety than that of the A and Y varieties.

Treatment with M resulted in lower pH (4.14 for D variety) compared to C, it was even lower (P<0.05) in other varieties (4.04 for A and 4.09 for Y). Treatment with all additives resulted in significantly higher (P<0.001) LA concentrations than C silages (Table 3). In addition, among the three varieties, when the A variety was ensiled with molasses and the D variety with wheat cracked, the LA concentration was found to be higher compared to other silages. PA concentrations in soybean varieties were also significantly (P<0.001) reduced by M and W additives; treatment with M and W was more effective (P<0.001) in reducing PA concentration in the Y variety than in the A and D varieties. Compared to AxC (0.69%), DxC (0.57%) and YxC (0.80%), concentrations of AA were lower (P<0.001) in additive-treated silages (range 0.32% to 0.52%).

3.2.3. Harvesting stage (S) x additives (A)

The interaction of harvesting stage and type of additives on silage chemical composition and quality is presented in Figure 1. Their interaction significantly affected silage DM, CP, LA, PA, BA and AA concentrations (P<0.001; Table 1). DM recorded in silage with no additives and, I additives in the R2 harvesting stage was significantly lower than others. With the progress of the harvest period, the DM content of all silages with additives increased compared to the silages without additives. CP content was significantly higher in M added silage in R2 stage and W added in R4 stage than others. The lowest CP was recorded in I added silage R2 harvesting stage (10.92%; Figure 2).

The effect of M and W additives on silage LA, PA, BA and AA content was stronger than that of I additive, and this also depended on the harvesting stage.

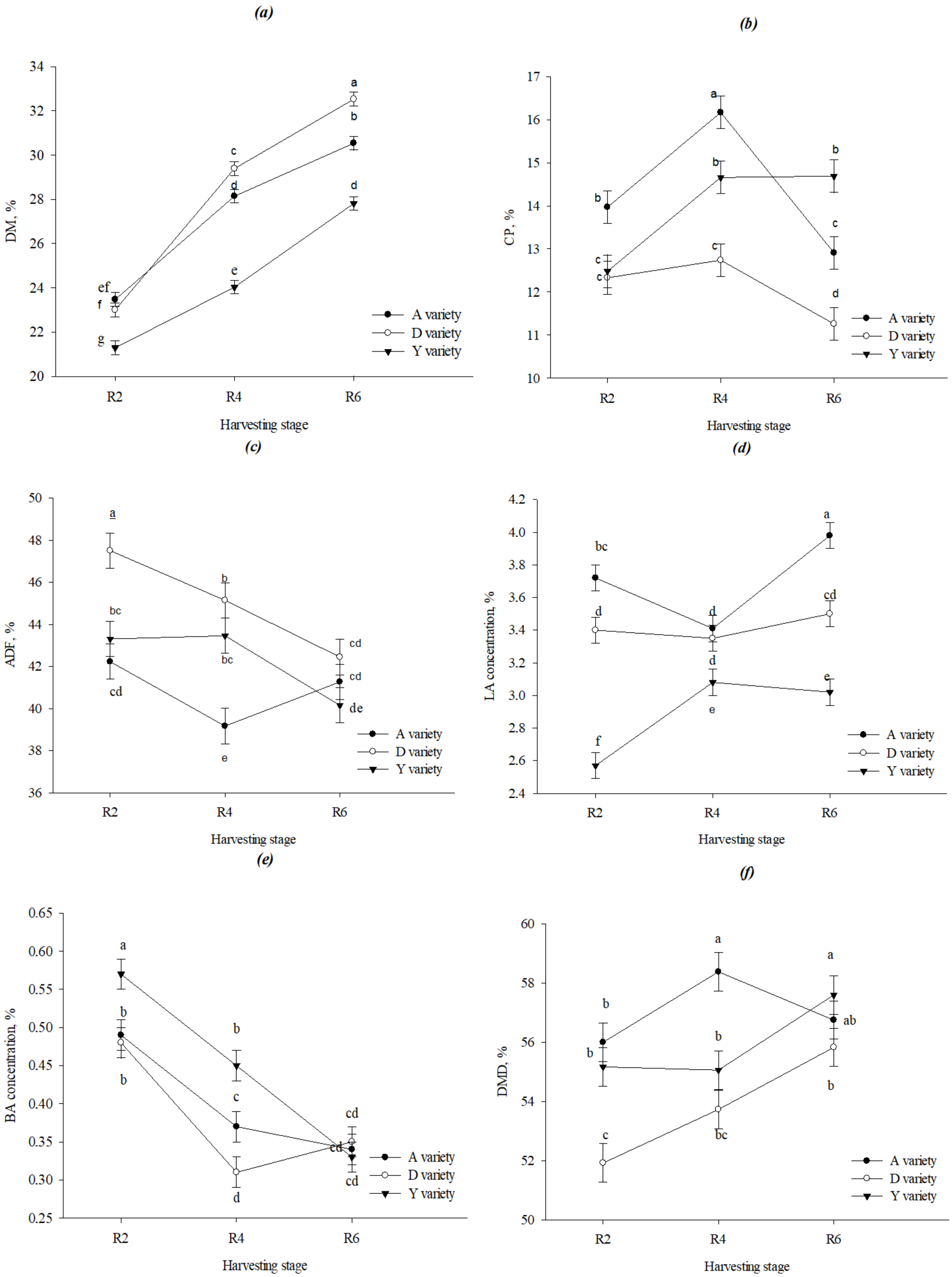


Figure 1- Differences in the chemical composition, silage fermentation quality and relative feed quality for the interaction of soybean variety and harvesting stage. Different letters in each figure mean significantly differences at P<0.05. A: Adasoy; D: Derry; Y: Yesilsoy; R2: full flowering; R4: full pod; R6: full seed

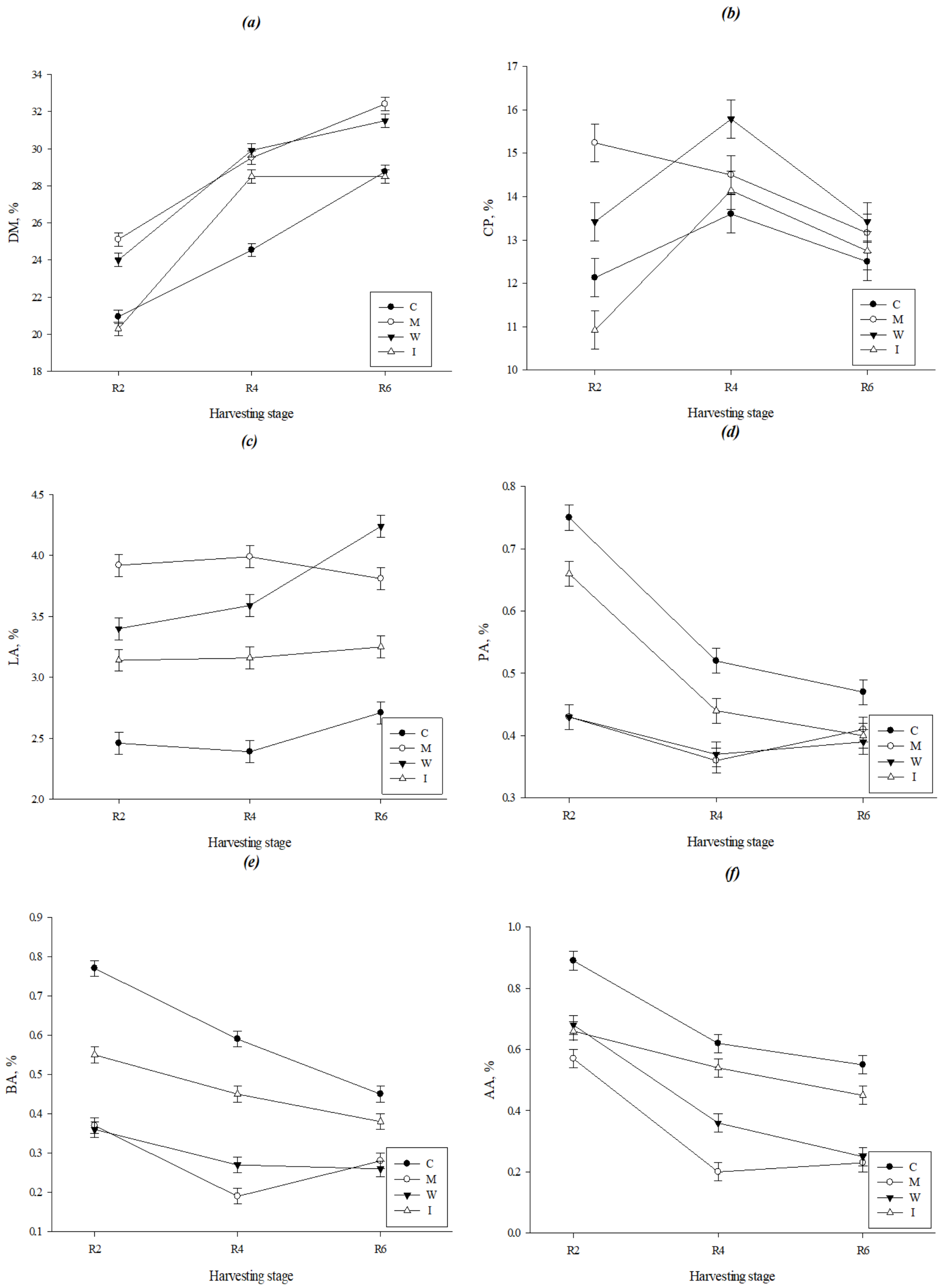


Figure 2- Differences in the chemical composition (a and b) and silage fermentation quality (c, d, e and f) for the interaction between harvesting stage and additives. R2: full flowering; R4: full pod; R6: full seed; C: control-additive-free treatment; M: molasses; W: cracked wheat; I: Pioneer11C33

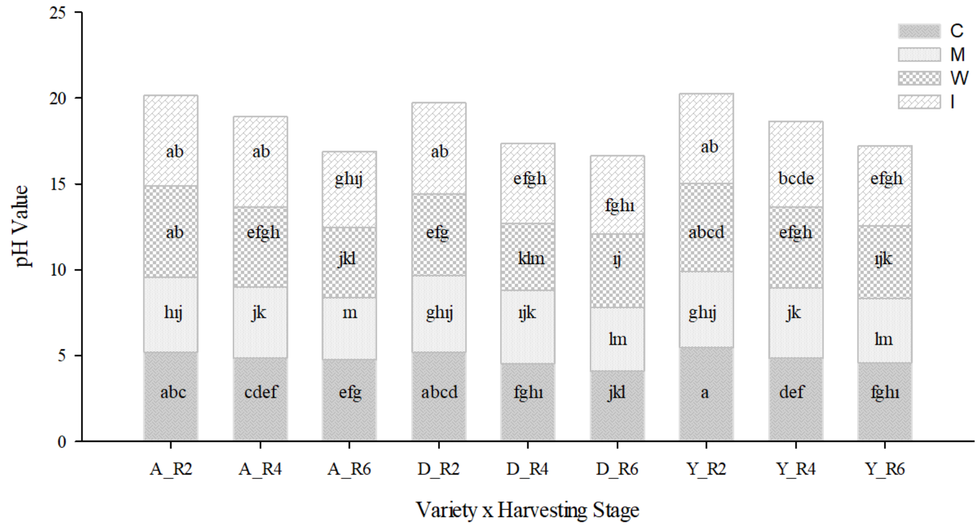
3.2.4. The interactions of the soybean variety, harvesting stages and additives

At the R6 stages, the silage pH of the A, D and Y soybean silages with molasses additives was significantly lower than that of other harvesting stages and additives. In all varieties and at the R2 harvest stage, the addition of inoculant caused a significant increase in pH, one of the silage fermentation criteria, followed by silage without additives. Molasses and cracked wheat used as additives exerted a significant effect in reducing pH and acetic acid concentration in all soybean varieties and in the R6 harvest period ($P<0.05$) (Figure 3a, b). In conclusion, the addition of molasses to all soybean silages, harvested at the R6 stage, improved the silage fermentation characteristics of silages, but had no positive effects on digestible dry matter.

3.2.5. Correlations between different soybean silage compositions

Silage pH was negatively correlated with DM content and LA concentration, but was positively correlated with AA, BA and PA concentrations. Among all the soybean silage composition parameters determined, DM concentration had the greatest correlation with silage pH (r=-0.68, P<0.001, Figure 4), and AA, BA, and PA concentrations were moderately correlated with pH (r=0.54, r=0.54, and r=0.41, respectively, P<0.001). CP content had positive correlations with DMD, DMI and RFV, but negative correlations with ADF and NDF content. A strong negative correlation was observed between ADF and NDF content of silages and DMD, DMI and RFV (r=-1.00, r=-0.81, r=-0.92; r=-0.82, r=-0.81, r=-0.98, r=-0.96, respectively, P<0.001, Figure 4).

(a)



(b)

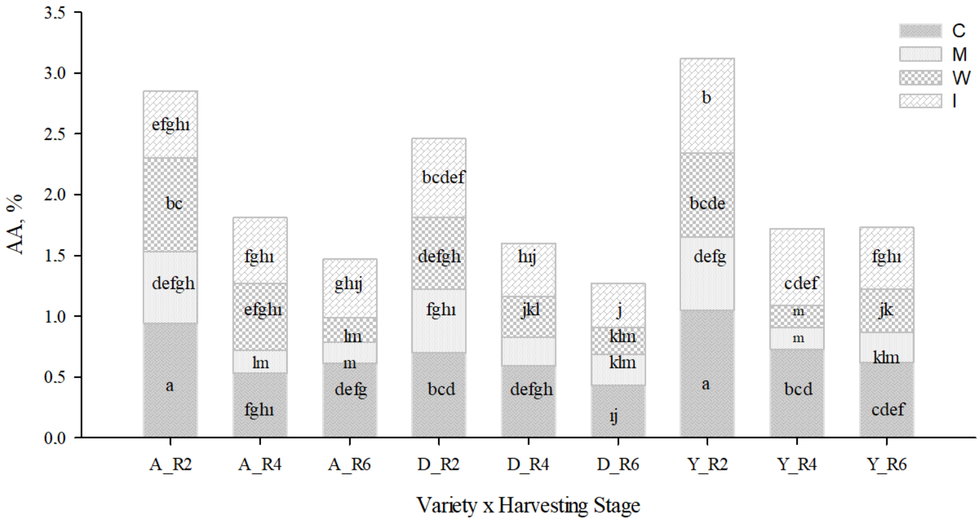


Figure 3- Differences in pH value (a) and acetic acid concentration (b) for the interaction between variety, harvesting stage and additives. Different letters in each figure mean significantly differences at P<0.05. A: Adasoy; D: Derry; Y: Yesilsoy; R2: full flowering; R4: full pod; R6: full seed; C: control-additive-free treatment; M: molasses; W: cracked wheat; I: Pioneer11C33 (*Lactobacillus buchneri, Lactobacillus plantarum, Enterococcus faecium*)

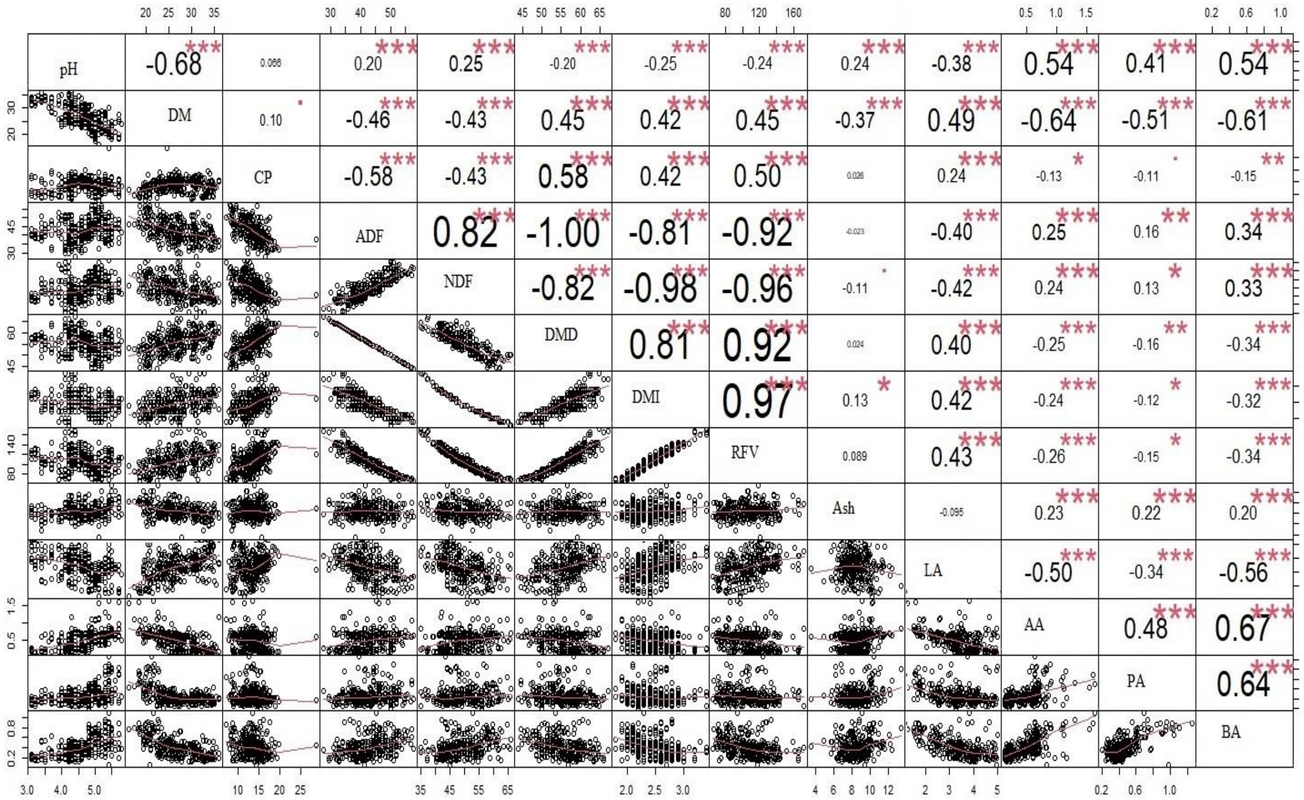


Figure 4- Correlation matrix of soybean silage nutrient composition and fermentation quality. "*, ** and *" indicate significant levels of P<0.05, P<0.01 and P<0.001, respectively**

4. Discussion

The chemical composition and fermentation quality of the silage was significantly influenced by soybean variety, harvesting stage and additives. The dry matter content of forage is an important parameter for optimal silage quality (Borreani et al. 2018). At dry matter contents below 30-35%, high moisture forages (especially legumes) are more susceptible to clostridial fermentation (high levels of butyric acid and/or ammonia-N) (Vissers et al. 2007). Conversely, the use of forage with a dry matter content >45-50% DM in silage should also be avoided, as ensiling and packaging is more difficult and can trap air in the silage mass, causing overheating and nutrient loss. Where conditions allow, the 30-35% DM content of forages makes it difficult for clostridia to dominate ensiling and limits total fermentation acids (Borreani et al. 2007; Kung 2009). In this study, the DM contents of soybean varieties harvested at the full pod and full seed varied from 27.19% to 30.29% (Table 2), which makes it possible to optimize silage quality. It is noteworthy that legume silages often show higher aerobic stability than maize silages, which are prone to aerobic degradation after feeding. However, the high buffering capacity and low soluble carbohydrate concentration in legumes are limiting factors for lactic acid synthesis and rapid pH drop, which we refer to as good fermentation (Bernardi et al. 2019). Silage pH is an indicator of the suitability of optimal fermentation. The higher the lactic acid content of the silage, the lower the pH of the silage. However, this decrease is related to the dry matter content of the silage (Coffey et al. 1995; Li et al. 2022). The composition of silage has a significant impact on the silage fermentation quality and relative feed value. Additionally, acetic and lactic acid concentrations were negatively related to DM content, as reported by Kung et al. (2018). For legume silages with 30-35% DM content, the silage pH is reported to be between 4.2-4.8. In addition, silages at the R6 harvest stage had the highest LA concentration, and the lowest BA concentration and pH value. It is seen that the A and D varieties are the best silage materials to produce good quality silage in terms of both nutrient contents such as DM and CP and fermentation criteria such as LA and BA concentrations during the R4 and R6 harvest periods (Figure 1). Spanghero et al. (2015), in their study on the effect of harvesting stages of soybeans on the chemical composition of silage, showed that the favourable stages for harvesting whole plants are from R4 to R6, as they have a high nutritional value for ensiling.

The addition of fermentable nutrients such as molasses and broken grains, and inoculants during ensiling lowers the pH, increases the LAB count and lactic acid content, and competitively inhibits harmful bacteria (Xia et al. 2018; Li et al. 2021; Wang et al.2021). The content of water-soluble carbohydrates required for proper fermentation in legume plants is low (Blount et al 2006). Therefore, the addition of a readily fermentable source of sugars, such as molasses, and/or the use of microbial inoculants can help to ensure adequate fermentation during the ensiling of soybeans. In this study, similar results were obtained, showing that the addition of molasses, cracked wheat and inoculant significantly increased the LA content and decreased the pH and AA content in silages of soybean varieties (Table 3), which agrees with Mahana and Chase (2003) and Rosa et al. (2018).

In the present study, when soybean varieties, harvest stages and additives were considered as single factors, differences in relative feed values were found to be significant and, in terms of quality class, variety A, R6 harvest stage and molasses application were found to be higher quality than other varieties, harvest and additives. Compared to other varieties, harvest period and additives, ruminants will have higher voluntary feed intake with the highest DMI in silages made with variety A, R6 harvest stage and molasses application. It was found that the RFV values of soybean silages were similar to the RFV values of corn and corn-soybean silages reported by Kızılşimşek et al. (2020), but lower than the RFV value of soybean and cowpea silages reported by Gülümser et al. (2021).

5. Conclusions

Different soybean varieties, harvesting stages, and additives had significant effects on the nutritional composition, and silage fermentation quality. The optimal harvesting stage for soybean varieties to produce quality silages was the full seed. Almost all additives improved the silage fermentation qualities of the soybean varieties. Molasses and cracked wheat additives were the best silages in terms of silage fermentation quality criteria. The A and D varieties of soybean are the best raw material to produce quality silages. Overall, a good quality silage could be produced by using the A and D varieties as silage material, harvesting at R6 stage and adding molasses and cracked wheat as additives.

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