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## An Approach to Color Change and Quality Relation in Roughages

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### ABSTRACT

In this work, it was aimed to associate CIELAB Scale ( $L^*$ ,  $a^*$ ,  $b^*$ ) with some quality parameters of roughages such as pH, crude protein (CP) and relative feed value (RFV). To this end, five different applications; haylages unwrapped on 25<sup>th</sup>, 40<sup>th</sup> and 55<sup>th</sup> days of fermentation, traditional silage unwrapped 55<sup>th</sup> days of fermentation and degraded haylage were examined for vetch-triticale (*Vicia sativa* L. - *Triticasecale wittmack*) and caramba-berseem clover (*Lolium multiflorum* cv Caramba-*Trifolium alexandrinum* L.) mixtures in the study. The tests were conducted according to randomized block design with three replications for both forage mixtures. The  $\Delta L$  and  $\Delta b$  color values and pH, CP, RFV were significantly correlated in the vetch-triticale mixture. The regression coefficients ( $R^2$ ) for  $\Delta L$  and  $\Delta b$  were 0.68 and 0.79 for pH, 0.40 and 0.38 for CP, 0.63 and 0.70 for RFV, respectively. In the mixture of the caramba - berseem clover, the regression coefficients for  $\Delta L$  and  $\Delta b$  were found to be 0.64 and 0.85 for pH, 0.14 and 0.12 for CP, 0.28 and 0.37 for RFV respectively. In both mixtures, the level of relation between  $\Delta a^*$  color value and quality parameters remained very low. Also, it was obtained that the best roughage in terms of RFV was haylages opened after 55<sup>th</sup> days. Decreasing the  $L^*$ ,  $b^*$  color values by 50% on average after fermentation can be regarded as an indication of roughage degradation and RFV reduction. From this point view, it can be possible to determine the quality change by following the colors before and after fermentation of roughage.

Keywords: Silage; Color change; Degradation; Quality; Roughage

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### 1. Introduction

Roughages is one of the largest expenses for livestock production and generally supplied as grass, hay and silage. Recently, a large proportion of forages for livestock feeding is conserved as silage due to its several advantages. These advantages include less field and harvest losses, many crop options, high mechanization of harvesting, storage and feeding, less likelihood of weather damage during harvesting

and low loss of nutrient. However, the silage quality is quite important for a profitable livestock farming.

Silage can be classified as good quality by physical characteristics like taste, smell, color and by chemical characteristics such as dry matter (DM) content, pH value, crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF). Good quality silage has vinegar smell, on acidic taste and a firm soft texture, leaves not

easily rubbed, light greenish or greenish brown in color (Uygur 2009). From the point of chemical characteristics, DM content of traditional silage varied in between 70%-85%. However, this value is around 40%-60% for haylage (bale silage). The pH value for good quality silage ranges between 3.8 and 4.0, however it can be higher (4.3-5.1) for haylage. Also, it is desirable that the CP value is as high as possible and the ADF and NDF values are low for pointing the silage as good quality.

Many researches have targeted to evaluate silage quality. In these studies, generally, it was aimed to determine the effects of storage period on chemical composition. It was reported that the pH value of silage decreased in long-term storage periods (Müller et al 2007; Shinnars et al 2009; Weinberg & Chen 2013).

There are many studies on changing pH, ADF, NDF, and RFV with fermentation duration in the literature. Shinnars et al (2009) determined that the pH, ADF and NDF values of the alfalfa silage incubated for 154 and 364 days were decreased from 5.9 to 5.13, from 34.4% to 29.4% and from 47.1% to 38.3%, respectively. Borreani et al (2008) found an opposite relationship for corn silage. The pH, ADF and NDF values of the corn silage stored for 154 and 364 days were increased from 3.74 to 3.80, from 22.95% to 26.2% and from 44.17% to 44.92%, respectively. However, they emphasized that the CP value decreased from 8.17% to 7.55%. Müller et al (2007) were found that the CP value of meadow grass stored for 2 and 14 months reduced from 4.88 to 4.50. In another work done by Weber & Kaiser (2006), it was obtained that the pH value of the sugar beet pulp silage has not changed for 14 and 183 days storage period. Moreover, Lee et al (2000) have determined that CP values decreased (from 10.03% to 3.04%) and ADF values increased (from 37.2% to 42.92%) according to 40, 50, 60, 65 days storage period for different forage species.

The extent of the effects of air ingress on bale silage is greatly important. The effect can be measure via color change. Color changing is one of the important parameters for quality of roughages

(Anonymous 1987). Also, it was reported that storage conditions affect color change in haylages and color values of the bale which is stored at protected areas were better than the others storage conditions (Toruk et al 2009; Toruk & Gonulol 2011). Silage color gives information about fermentation. Color evaluation of silages is considered as a physical criterion in literature and scored according to color change. In this context, relating color change to the quality parameters which are determined according to chemical analyzes is an important issue and there is limited studies focused on obtaining the relation between roughage quality and color change in the literature. The quality of roughage is foremost parameter for purchasing and adding to the feeding ration. DM, pH, CP and RFV can be listed as most important quality parameters. Although there are a lot of methods for determination of quality, it is another necessity for farmers to use fastest methods. Because, chemical analysis are costly and take times. In this case, affects the livestock economy negatively by increasing the cost of roughage. However, there are methods free from chemical analysis, but the results of these methods can change relatively depends on the person who makes decision. The significant difficulties occur in determining quality of forage for farmers due to there is no standard for the estimation of roughage quality. From this point of view, the objective of this current work was to associate CIELAB Scale (L\*, a\*, b\*) with some quality parameters of roughages such as pH, CP and RFV. Thus, practical methods can be developed to estimate the quality of forage using color change.

## 2. Material and Methods

Two roughages mixtures namely, vetch - triticale (*Vicia sativa* L. - *Triticasecale wittmack*) and caramba - berseem clover (*Lolium multiflorum* cv Caramba - *Trifolium alexandrinum* L.) were used as plant material in the experiment. Mixture rates were 70% and 30% for vetch-triticale and 50% and 50% for caramba-berseem clover, respectively. Plants were harvested at the end of the flowering stage. Materials have been stored as haylage and traditional silage. Haylage bales were wrapped with

0.025 mm plastic film in white color as four layers. Haylage bales were opened at the days of 25, 40 and 55 and the color changing was compared with traditional and degraded silage in terms of pH, CP and RFV.

The silage samples color was examined by using Minolta CR-100 Chromameter color measurement device. In the CIE 1976 ( $L^*$ ,  $a^*$ ,  $b^*$ ) color space, abbreviated CIELAB, the lightness coefficient,  $L^*$ , ranges from black = 0 to white = 100 and is roughly analogous to the Munsell value scale times 10. For any measured color of lightness,  $L_i^*$ , the coordinates ( $a^*$ ,  $b^*$ ) locate the color on a rectangular-coordinate grid perpendicular to the  $L^*$  axis at  $L_i^*$ . The color at the grid origin ( $a^* = 0$ ,  $b^* = 0$ ) is achromatic (gray). On the horizontal axis, positive  $a^*$  indicates hue of red-purple; negative  $a^*$ , of bluish-green. On the vertical axis, positive  $b^*$  indicates yellow and negative  $b^*$  blue (Figure 1) (McGuire 1992).

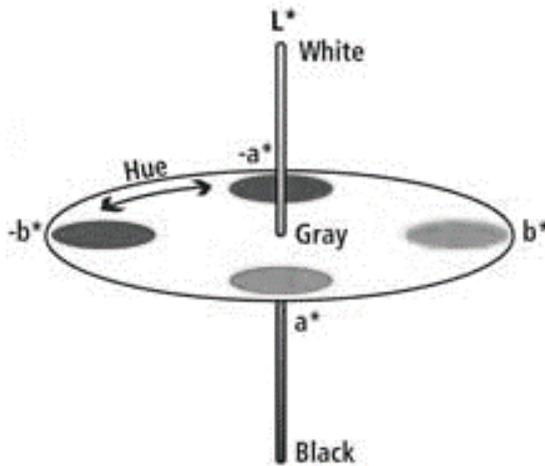


Figure 1- CIELAB color space

For determination of color changing, material color was measured before storage and after unwrapped and calculated by subtracting second (2) measurement from first one (1) (Snell et al 2002).

$$\Delta L = L_1^* - L_2^* \quad (1)$$

$$\Delta a = a_1^* - a_2^* \quad (2)$$

$$\Delta b = b_1^* - b_2^* \quad (3)$$

The quality of the material was evaluated in terms of pH, CP, ADF, NDF and RFV. Nitrogen (N) content was measured using the Kjeldahl Method. The pH values of plants were obtained as reported by Chen et al (1994). The CP was calculated as  $N \times 6.25$  (AOAC 1990). The NDF and ADF were determined as suggested Van Soest et al (1991) by using ANKOM fiber analyzer. The RFV was calculated by using equation given below (Mayouf & Arbouche 2014).

$$RFV = (88.9 - (0.779 \times ADF\%)) \times ((120 / NDF\%) / 1.29) \quad (4)$$

To investigate the effect of color changing on quality parameters, the tests were conducted according to randomized block design with three replications for both forage mixtures. Five different applications were tested in the study (Table 1). The Jump 7 statistical program was used in evaluating the data and groupings were made according to LSD (Yurtsever 1984).

Table 1- Applications tested in the study

Abbreviation	Applications
H1	Haylage, unwrapped on the 25 <sup>th</sup> day of the fermentation
H2	Haylage, unwrapped on the 40 <sup>th</sup> day of the fermentation
H3	Haylage, unwrapped on the 55 <sup>th</sup> day of the fermentation
TS	Traditional silage, opened on the 55 <sup>th</sup> day of the fermentation
DH	Degraded haylage

### 3. Results and Discussion

A review of Table 2 shows that the color values of both mixtures did not differ statically before wrapping but they were found significant at 1% level after opening.

It is desirable that the color values after opening as silage quality indicators do not differ much from the initial color values. Table 3 presents the color changing values according to applications. When the color values are examined after both mixtures

**Table 2- Coefficients of variation for according to mixtures**

Parameters	<i>Vetch-Triticale</i>			<i>Caramba-Berseem Clover</i>		
	<i>P value</i>	<i>LSD</i> <sub>(0.05)</sub>	<i>CV</i> (%)	<i>P value</i>	<i>LSD</i> <sub>(0.05)</sub>	<i>CV</i> (%)
$L_1^*$	0.3526 <sup>ns</sup>	-	5.08	0.2893 <sup>ns</sup>	-	2.41
$a_1^*$	0.9211 <sup>ns</sup>	-	13.0	0.3040 <sup>ns</sup>	-	8.06
$b_1^*$	0.1507 <sup>ns</sup>	-	7.37	0.8241 <sup>ns</sup>	-	8.73
$L_2^*$	<0.0001 <sup>**</sup>	4.20	5.34	0.0010 <sup>**</sup>	6.15	7.79
$a_2^*$	0.0047 <sup>**</sup>	1.45	23.24	<0.0001 <sup>**</sup>	0.53	6.52
$b_2^*$	<0.0001 <sup>**</sup>	1.94	4.80	0.0001 <sup>**</sup>	2.81	7.44

\*, \*\*, <sup>ns</sup> significant at the levels of 5%, 1%, and not significant respectively

are opened, it was determined that the  $L^*$  value before wrapping was 52.7 for the vetch-triticale mixture and 50.2 for the caramba-berseem clover mixture on average for silage and haylage (H1, H2, H3 and TS). It can be seen that the best color values in terms of  $L^*$  are obtained from the H3 application in the study. It was determined that the  $L^*$  values decreased in both mixtures when comparing H3 and TS applications. This value decreased from 47.70 to 43.56 in the case of the vetch-triticale mixture and from 42.50 to 49.85 in the case of the caramba-berseem clover mixture. This is an indication that the storage conditions affect the  $L^*$  value. After opening, these values decreased 45.0 and 44.5 in average for the vetch-triticale mixture and the caramba-berseem clover mixture, respectively. The  $L^*$  value was found to be 28.5 for the vetch-triticale mixture and 31.4 for the caramba-berseem clover mixture, on average, for the degraded haylage (DH) samples. It was found that the average  $L^*$  value of the degraded roughage decreased by 45.6% in the vetch-triticale mixture and by 37.6% in the mixture of caramba-berseem clover according to the initial  $L^*$  value. That means, the degraded samples lost their brightness compared to the other roughage samples. When the  $b^*$  (yellow-blue) value before wrapping was examined, it was determined that this value was 23.3 in the vetch - triticale mixture and 21.0 in the caramba-berseem clover mixture. After opening the silage and haylage, this value increased slightly, reaching 23.9 for the vetch-

triticale mixture and 21.8 for the caramba-berseem clover mixture. This situation can be interpreted as an indication that the  $b^*$  color value is not affected by the storage duration. In the degraded specimens,  $b^*$  decreased by an average of 50% and it was found to be 12.1 and 13.1 for the vetch-triticale mixture and the caramba-berseem clover mixture, respectively. The  $a^*$  (red-green) value was found as 11.4 for the vetch-triticale mixture and 11.9 in the caramba-berseem clover before wrapping, then decreased to 4.1 and 4.7 after opening, respectively. In deteriorated samples, this value decreased to 2.2 and 2.9, respectively. Snell et al (2003) found a relation between applications and color values of  $L^*$  and  $a^*$ . Ball et al (2017) also emphasized that a bright green color can be an indicator of storage conditions which shows good protection during storage. However, statistical mean value comparison regarding the  $b^*$  values did not result in any significant difference. This study also gave similar results.

Table 4 illustrates that in the vetch-triticale mixture, all applications significant at 1% level on pH, ADF, NDF, CP and RFV. In the case of the mixture of caramba-berseem clover, the applications have a significant effect on pH, ADF, CP at 1% level, but at 5% level on RFV and no effect on NDF.

It was determined that the pH value was highest in deteriorated haylage and the lowest in traditional silage. In both mixtures, it was obtained that the best application among haylages was roughage which was left for 55 days fermentation (H3). In terms of

**Table 3- Color changing values according to applications**

Mixture		H1	H2	H3	TS	DH
Vetch- triticale	L <sub>1</sub> *	52.70	50.05	54.85	52.43	53.46
	a <sub>1</sub> *	11.35	11.40	12.00	11.40	10.86
	b <sub>1</sub> *	24.55	24.65	23.85	22.83	21.03
	L <sub>2</sub> *	46.30 <sup>ab</sup>	42.60 <sup>b</sup>	47.70 <sup>a</sup>	43.56 <sup>ab</sup>	28.56 <sup>c</sup>
	a <sub>2</sub> *	6.05 <sup>a</sup>	2.65 <sup>c</sup>	3.50 <sup>bc</sup>	4.43 <sup>ab</sup>	2.26 <sup>c</sup>
	b <sub>2</sub> *	24.75 <sup>a</sup>	22.10 <sup>b</sup>	24.75 <sup>a</sup>	24.00 <sup>ab</sup>	12.10 <sup>c</sup>
Caramba- Berseem clover	L <sub>1</sub> *	50.25	50.30	49.70	51.53	49.25
	a <sub>1</sub> *	10.90	11.65	12.50	12.46	12.10
	b <sub>1</sub> *	20.60	21.90	20.60	21.63	20.60
	L <sub>2</sub> *	39.35 <sup>c</sup>	47.55 <sup>ab</sup>	49.50 <sup>a</sup>	41.83 <sup>bc</sup>	31.40 <sup>d</sup>
	a <sub>2</sub> *	4.70 <sup>b</sup>	4.10 <sup>c</sup>	4.50 <sup>bc</sup>	5.56 <sup>a</sup>	2.90 <sup>d</sup>
	b <sub>2</sub> *	19.35 <sup>b</sup>	22.05 <sup>ab</sup>	24.65 <sup>a</sup>	21.26 <sup>b</sup>	13.10 <sup>c</sup>

Means in each column with the same letters are not significantly different (P<0.05)

**Table 4- Coefficients of variation pH, ADF, NDF, CP and RFV according to mixtures**

Parameters	Vetch-Triticale			Caramba-Berseem Clover		
	P value	LSD <sub>(0.05)</sub>	CV(%)	P value	LSD <sub>(0.05)</sub>	CV(%)
pH	<.0001**	0.12	1.34	<.0001**	0.41	4.26
ADF	0.0003**	4.11	4.58	0.0071**	6.54	7.63
NDF	0.0014**	5.67	5.34	0.3322 <sup>ns</sup>	-	4.74
CP	0.0005**	0.89	5.73	0.0031**	0.93	6.39
RFV	0.0012**	14.55	8.25	0.0372*	15.05	8.65

\*, \*\*, <sup>ns</sup> significant at the levels of 5% , 1%, and not significant respectively

ADF, the H3 application has the lowest value in both mixtures, and this value was highest in the degraded haylage. Shinnars et al (2009) also obtained similar results in their studies. It can be said that the fermentation period has the positive effect on ADF and pH. This may be explained by the increase in the amount of sugar used by Lactic Acid Bacteria in the length of time. When the HP values were compared according to the applications, it was determined that the HP ratio in the caramba-berseem clover was not affected statistically by the fermentation period and remained in the same group. However, the CP ratio for the conventional silage remained low compared to haylages. In the vetch-triticale mixture, the CP

value was slightly increased with the fermentation time. In both mixtures, the CP value was lower in the degraded silage than the other applications (Table 5).

The relationship between color values ( $\Delta L$ ,  $\Delta a$ ,  $\Delta b$ ) and quality parameters (pH, CP and RFV) for both mixtures was determined and graphically presented in Figure 2, 3 and 4. It was determined that  $\Delta L$  and  $\Delta b$  color values and pH, CP, RFV were significantly correlated in the vetch-triticale mixture. The regression coefficients ( $R^2$ ) for  $\Delta L$  and  $\Delta b$  were 0.68 and 0.79 for pH and 0.63 and 0.70 for RFV, respectively. However, for CP these coefficients were calculated lower as 0.40 and

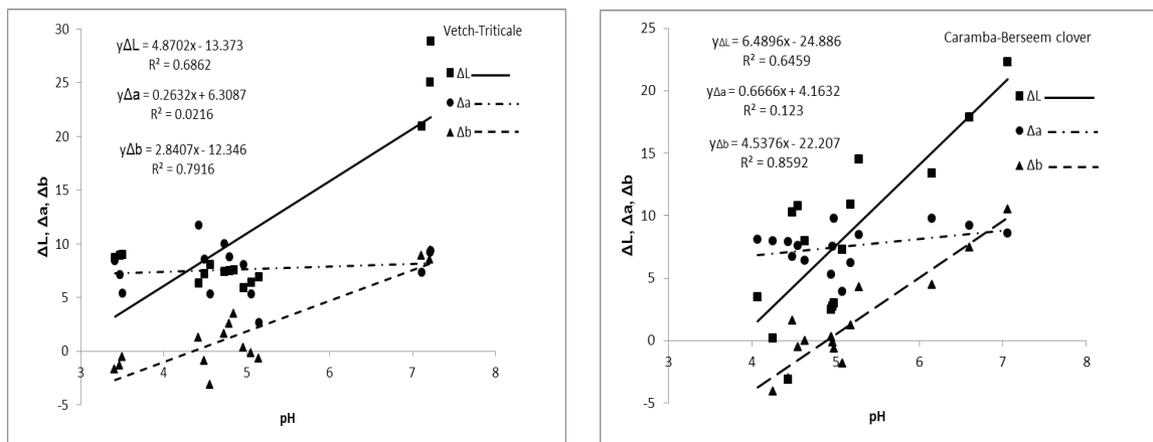
**Table 5- Quality values according to applications**

Parameters	H1	H2	H3	TS	HD	
Vetch-triticale	pH	5.05 <sup>b</sup>	4.79 <sup>c</sup>	4.49 <sup>d</sup>	3.46 <sup>c</sup>	7.18 <sup>a</sup>
	ADF(%)	46.70 <sup>b<sup>c</sup></sup>	49.50 <sup>b</sup>	41.35 <sup>d</sup>	44.06 <sup>cd</sup>	56.36 <sup>a</sup>
	NDF(%)	56.40 <sup>b</sup>	54.60 <sup>b</sup>	52.35 <sup>b</sup>	51.36 <sup>b</sup>	67.03 <sup>a</sup>
	CP(%)	7.90 <sup>bc</sup>	8.80 <sup>b</sup>	10.16 <sup>a</sup>	8.34 <sup>bc</sup>	7.01 <sup>c</sup>
	RFV	93.14 <sup>b</sup>	92.59 <sup>b</sup>	108.34 <sup>a</sup>	106.83 <sup>ab</sup>	67.21 <sup>c</sup>
Caramba-Berseem clover	pH	5.18 <sup>b</sup>	4.96 <sup>bc</sup>	4.25 <sup>d</sup>	4.55 <sup>cd</sup>	6.60 <sup>a</sup>
	ADF(%)	42.30 <sup>b</sup>	43.50 <sup>b</sup>	42.10 <sup>b</sup>	45.16 <sup>b</sup>	55.60 <sup>a</sup>
	NDF(%)	57.50	56.45	56.00	59.53	60.15
	CP(%)	8.62 <sup>a</sup>	8.47 <sup>a</sup>	8.07 <sup>a</sup>	6.94 <sup>b</sup>	6.60 <sup>b</sup>
	RFV	97.30 <sup>a</sup>	97.42 <sup>a</sup>	100.16 <sup>a</sup>	90.52 <sup>ab</sup>	76.38 <sup>b</sup>

Means in each column with the same letters are not significantly different (P<0.05)

0.38, respectively. In the mixture of the caramba-berseem clover, the regression coefficients for ΔL and Δb were found to be 0.64 and 0.85 for pH, 0.14 and 0.12 for CP, 0.28 and 0.37 for RFV respectively. In both mixtures, it was obtained that R<sup>2</sup> which was calculated to determine the level of relation between Δa color value and quality parameters remained very low. It was found that the values of ΔL and Δb were significantly related to pH in both mixtures, while the value of L\* before wrapping decreased gradually after opening, while pH

value increased and RFV decreased. The similar situation was obtained for b\* color value. It has been determined that the best roughage in terms of RFV was obtained from haylages opened after 55 days (H3). In this period the ΔL and Δb values for the vetch-triticale mixture were 7.15-0.9 and 0.2-4.05 for the mixture of caramba-berseem clover, respectively. This shows that when the difference between the values of L\* and b\* before and after fermentation approaches the value of 0, RFV increases.



**Figure 2- Relationship between color changes and pH**

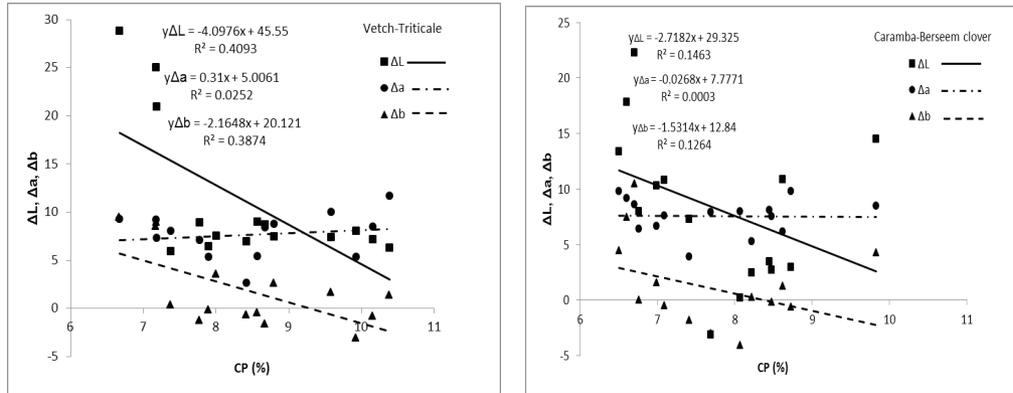


Figure 3- Relationship between color changes and CP

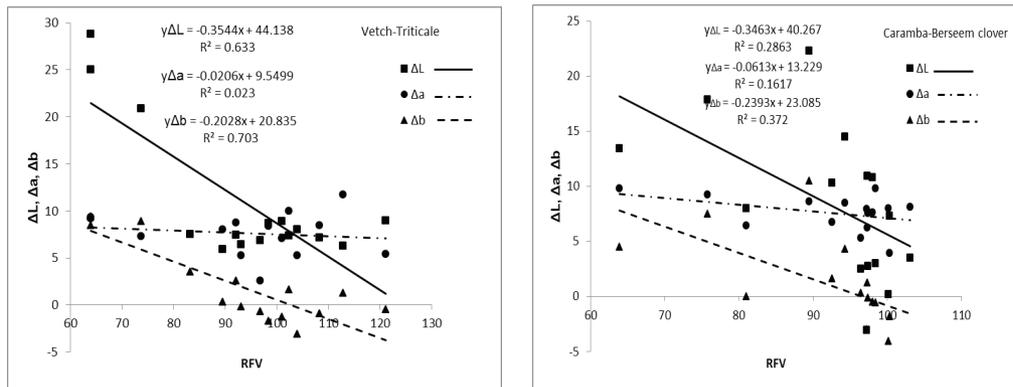


Figure 4- Relationship between color changes and RFV

#### 4. Conclusions

In the study, some quality criteria were tried to be associated with the change of  $L^*$ ,  $a^*$ ,  $b^*$  color values in roughage. It has been determined that these values have an acceptable correlation with pH and RFV in both mixtures. Decreasing the  $L^*$ ,  $b^*$  color values by 50% on average after fermentation can be regarded as an indication of roughage degradation and RFV reduction. From this point view, it can be possible to determine the quality change by following the colors before and after fermentation of roughage. It can be said that this study is important because of showing possibility to have information about the quality of roughages by following the color change. Further, similar studies can be performed on other roughage to obtain color change scales, which can be used for quality estimation.

Abbreviations and Symbols	
CIELAB ( $L^*$ , $a^*$ , $b^*$ )	Color space
pH	Potential of hydrogen
CP	Crude protein, %
RFV	Relative feed value
$\Delta L$	Color change of $L^*$
$\Delta b$	Color change of $b^*$
$\Delta a$	Color change of $a^*$
$L^*$	Lightness
$a^*$	Red-green
$b^*$	Yellow-blue
DM	Dry matter, %
ADF	Acid detergent fiber, %
NDF	Neutral detergent fiber, %
N	Nitrogen
CV	Coefficient of variation, %
P	Probability
LSD	Least significant difference

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