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Chemical Properties of Silage and Grain Sorghum (*Sorghum bicolor* L.) Varieties

Mehmet OTEN[1]Orçun CINAR[1]
Bati Akdeniz Agricultural Research Institute Antalya/Turkey
Corresponding author:Mehmet OTEN

--***ABSTRACT***--
Yield, in sorghum breeding program has come to the fore, researchers little attention have been given tochemical composition of sorghum.The aim of the study was to evaluation of the chemical properties of some silage and grain sorghum varieties. Hydrocyanic acid (HCN) content, tannin content, protein ratio, sugar and some elements content of sorghum was determinated. The experiment was conducted at Bati Akdeniz Agricultural Research Institute in 2017. The plant materialwhich are widely used in the Mediterranean region and registered by BATEM, as silage sorghum varieties; Rox, Leoti, Early Sumac and Nes, as grain sorghum varieties; Aldari, Akdari, Beydari, Ogretmenoglu and IS407 were used in this study. After harvesting, the seed samples were analyzed to evaluation of the defined properties. The collected data were analyzed and the means were done according to multiple range test at 1% level of probability by Duncan's. As a result of the study; the highest HCN content of grain (HCNG) was detected in Esumac variety, HCN content of sprout (HCNS) was detected in IS407 variety, tannin content was ranged from 131.66 ppm to 1405.83 ppm, the highest crude protein ratio were found in Leoti, Aldari and IS407. Furthermore, large differences were observed in some elements in all varieties. The obtained results revealed that, HCNG has significant positive correlation with tannin and Cu but HCNS has negative significant correlation with sucrose.

Key words: *Element, Sorghum bicolor, hydrocyanic acid, protein, silage sorghum, tannin,*

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I. INTRODUCTION

Sorghum is very important plant for human, poultry and cattle, for food, feed and fodder, respectively. It is cultivated in Turkey during the summer season especially in Mediterranean and Southeastern Anatolia regions, in order to meet both green as well dry fodder requirements of the livestock (Ahmad *et al*., 2007). Because of sorghum's rapid growth rate, high biomass yield, early maturity (90-180 days), and wide adaptability, it plays a significant role in providing nutritious feed to dairy and farm animals. (Prasad *et al*., 2007; Dimple *et al*., 2010; Ghasemi *et al*., 2012). Variously classified sorghum is known as a tropical crop and can be an alternative to corn (*Zea mays* L.) in arid and semiarid climates (Tiryaki, 2005; Black *et al*., 2005; Barba *et al*., 2012). Because of the sorghum is grown under diverse agroclimatic conditions, it's variability is large in grain composition. Climate conditions, varieties, soil types, fertilizers and fertilization method are recorded among the factors related to the chemical composition and nutritional value of sorghum (Ebadi *et al*. 2005). For the nutritional value of sorghum is associated with its chemical composition and the utilization level of nutrients. The contents of the HCN in sorghum vary depending on plant growth stage, variety and environmental conditions that harvesting time, cutting high, drought and frost etc. (Zahid*et al*. 2012; Oten, 2017). The content of HCN in seeds and plant is harmful to animals. Any stress that disrupts normal growth can contribute toward increased HCN toxicity. Tannin content in the pericarp is one of the most important factors affecting the feeding value of sorghum grain and adversely affects its metabolizable energy and protein utilization (Selle *et al*., 2010). Sedghi *et al*. (2012) stressed that the color of sorghum grain was associated with the tannin content additionally, varies greatly due to pericarp color and thickness, presence of testa, and endosperm texture and color. Palavecino *et al*. (2016) reported that sorghum crude protein ranged from 8.47 to 17.08%. However, sorghum proteins are deficient in the essential amino acid, because both genetic and environmental factors affect the protein content of sorghum(Proietti *et al*. 2015). Although sorghum is rich in minerals, compared with barley and rye, it has low levels in point of P, K, Mg, Ca, Na, Zn, Fe, Mn, and Cu (Ragaee *et al*., 2006).

In sorghum breeding programmes have increased yields of sorghum forage and grain but little attention has been paid to the quality. Because of the attributes normally emphasized are yield and quality, researchers little attention have been given to mineral concentration in sorghum breeding programs. Therefore, with the present study to evaluate 10 different sorghum varieties, to determine their HCN, tannin, crude protein and some element contents, additionlly to revealed association among of them. For this purpose it was carried out to study to evaluation chemical composition of sorghum, as this aspect relates directly to the nutritive value of the plant.

II. MATERIALS AND METHODS

The study was conducted in the experimental area of Batı Akdeniz Agricultural Research Institute in Turkey in 2017. The climate data of experimental area, average temperatures, rainfall and relative humidity in 2017 and long periods are shown in the Table 1.

Table 1. Climate Data for Long Period and 2017

Months	Precipitation (mm)		Temperature (^{0}C)		Relative Humidity (%)	
	Long period	2017	Long period	2017	Long period	2017
January	245.7	114.0	10.2	8.8	67.2	64.6
February	133.2	8.8	11.1	11.1	67.1	63.6
March	48.2	95.0	13.7	13.3	66.4	69.7
April	55.8	34.0	16.4	16.9	67.1	66.4
May	49.8	59.6	21.0	20.7	66.6	70.7
June	4.2	0.2	25.9	26.2	61.2	63.9
July	3.0	0.0	28.9	29.9	60.3	54.0
August	1.8	0.0	28.8	28.9	62.9	53.8
September	27.0	0.4	25.1	25.3	61.3	70.3
October	134.4	44.6	20.3	20.2	62.7	59.8
November	77.8	80.7	15.4	16.5	66.5	64.6
December	182.5	75.4	11.6	12.8	66.2	61.3

Except from precipitation, the climate values of 2017 was similar to the long period. The irrigation was applied six times in growing seasons when need it. Chemical properties of the soil of the trial area have silty-clay-loamy soil with organic matter content adequately with 2.3% and strong alkaline with 8.2 pH level. Weeds were controlled with hand by hoeing when needed throughout the growing seasons. There were no pests or diseases in the study, so no chemical was applied. It was harvested in September 17th, 2017. After harvesting, the seed samples were analyzed to evaluation of the defined properties.

Sorghum cultivars; Rox, Leoti, Early Sumac, Nes, Aldari, Akdari, Beydari, Ogretmenoglu and IS407 were procured from the Bati Akdeniz Agricultural Research Institute in Antalya in Turkey. Analysis for sprout HCN (HCNS), grain HCN (HCNG), tannin, crude protein and some elements (Ca, Cu, K, Mg, Fe, P and Zn) of sorghum were carried out with standard methods of AOAC (2000). It was done asrandomized complete block design with 3 replications on April 21th, 2017.

Extraction of the samples

The samples were grinded in grinder firstly. Then, 0.5 g sample was weighed and put to the 50 ml falcon tube. 9.5 ml 80% MeOH was added to the tube. It was mixed with vortex device and it was put to the orbital shaker for the extraction. After 1 hour, tubes were taken from the shaker and put into the centrifuge device. It was centrifuged for 5 min. at 5000 rpm. Then, the upper phases taken and put to another tube. 10 ml 80% MeOH was added onto the tubes which the samples were in it and it was put to the shaker again. This procedure was repeated 3 times. Finally, the upper phases were collected.

HCN content analysis

HCN levels in the samples were determined using the colorimetric method that is suggested by Lambert *et al*. (1975) and adapted by Pirincci & Tanyıldızı (1994). According to this method; calibration curve was prepared using by 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1, 2, 3, 4, 5, 6, 8, 10 and 20 ppm potassium cyanide solutions (*Sigma-Aldrich Cat. No:31252-100 g*). 1 ml water samples, 1 ml N-chlorosuccinimide-succinimide solution (10 g succinimide *Sigma-Aldrich Cat. No: 59381-500 g* was dissolved in 200-300 ml distilled water, afterwards, 1 g N-chlorosuccinimide *Sigma- Aldrich Cat. No: 109681-100* g was added and the solution was completed to the 1 lt with distilled water) and 1 ml barbituric acid-pyridine solution (3 g barbituric acid *Sigma-Aldrich Cat. No:185698-25 g* was dissolved in 10 ml distilled water, afterwards, 15 ml pyridine *Sigma-Aldrich Cat. No:360570-100 ml* was added and the solution was completed to the 50 ml with distilled water) were mixed and dropped to the 25 ml flasks, afterwards, the solution was completed to the 25 ml with distilled water. The solution was held on 15 minutes in the darkness for the colour formation. After that, HCN levels of the samples were determined as ppm by observation of absorbance in 575 nm in the spectrophotometer (Shimadzu, UV 1600).

Tanin analysis

Tannins were assayed in a reaction solution containing 4 mL of the diluted (1/25) methanolic extract, 2 mL ethanol, 4 mL vanillin solution (1% vanillin in 70% sulphuric acid). Samples were compared to a control with 4 mL of sulphuric acid instead of vanillin solution and absorbance was detected at=500 nm. Tannin concentration was calculated following a calibration curve with pure catechine solution of known concentrations. The samples are diluted 1:5, 1:10 or more proportion with distilled water. Then homogenized during one minute.Homogenized samples were centrifuged for 10 min, at 5000 rpm. Then, the upper phases take and read at UV-Vis spectrophptometer device.

Mineral matter analysis

The seed samples of the sorghum varieties were washed by distilled water and then it was dried in a drying oven at 65°C for becoming constant weight. The seed samples were sieved from 20 mesh screen and it was waited in polyethylene bags until analysis. 0.5 g of the seed samples were weighed and 10 ml $HNO_3/HClO_4$ (4:1) acid solution was added in. The samples were heated until the dissolution was completed. The same procedure was repeated several times. The seed samples were filtered and diluted to 100 mL with distilled water. The mineral elements (K, Ca, Mg, Fe, Zn, Mn and Cu) concentration of the samples were determined with ICP-OES device (Kacar & Inal, 2008). The phosphorus content was determined by spectrophotometric method (Kacar & Kovancı, 1982) .

Protein analysis

The nitrogen content was determined by a modified Kjeldahl procedure (Kacar & Inal, 2008) and then the protein ratio was calculated by using nitrogen values. Obtained data in the experiment, was evaluated with analysis of variance by SAS (1998) statistical software. The differences between means were compared according to Duncan's test (Duzgunes *et al*., 1987).

III. RESULTS AND DISCUSSION

In this experiment; Rox, Gozde, Leoti, E.Sumac, Nes, Aldari, Akdari, Beydari, Ogretmenoglu and IS407 sorghum varieties were used as plant material which are registered by Bati Akdeniz Agricultural Research Institute. According to the results of analysis of variance there was found highly significant at the 0.01 level for all investigated traits (Table 2).

Table 2. Variance analysis of investigated traits

Source	DF	HCNS	HCNG	Tannin	Crude Protein	Sucrose	Glucose	Fructose	Ca	Cu	K	Mg	Fe	P	Zn
Replication	2	54.5	9.1	33.8	0.9	0.0000037	0.000004	0.0000009	0.00002	0.001	0.00000645	0.00000678	0.3	0.44	1.74
Varieties	9	20848.7**	1333.5**	4620550.0**	42.5**	1.0698534**	10.990569**	8.08162**	0.005**	14.6**	0.03705841**	0.01188916**	3293.1**	1.82**	470.27**
Error	18	285.3	39.2	63241.3	11.8	0.0000026	0.000007	0.0000023	0.000009	0.04	0.00006687	0.00002857	25.0	3.99	6.67
Total	29	21188.6	1381.9	4683825.0	55.2	1.0698597	10.990580	8.0816305	0.004	14.6	0.03713174	0.01192450	3318.5	6.26	478.70

** Significant at 0.01 probability level

Duncan test for means of HCN levels was stated at Table 3. The HCN analysis were done both on grain and on sprouts. Sprouts of the same cultivars were grown for 3 days in the dark at 30°C (Oksana & Danold, 1984). While the highest HCN content of sprout was detected in IS407 among the varieties, the lowest HCN content of sprout was detected in Ogretmenoglu variety. HCN content in sprout varied from 10.31 ppm to 90.66 ppm. Oksana & Danold (1984) reported that while sorghum seeds contained only traces 1 or 2 ppm to 29 ppm of potential hydrocyanic acid however sprouts of the same cultivars contained from 258–1030 ppm potential HCN relative to the weight of the ungerminated, dry seed. The present results were parallel with our results by high HCN content of the sorghum sprouts. While Christopher *et al*.(1988) reported that the HCN content increased about 4000-7000% in sprouted sorghum after 2-6 days' sprouting,however it was not so high in our study. Furthermore, the content of HCN in the grain was lower than in the sprout. HCN content of grain ranged 5.41 ppm to 23.80 ppm. Similar observations have been recorded by Oksana & Danold (1984). The highest HCN content of grain was detected in Esumac variety in the experiment. While the highest HCN was detected in the sprout of IS407 variety, the opposite was observed in the grain. Moreover, HCN ratio of grain sorghum was detected in silage sorghum varieties higher than the grain varieties. It was found significant differences in terms of tannin levels among the varieties. While the tannin content ranged from 131.66 ppm to 1405.83 ppm, the lowest tannin were detected in Beydari and IS407 varieties with 131.66 ppm, 134.16 ppm respectively. The highest tannin content were found in Rox and Gozde varieties.Previous studies found that sorghum tannins content ranged from 10 to 2056 mg/100 g (El-Moneim *et al*. 2012). In the other study they had found the highest tannic acid concentration as 100 mg/100 g in the sample (Mulimani & Supriya, 1994). Our results are different from other results, because of used different varieties. In their study similarly; Dicko *et al*. (2002) reported that tannin content of sorghum has changed according to the varieties, even most of the varieties (82%) had a tannin content less than 0.25%. There is linked to the negative effects of the tannins on proteins considering Emmambux & Taylor (2003), however, it was found statistically insignificant effects in our study.The highest crude protein ratio were found in Leoti (14.12%), Aldari (13.44%) and IS407 (12.14%) varieties. The crude protein was found 9.35-11.05% by Adebiyi *et al*. (2005), 10.43% by Patekar *et al*. (2017). As the reason for differences in protein ratio between the studies it can be said that different varieties have been used. As it is known sweet sorghums are accumulate sugar (>8°Brix) in the stalks and produces in seed however, the varieties used in the experiment were not sweet sorghum because they had little sugar content according to sweet sorghum.There were found that Rox variety had highest sucrose with 0.635%, Akdari variety had highest glucose and fructose with 2.37% and 1.99% respectively. In Table 2; different minerals in sorghum were observed that Gözde had the highest Ca value with 0.131%. Rox had lowest Ca level and it was followed by Akdari with 0.086%. While Rox and Gozde varieties were determined as highest Cu level by 5.19, 5.33 ppm respectively, Leoti and Nes varieties were determined as lowest Cu level by 3.46, 3.40 ppm respectively. Large differences K ratio were observed in all varieties. It was observed that K ratio high in Aldari by 0.45%, low in Nes variety by 0.33%. Table 2 shows the result for the determination of Mg ratio of the various cultivars. The Mg ratio for the variety ranged from 0.13% to 0.19%. Rox variety had the highest Mg level (0.199%) followed by those of Aldari variety (0.197%). While the highest Fe value was determinated in Beydari variety with 169.75 ppm, the lowest Fe value were determinated in Akdari (136.12 ppm) and Aldari (136.16 ppm). It was observed that Aldari variety had the highest P value with 0.43% and Esumac variety had lowest P level with 0.28%. Zn value varieties was found ranged from 15.13 ppm (Nes) to 29.56 ppm (Akdari) in sorghum. Pontieri *et al* (2014) emphasized that contents of the potassium, calcium, phosphorus, magnesium, iron were between 3434.46 and 6957.67 mg kg^{-1}, 233.84 and 411.83 mg kg^{-1}, 2148.60 and 2963.40 mg kg^{-1}, 1454.92 and 2862.00 mg kg^{-1},39.36 and 77.03 mg kg^{-1}, respectively. In the other study Patekar et al. (2017) were found calcium with 20.357mg/100 g, phosphors with 463.33 mg/100 g, iron with 4.0086 mg/100 g and zinc with 4.0086 mg/100 g. The mineral content of grain crops is affected by both genotype and environment (Hussain *et al*., 2010; Zhang et al., 2010). For this reason differences between the results were owing to genotype and environment.

Table 3. Chemical contents in different varieties of sorghum

VARIETIES	HCNS	HCNG	Tannin	CrudeProtein	Sucrose	Glucose	Fructose	Ca	Cu	K	Mg	Fe	P	Zn
ROX	48.22 c	18.03 b	1276.66 a	10.57 c	0.635 a	0.059 ı	0.108 j	0.086 d	5.19 a	0.39 c	0.19 a	147.26 d	0.34 d	20.39 d
GOZDE	76.92 b	27.13 a	1405.83 a	10.87 c	0.137 h	0.467 g	0.519 f	0.131 a	5.33 a	0.40 b	0.18 b	145.96 de	0.35 b	22.69 c
LEOTİ	56.04 c	13.59 c	615.83 d	14.12 a	0.376 e	0.577 f	0.649 d	0.098 c	3.46 e	0.36 e	0.17 c	165.24 b	0.32 e	19.04 d
ESUMAC	19.27 d	23.80 a	912.50 b	10.51 c	0.460 d	0.818 d	0.683 c	0.101 c	4.99 b	0.34 g	0.16 d	153.25 c	0.28 j	24.72 b
NES	9.73 d	15.09 bc	894.16 b	11.06 c	0.280 f	0.624 e	0.631 e	0.089 d	3.40 e	0.33 h	0.15 e	143.30 e	0.30 g	15.13 e
ALDARI	48.39 c	6.66 de	796.66 bc	13.44 ab	0.118 j	0.881 c	0.484 g	0.098 c	4.44 c	0.45 a	0.19 a	136.63 f	0.43 a	22.45 c
AKDARI	44.50 c	14.44 bc	700.83 cd	11.42 bc	0.591 b	2.372 a	1.99 a	0.086 d	4.98 b	0.35 f	0.17 c	136.12 f	0.29 h	29.56 a
BEYDARI	70.55 b	10.91 cd	131.66 e	10.62 c	0.222 g	0.996 b	1.106 b	0.102 c	3.75 d	0.34 g	0.13 g	169.75 a	0.29 ı	16.05 e
OGRETMEN OGLU	10.31 d	5.41 e	706.66 cd	11.30 bc	0.544 c	0.174 h	0.153 ı	0.114 b	4.45 c	0.37 d	0.15 de	152.86 c	0.31 f	19.90 d
IS407	90.66 a	9.20 de	134.16 e	12.14 ab	0.133 ı	0.622 e	0.358 h	0.098 c	3.78 d	0.39 c	0.14 g	154.30 c	0.34 c	20.49 d
CV	8.38	10.2	7.8	6.9	0.001	0.0008	0.0005	1.2	1.1	0.5	0.7	0.7	0.07	2.8

Significant correlations between the investigated traits are presented given in Table 4. It was observed that a significant positive correlation (r=0.41) between HCNF and K, however, negative correlation (r=0.53) between sucrose. HCNT was positive correlated with tannin and Cu ratio. Moreover, tannin had negative correlation on Fe ratio. Crude protein correlated with K ratio. Additionally, the significant and positive correlations were found between grain minerals, Mg-Cu (r=0.56), Mg-K (r=0.6), Mg-Zn (r=0.38) and negative correlation was found between Fe-Cu (r=-0.48).This results confirmed the result found byPontieri *et al* (2014).

Table 4. Correlation coefficient of chemical contents in different varieties of sorghum

	HCNS	HCNG	Tannin	Crude protein	Sucrose	Glucose	Fructose	Ca	Cu	K	Mg	Fe		Zn
HCNS	-													
HCNG	ns	-												
Tanin	ns	0.64**	-											
Crude protein	ns	ns	ns	-										
Sucrose	-0.53**	ns	ns	ns	-									
Glucose	ns	ns	ns	ns	ns	-								
Fructose	ns	ns	ns	ns	ns	0.95**	-							
Ca	ns	ns	ns	ns	-0.39*	-0.36*	ns	-						
Cu	ns	0.53**	0.67**	ns	0.37*	ns	ns	ns	-					
K	0.41*	ns	ns	0.39*	-0.40*	ns	-0.41**	ns	ns	-				
Mg	ns	ns	0.69**	ns	ns	ns	ns	ns	0.56**	0.65**	-			
Fe	ns	ns	-0.51**	ns	ns	ns	ns	ns	-0.48**	-0.36*	-0.52**	-		
P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	
Zn	ns	ns	ns	ns	ns	0.61**	0.48**	ns	0.69**	ns	0.38**	ns	ns	-

According to the results, it can be said that, variation of climatic conditions during crop growth period would not effect all content concentration. It was found that the content of HCN in the sprout was higher than in the grain. HCN content of silage sorghum varieties have higher value than grain sorghum varieties. However, in view of that all the varieties studied contained HCN contents less than critical limit because safe limit of HCN for livestock is 500 ppm. Results indicated that highly relationships between chemical parameters of sorghum grain. There were detected varied significant differences among the elements and also detected significant correlations between the investigated traits. Also, it was detected that HCNG has significant positive correlation with tannin and Cu but HCNS has negative significant correlation with sucrose. It can be said that the dark coloured seeds are rich in terms of both tannin and HCN. The varieties used in this study have low HCN and tannin, high protein ratio. For this reason, these varieties can be preferred in agricultural areas.

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