Silage Quality Characteristics of Quinoa Varieties Grown in Different Row Spacings

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Abstract: Changing climatic conditions, agricultural lands becoming barren, losing their qualities and decreasing feed resources have led people to search for alternative feed sources. Quinoa (*Chenopodium quinoa* Willd.) is increasing in importance as an alternative feed source because it is a plant resistant to arid, salty and cold conditions. The quinoa plant can be considered an alternative plant for silage, which is an important feed source for animals. In this study, the effects of sowing Cherry Vanilla and Read Head quinoa varieties at 4 different row spacings (17.5, 35.0, 52.5 and 70.0 cm) on silage quality were investigated. In the research, pH, dry matter ratio, fleig score, ammonia production, sensory analyses, lactic acid, acetic acid, propionic acid and butyric acid contents of quinoa silage were determined. While the effect of row spacing on dry matter ratio, ammonia production, sensory analysis, lactic acid, acetic acid, propionic acid and butyric acid ratios was found to be significant, its effect on pH value was insignificant. The quinoa varieties used in the research had a significant effect on the dry matter ratio and propionic acid content. In the light of the results obtained from the research, it was concluded that in order to obtain quality quinoa silage, the plants should be sown in 52.5 cm of row spacing and the Cherry Vanilla variety should be preferred.

Keywords: Quinoa, row spacing, silage, quality, organic acid, lactic acid

Farklı Sıra Aralığında Yetiştirilen Kinoa çeşitlerinin Silaj Kalite Özellikleri

Öz: Değişen iklim koşulları, tarım arazilerinin çoraklaşması, niteliklerini kaybetmesi ve yem kaynaklarının azalması insanları alternatif yem kaynakları arayışına sürüklemiştir. Kinoa (Chenepodium quinoa Willd.) kurak, tuzlu ve soğuk şartlara dayanıklı bir bitki olması nedeniyle alternatif yem kaynağı olarak önemi artmaktadır. Hayvanlar için önemli bir yem kaynağı olan silaj için de kinoa bitkisi alternatif silaj bitkisi olarak düşünülebilir. Bu çalışmada Cherry Vanilla ve Read Head kinoa çeşitlerinin 4 farklı sıra aralığı mesafede (17.5, 35.0, 52.5, 70.0 cm) ekilmesinin silaj kalitesi üzerine etkileri araştırılmıştır. Araştırmada kinoa silajının pH, kuru madde oranı, fleig puanı, amonyak üretimi, duyusal analizler, laktik asit, asetik asit, propiyonik asit, bütirik asit içerikleri belirlenmiştir. Araştırma sonucunda sıra aralığı mesafesinin, kuru madde oranı, amonyak üretimi, duyusal analizler, laktik asit, asetik asit, propiyonik asit ve bütirik asit oranları üzerine etkisi önemli bulunurken, pH değeri üzerine etkisi önemsiz olmuştur. Araştırmada kullanılan kinoa çeşitlerinin kuru madde oranı, propiyonik asit içeriği üzerine önemli etkisi olurken diğer özellikler arasında önemli bir fark görülmemiştir. Araştırma sonucunda elde edilen sonuçlar ışığında kaliteli bir kinoa silaj elde etmek için bitkilerin 52.5 cm sıra aralığında ekilmesi Cherry Vanilla çeşidinin tercih edilmesi gerektiği sonucuna varılmıştır.

Anahtar kelimeler: *Kinoa, sıra aralığı, silaj, kalite, organik asit, laktik asit*

INTRODUCTION

The feed obtained because of the plants being harvested green, broken down and fermented in an oxygen-free environment is called silage. The most preferred plant for silage purposes is corn. On the other hand, clover, vetch, barley, sudangrass and oat plants are among the plants preferred for silage making. Droughts have increased as a result of significant changes in climate conditions as a result of global warming. On the other hand, salinity in agricultural areas is increasing day by day as a result of the increase in temperature and unconscious agricultural practices.

Plants preferred for silage making are not resistant to extreme climate and soil conditions. It is important to identify alternative forage plants that are resistant to extreme climate and soil conditions, to make silage from these plants and to determine their silage quality. Studies have shown that the quinoa plant is resistant to arid, salty and cold conditions (Jacobsen et al., 2003; Jacobsen et al., 2005; Geerts et al., 2009; Razzaghi, 2011; Pulvento et al., 2012; Zerrouk et al., 2012). al., 2020; Keskin et al., 2023).

Quinoa plant, belonging to the Chenopodiaceae family, is an annual and C3 plant. It is a plant that has been cultivated in Peru and Bolivia for 5000 years and has been cultivated in South America for thousands of years and consumed by people as grain (Jacobsen, 2003; Tan and Temel, 2019). Its production has been increasing in recent years due to the fact that it does not contain gluten, has a high nutritional content, and has a high yield of seeds and grass per unit area (Van Schooten and Pinxterhuis, 2003; Tan and Yöndem, 2013; Önkür and Keskin, 2019a; Önkür and Keskin, 2019b; Temel and Keskin, 2019a; Temel and Keskin, 2019b). It is an alternative forage plant that can be used as a silage plant due to its high crude protein and dry matter ratio (Temel and Keskin, 2020).

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This study was carried out to determine the silage quality of quinoa varieties in different row spacings.

MATERIAL AND METHODS

Material

The material to be used in silage quality was taken from the plants of Cherry Vanilla and Read Head quinoa varieties planted at 17.5, 35.0, 52.5 and 70.0 cm row spacings at the Iğdır University Agricultural Application and Research Center trial site (450 05'42.63'' E, 390 55'43.59" N) in 2021. Plants were harvested at the beginning of flowering.

Method

When the plants reached the harvest period (June 21, 2021), after leaving the edge effects, the remaining plants were harvested from 5 cm above the soil level. After the harvested fresh herbage was shredded with a shredding machine, approximately 500 g was taken and placed in transparent polyethylene bags for fermentation. After the air in the bags was removed with the vacuum machine, the mouths of the plastic bags were automatically closed by the vacuum machine. The prepared silages were left to ferment for 60 days. The following silage quality analyzes were performed on the silage samples whose fermentation was completed.

Silage pH: Silage pH: 20 grams of silage samples were placed in a blender and 180 ml of pure water was added and mixed at high speed. The material crushed in the blender was filtered through coarse filter paper. The pH of the silage was determined by measuring it with a pH meter (AOAC, 1990).

Dry Matter Ratio: After the silage material was weighed as wet, it was dried a little in the open air and then kept in a drying oven at 65°C for 48 hours. The oven-dried material was weighed. The dry matter ratios (%) were determined by proportioning the obtained dry weights to the wet weights (AOAC, 1990).

Fleig Score: The fleig score was calculated according to the formula below and the obtained values were defined as the quality characteristic of silage according to the scale in Table 1 (Kılıç 1986).

Fleig score = 220 + (2 x % dry matter - 15) - 40 x pH

Table 1. Scale values.

81-100	Very good
61-80	Good
41-60	No bad
21-40	Middle
0-20	Very bad

Ammonia Production: Ammonia content was determined by taking 100 ml of the silage sample used in pH measurement and performing distillation and titration processes in protein determination according to the Kjeldahl method (AOAC, 1990).

Sensory Analyzes: The color, smell and structure of the silage were evaluated by 3 people and scored according to Table 2, and the quality of the silage material was classified according to the total scores (Kılıç 1986).

Organic Acids: The silage liquid obtained from pH measurement was passed through a 0.45 micron (µm) filter attached to the syringe tip and placed into the vial. The HPLC-DAD device used H_2SO_4 mobile phase at 41 °C, 0.6 ml/min flow rate, 50 µl sampling amount, and organic acids (acetic acid, buturic acid, propionic acid and lactic acid) were determined in mg and then converted to %. (De Baere et al., 2013).

Statistical Analysis

Variance analyzes were performed on the research data using the JMP 5.0.1 statistical package program. The averages of the factors and interactions that were found to be significant were grouped according to the LSD test.

RESULTS AND DISCUSSION

pH value

pH value is an important criterion in determining silage fermentation. The more fermentation, that is, sourness, in silage, the better the silage quality. The effect of variety and row spacing on the pH value was found to be insignificant, but the effect of their interaction was significant (Table 3). The pH values of the varieties were 4.56 (Red head) and 4.63 (Cherry vanilla), and the pH values in the row spacings of 17.5, 35, 52.5 and 70 cm were 4.56, 4.71, 4.58 and 4.53, respectively. Depending on the varieties and row spacing, the pH values of quinoa silage were found to be slightly high. The highest pH value of quinoa silages was observed in Cherry Vanilla varieties sown in 35 cm of row spacing and Read Head varieties planted in 52.5 cm row spacing. The lowest pH value (4.23) was seen in the Cherry Vanilla variety planted in 52.5 cm row spacing. In order for lactic acid bacteria to be effective in silage, the pH must be between 3.8-4.2. While the appropriate pH value was obtained if the Cherry Vanilla variety was sown in 52.5 cm row spacing, the pH values in other applications were found to be higher than the desired pH values in silages. In this case, the varieties showed different reactions to different sown densities and an interaction occurred. In studies conducted to determine the pH of quinoa silage, pH values were determined to be between 4.1 and 6.7 (Podkòwka et al., 2018; Salama et al., 2021; Yacout et al., 2021; Fang et al., 2022; Güner and Temel, 2022). While the pH values obtained in the current study were found to be higher than the values obtained in some studies, they were found to be lower than the pH values obtained in some studies.

Table 2. Scoring used in sensory analysis

SMELL OF SILAGE	Point
No butyric acid, slightly acidic	14
There is very little butyric acid, a pungent acid smell, and a slight musty smell	8
Moderate butyric acid smell, pungent musty odor	4
Strong butyric acid and ammonia smell	2
Bad and pungent musty smell	0
SILAGE STRUCTURE	
Leaf and stem tissue are normal	4
Leaf and stem textures are slightly distorted	2
Leaf and stem tissue is very deteriorated, dirty and moldy	1
Leaves and stems are cousing, excessive pollution and excessive mold growth.	0
SILAGE COLOR	
Green color	2
Yellow or brown	1
Discolored, light yellow or dark	0
TOTAL EVALUATION	
Very googd	18-20
Good	14-17
Middle	10-13
Bad	5-9
Very bad	0-4

Table 3. Some silage quality values of quinoa varieties grown in different row spacings.

Variety (V)	**pH value**				
	Row spacing (R)				Variety avg.
	17.5	**35**	**52.5**	**70**	
Cherry Vanilla	4.76 ab	4.90 a	4.23 d	4.63 bcd	4.63
Red Head	4.36 cd	4.53 b-d	4.93 a	4.43 bcd	4.56
Row spacing avg.	4.56	4.71	4.58	4.53	
F value and significance	R: 0.95 ns	V: 0.65 ns	V×R: 9.83**		
	Dry Matter Ratio				
Cherry Vanilla	18.2 d	17.7 d	22.6 a	19.9 bc	19.5 a
Red Head	18.8 bcd	18.0 d	18.3 cd	20.0 b	18.7 b
Row spacing avg.	18.4 b	17.8 b	20.4 a	19.9 a	
F value and significance	R: 10.17**	V: 4.61*	R×V: 9.52**		
	Fleig Point				
Cherry Vanilla	49.7 cd	43.7 d	80.7 a	59.3 bc	58.3
Red Head	68.0 ab	60.2 bc	44.0 d	67.0 ab	59.8
Row spacing avg.	58.9	51.9	62.4	63.1	
F value and significance	R: 2.15 ns	V: 0.18 ns	R×V: 13.84**		
	Ammonia Production				
Cherry Vanilla	5.20 bc	6.33 a	3.93 d	3.63 d	4.77
Red Head	3.90 d	5.56 ab	4.20 cd	5.86 ab	4.88
Row spacing avg.	4.55 b	5.95 a	4.06 b	4.75 b	
F value and significance	R: 10.12**	V: 0.19 ns	R×V: 9.60**		
	Sensory Analysis				
Cherry Vanilla	17.9 a	14.0 d	11.8 c	17.0 ab	15.2
Red Head	16.6 b	17.2 ab	10.5f	15.4 c	14.9
Row spacing avg.	17.3 a	15.6 b	11.2 c	16.2 b	
F value and significance	R: 94.34**	V: 0.52 ns	R×V: 17.23**		

* P<0.05 is significant at the probability level, ** P<0.01 is significant at the probability level, ns is not significant. The difference between the data represented by the same letters is not significant.

Dry Matter Ratio

The effect of varieties and row spacing on the dry matter ratio of quinoa silage was significant (Table 3). The highest dry matter ratio was reached in Cherry Vanilla variety. The highest dry matter rate was seen in plantings with row spacings of 52.5 and 70 cm. Güner and Temel (2022), in their study conducted under dry conditions, determined that the dry matter ratio of silage obtained from quinoa varieties varied between 23.45% and 24.38%, depending on the row spacing. It is thought that the difference in dry matter ratio was caused by the fact that the quinoa varieties used by Güner and Temel (2022) were different, and the research was conducted in arid conditions. As a result of the combined effects of row spacing and

varieties, dry matter rates varied between 17.7% and 22.6%. It was observed that the dry matter ratios of Cherry Vanilla and Red Head varieties changed significantly when quinoa plants were sown at 52.5 cm. For a quality silage, the dry matter ratio must be 25% or higher (Huhtanen et al., 2007). However, it was observed that the dry matter ratios obtained in our current study were lower than this value. Therefore, it can be said that the pH of the silage is not low enough. As a matter of fact, the high dry matter ratio in silage material facilitates the decrease of silage pH. It has been determined that it may be appropriate to use additives to increase the dry matter ratio in quinoa silage.

Fleig Point

Fleig score, determined by using pH and dry matter ratios, is a method used to determine the qualities of silo feeds (Woolfort, 1984; Kılıç, 1986). While the effect of variety x row spacing interaction on the Fleig score was significant, the effect of variety and row spacings were found to be insignificant (Table 3). Fleig scores obtained in different row spacing ranged between 51.9 and 63.1. Fleig scores of the varieties varied between 58.3 and 59.8. While it was observed that the Red Head variety had a higher Fleig score in the 17.5, 35, and 70 cm row spacings, the Cherry Vanilla variety had a higher Fleig score in the 52.5 cm of row spacing (Table 3). Varieties showed different reactions depending on row spacing. The highest fleig score was seen in the Cherry Vanilla variety sown in 52.5 cm row spacing, and the lowest fleig score was seen in the Cherry Vanilla variety sown in 35 cm row spacing. When the Fleig scores of quinoa silages were reported in the study of Kılıç (1986), it was reported as "satisfactory".

Ammonia Production

Ammonia production of the varieties was similar. However, the effect of row spacing and variety x row spacing interaction on ammonia production is important (Table 3). Ammonia production of Cherry Vanilla and Red Head varieties was 4.77 and 4.88%, respectively. While the lowest ammonia production was obtained in the 52.5 cm row spacing, the highest ammonia production was obtained in the 35 cm row spacing. In a study, they reported that ammonia production increased as the row spacing increased (Güner and Temel, 2022). As a result of the combined effects of variety x row spacing, ammonia production varied between 3.63 and 6.33.

Sensory Analysis

While the sensory analysis of the varieties was similar in the study, the effects of row spacing and factors together on the sensory analysis were found to be significant (Table 3). When quinoa varieties were sown at 17.5, 35.0, 52.5 and 70.0 cm row spacing, sensory analysis values were found to be 17.3 (very good), 15.6 (good), 11.2 (medium) and 16.2 (good), respectively. Sensory analysis values also decreased as the row spacing increased. However, the sensory analysis values obtained at 70 cm of row spacing were similar to the sensory values obtained at 35 cm of row spacing. Varieties responded differently to row spacing. According to the sensory analysis data, the highest sensory analysis score was seen in the Cherry Vanilla variety sown in 17.5 cm row spacing, and the lowest sensory analysis score was in the Read Head variety sown in 52.5 cm row spacing. It was determined that quinoa silage was of good quality in terms of sensory analysis evaluation. In studies conducted on quinoa silage, sensory analysis values were found to be in the range of 15.6-16.1 (Güner and Temel, 2022).

Lactic acid

While the effect of row spacing on lactic acid ratio was significant, the effect of variety and variety x row spacing interaction was not significant. The lactic acid rates of Cherry Vanilla and Read Head varieties were 0.99% and 1.12%, respectively. While the highest lactic acid ratio was obtained in plants sown in 52.5 cm of row spacing, the lactic acid ratios obtained in other row spacings were in a similar group (Table 4). Filya (2021) reported that the fermentation quality of silages is determined by the amount and composition of organic acids they contain. As a matter of fact, it has been reported that the lactic acid rate in quality silage feeds should be 2% and above (McDonald et al., 1991; Weinberg and Ashbell, 2003). On the other hand, it has been reported that the lactic acid rate in quinoa silage is between 1.05% and 2.11% (Yacout et. al., 2021; Dong et. al., 2022). In the light of this information, the lactic acid rates obtained in the current study were found to be lower than in previous studies. In the current study, it can be said that high silage pH also causes low lactic acid formation.

Acetic acid

The varieties' acetic acid ratios did not significantly differ from one another. However, row spacing and variety x row spacing interactions had significant effects on acetic acid rates. While the amount of acetic acid was higher in the silages of plants sown in 35 cm of row spacing, the acetic acid rates in the silages of plants sown in other row spacings were similar. When looking at the variety x row spacing interaction, the acetic acid rate of the Cherry Vanilla variety was found to be higher than the Read Head variety at 17.5 cm row spacing. When the row spacing was increased to 35.0 cm, there was an increase in the acetic acid content of both varieties, but the increase rate of the Read Head variety was higher than that of the Cherry Vanilla variety. Afterwards, when the row spacing was increased to 52.5 cm, it was observed that there was a decrease in the acetic acid ratio in both varieties, and it was concluded that the acetic acid ratio of the varieties increased again with the row spacing of 70.0 cm. Differences in the acetic acid ratios of the varieties at different row spacings caused the formation of a binary

interaction (Table 4). High acetic acid levels in silages are undesirable. The acetic acid rate in quinoa silage was determined as 0.77% and 0.80% (Podkòwka et al., 2018; Yacout et. al., 2021). It was reported that the acetic acid content of quality silage should be between 0.3-0.7% (Menke and Huss, 1975; McDonald et al., 1991). In the current study, acetic acid rates were determined to be above the desired values.

Table 4. Some organic acid values of quinoa varieties grown in different row spacings.

Variety (V)	Ros spacing (R)				Variety avg.
	17.5	35	52.5	70	
Lactic acid					
Cherry Vanilla	0.98	0.86	1.25	0.89	0.99
Red Head	1.12	1.13	1.24	0.97	1.12
Row spacing avg.	1.05 b	0.99 b	1.24 a	0.93 b	
F value and significance	R: 4.97**	V: 3.97 ns	R×V: 0.91 ns		
Acetic acid					
Cherry Vanilla	1.27 a	1.34 a	0.79 c	1.31 a	1.18
Red Head	0.79 c	1.35 a	1.14 ab	1.03 b	1.08
Row spacing avg.	1.03 bc	1.34 a	0.97 c	1.17 b	
F value and significance	R: 9.50**	V: 3.39 ns	R×V: 11.02**		
Propionic Acid					
Cherry Vanilla	0.26 bc	0.17 d	0.16 d	0.29 b	0.22 b
Red Head	0.18 d	0.40 a	0.24 c	0.18 d	0.25 a
Row spacing avg.	0.22 bc	0.28 a	0.20 c	0.24 b	
F value and significance	R: 8.33**	V: 6.69*	R×V: 39.76**		
Butyric acid					
Cherry Vanilla	0.27	0.26	0.30	0.26	0.27
Red Head	0.29	0.29	0.30	0.27	0.29
Row spacing avg.	0.28 ab	0.27 b	0.30 a	0.27 b	
F value and significance	R: 3.65*	V: 3.65 ns	R×V: 0.55 ns		

* P<0.05 is significant at the probability level, ** P<0.01 is significant at the probability level, ns is not significant. The difference between the data represented by the same letters is not significant.

Propionic Acid

The propionic acid ratios of the quinoa varieties used in the research were different. Row spacing and variety x row spacing interaction had significant effects on the propionic acid amount of quinoa silage. The amount of propionic acid was higher in the Red Head variety. It was determined that if the quinoa plant was sown at a 35 cm row spacing, there would be more propionic acid in the quinoa silage. No significant difference was found between propionic acid values in other row spacings. It was determined that the amount of propionic acid reached its highest value when the red head variety was planted at 35 cm of row spacing (Table 4). It is undesirable to have an excessive amount of propionic acid in silage, which causes a sharp and unpleasant odor. The amount of propionic acid is expected to be low in well-preserved and fermented silages. Dong et. al., (2022) reported in their study that the amount of propionic acid in quinoa silage was 0.85%. This rate was found to be higher than the current study.

Butyric Acid

The effect of row spacing on butyric acid ratio was found to be significant. It was determined that the butyric acid ratios in the silages of quinoa varieties were similar. The highest amount of butyric acid was detected in quinoa silages planted at a 52.5 cm row spacing (Table 4). Butyric acid is not a desired organic acid in silages, in fact it is not desired to be present at all. Butyric acid bacteria break down the proteins in silage, causing their biological values to decrease. Weinberg and Ashbell, (2003) reported that the butyric acid rate in silages is generally between 0.1-0.7%. This supports the butyric acid rates we obtained in the current study.

CONCLUSION

The effect of different row spacings on the silage quality characteristics of quinoa under irrigated conditions was investigated. The dry matter ratio and acetic acid ratio of silages in quinoa varieties differed. Row spacing had significant effects on the dry matter ratio, ammonia production, sensory analysis, lactic acid ratio, acetic acid ratio, propionic acid ratio and butyric acid ratio of quinoa silage. In order to obtain quinoa silage of the desired quality, it is necessary to lower the pH value, increase the dry matter ratio, increase the lactic acid ratio and reduce the acetic acid ratio. For this purpose, additives should be added to quinoa silage and silage should be made.

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