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A Study on the Decay Rate of Vacuum-Packaged Refrigerated Beef Using Image Analysis

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ABSTRACT

In this study the amount of total volatile nitrogen for beef stored at temperature of +4 at 0, 4, 8 and 12 days was measured using image processing techniques. For this matter, the level of this index measured by conventional laboratory methods and then compared with colorimetric parameters of the image processing system. The results indicated that the due to decay, vacuum and non-vacuum samples showed a significant increase in total volatile nitrogen level in all the maintenance days. Samples of non-vacuum meat at the early of second day and samples of vacuum meat at early of the fourth day were over the allowable limits. Colorimetric study of the stored samples showed that due to amino corruption, all samples lost their red and bright colour and tends to darkness. Examining the regression equations between total volatile nitrogen and colorimetric parameters of vacuum meat, the following regression model was obtained:

TVB-N(VAC) = 71.87 -1.57 a* - 1.06 b* -0.60 L* ($R^2_{adjusted}$ = 98.2 $P<0.05$).

Regarding the applicability of the results obtained by image processing of the samples and benefits such as easy and fast process, this technique and predictive models could be used as an auxiliary method to estimate the level of corruption of beef.

KEYWORDS: Image Processing, Red meat, Storage time, Total volatile base nitrogen.

INTRODUCTION

Meat is one of the most important resources of protein. Rich rate of precious proteins-including necessary amino acids for body, minerals such as iron, zinc, different types of vitamins and calorie cause to be arranged in the level of best and perfect foods [1]. This precious nutrition environment made it appropriate for bacteria and other decay factors and would cause on decreasing the durability [2]. Economic and nutritional importance of meat results on wide researches to delay the decay and increase its quality from slaughter to consume. The most important researches includes using gamma exposure technology on beef [3], high pressure technology on ham meat [4], anti-oxidant on ground beef [5] and different packaging methods such as modified atmosphere on beef steaks [6].

The purpose of food packing is to preserve the quality and increase the Retention time from production to consume. It should use as a protective factor for the product against the physical, chemical and biological damages [7]. In packing the fresh meat, the most important points from the opinion of producers and consumers includes microbial pollution prevention, delaying the decay, possibility of some enzymes activity which causes friability of the meat, prevention of weight loss and also creation of oxymyoglobin pigment which results on desired red color on red meats. High surfaces contacting oxygen in packing the meat, increases the risk of oxidation of fats exist in the meat and could be as an important deterrent factor of odor and color of fresh meat [8]. Nowadays, vacuum packaging is widely using as a useful method for packaging the fresh meat [1]. Limiting the oxygen amount in packaging process will decreases the growth of bacteria and decay factors in the meat. The

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vacuum packaging is a kind of modified atmosphere packing which just the oxygen removes from the meat environment and meat places in an environment with the minimum level of oxygen [9]. The main effecting factors on determining meat quality by consumer include the meat appearance, tissue, odor and taste. Among the effecting factors on appearance of the meat, color has the most important on consumer purchasing [8]. The meat color is the most dominant factor in the quality of the meat which is caused by the presence of color pigments such ascytochromes, catalase, flavins and myoglobin in the muscle.

Among the above-mentioned pigments, myoglobin is the major color pigment of the meat which the color of the meat is dependent to this pigment. The Myoglobin level in muscle could be depends on various factors such as the species, age and animal activity. Color changes due to the storage of meat depend on the myoglobin in the raw meat, storage conditions and type of packaging [1]. Myoglobin could exist in three forms of Deoxymyoglobin, oxymyoglobin of metmyoglobin in the red meat.

Differences in meat color originate from the chemical changes in these pigments. As in the presence of the air, Deoxymyoglobin absorbs an oxygen molecule by its iron and changes into the red oxy-myoglobin [8]. Over time and under atmospheric conditions the amount of oxy-myoglobin increased and changes into Matt myoglobin which is the reason of the meat brown color. At this point, the meat is inedible. The rate formation of Matt myoglobin depends on different factors such as storage temperature, the meat PH level and the microbiological level of the meat which increasing in each of these factors would increase the rat of the color change [1].

Color forms due to the collision of light wave with the wavelength in the range 390 to 760 nm to the human retina and transmission of related messages from the retina to the optic nerve cells in the brain and its reciprocal response. While it speaks about the color measurement, the purpose is to express the color position in its three-dimensional space coordinate. Color is a very important characteristic in determination of the quality of the nutrition and its measurement can help to identify compounds of the food. This can be used to check the quality [10]. The human eye is capable to detect the negligible color changes (even changes in the brightness). Unfortunately, the color evaluation of human eye is not general and may be very different depending upon the assessor person. So it recommends to use the color test devices [11, 12]. Nowadays color spaces and numerical values are using to create, express and specifying of color in three-dimensional space. According to application, different color spaces including RGB , CMYK , L*a*b* use to determination of the color of the food. The L*a*b* space have the most complete and uniform color range relative to other spaces.

In Euclidean viewpoint, the distance between two color points in L*a*b* model is approximately equal to the distance the human eye recognize it. This space is independent form the coordinate and color describes as a constant number, so it mostly uses in the food industry researches [13, 14]. Such studies in order to assess the meat quality by colorimetric method includes studies on changes in the color of raw meat in beef [15], pork [16] and fish meat [17]. Considering the importance of spoilage fresh cow meat and discoloration caused by decay during storage and the effect of the vacuum packaging on its color, color changes in both conventional packaging and vacuum packaging during storage at refrigerator temperature is investigated. Also according to importance of fresh meat chemical spoilage, the levels of total volatile base nitrogen is measured parallel to the color change. The aim of this work is to achieve a predictive regression model to predict this index by means of parameter which obtained by colorimetric analysis.

Nomenclature

L* = lightness in L*a*b color space
a* = redness in L*a*b color space
b* = yellowness in L*a*b color space
R = coefficient of correlation
$R^2_{Adjusted}$ = modified R-square
ANOVA = Analysis of variance
TVB-N = Total volatile base nitrogen

MATERIAL AND METHODS

Preparation of samples

15 big piece of fresh meat were purchased from a beef supermarket of Mashhad city (East of Iran). In the laboratory of Ferdowsi University of Mashhad, pieces flaked in the 15 x 15 cm segments and 24 pieces were randomly selected. 12 Number of pieces of selected meat packed using vacuum machine and the rest were packed normally. Then specimens were coded and were kept for 12 days at 4 centigrade temperature. At 0, 4, 8 and 12 days after this process, vacuum and non-vacuum samples tacked out of the refrigerator, and after photographing by the imaging system, the total amount of volatile nitrogen was measured.

Measuring of total volatile base nitrogen

Total volatile basic nitrogen (TVB-N) was determined in a distillatory system, as described by Connell, 1975 [18].

Image processing system

Imaging system

Imaging system was formed of four components including lighting system, cameras, hardware and software. Four fluorescent lamps were used to provide room light shooting. The lamps direction was set in a way which has a uniform and adequate light intensity on the sample and also the minimum light reflection occurs from the sample's surface.The picturing room walls and its background was covered by matte black color. After placing the sample in the devised room, the picturing process was done using a Canon camera which was connected to a computer. In the next step, the images were saved in JPEG format and the obtained images were processed using Image J software [19].

Image processing

Obtained Photos were opened in the Image J software. Photos were taken in the RGB space which due to their dependency on the used camera, photos was transformed to the L*a*b* independent space. The data were measured and expressed based on the L *, a * and b * system which based on the proposal of the International Commission on Illumination, each one represents brightness level, redness, and yellowness of the samples, respectively which is considered as a universal standard [17, 20].

Sensory evaluation

The level of color change in the meat was evaluated by 7 panelists. Sensory evaluation was performed by two tests. The first test was about the color change during the storage period observed by Panelists whicha 8 unite scale was used which is shown in the Table 1. For the purpose of acceptability of the samples color, another scale was used which is shown in Table 1 of section B. Sensory evaluation was performed under the same conditions of light, temperature and humidity which these conditions were fixed in all of the tests [21].

Statistical Analysis

One-way ANOVA test was used for comparison of variance and Duncan test was used for studying the presence or absence of significant differences between treatments through SPSS software version 18 ($p<0.05$). Experiments were conducted in a completely randomized design (CRD) with three replications. In order to present a model, the regression relation was investigated between the decay parameters level and the color indices. To study the correlation between the indices, the Pearson correlation test in 5% reliance level was used.

RESULTS

Total volatile base nitrogen

Results of the measurement of total volatile base nitrogen during storage period are shown in Figure 1. Statistical test of analysis of variance showed that for vacuum and non-vacuum meat within twelve days, at refrigerator temperatures, there was a significant increase in the mean values of total volatile base nitrogen ($P < 0.05$). The total volatile base nitrogen level in the first days of storage at refrigerator temperature was 18.33 and 17.5 mg for vacuum and non-vacuum meat, respectively. Increasing the storage time results in significant statistically increasing of the total volatile base nitrogen in at all days. However, this statistically increase in the vacuum meat was significant from the fourth day onwards and reaches 52.4 mg in twelfth day. The highest difference between treated and non-treated samples was in the eighth day which was about 25 mg. Studying on the regression equations of the change of total volatile base nitrogen in vacuum and non-vacuum meat relative to time results in the following equations. TVN (vacuum)= 19.04 -1.53DAY + 0.35 DAY^2 ($R^2_{adjusted}$= 95.91 ; $P<0.05$) ; TVN (non-vacuum)= 12.61+ 5.008 DAY ($R^2_{adjusted}$= 94.1 ; $P<0.05$).

Colorimetric parameters

Color changes of beef stored at refrigerated temperature analyzed by measuring the colorimetric parameters. The results of the measurement of the changes in colorimetric parameters are shown in figures 2, 3 and 4. Concurrent with the increase of storage time and spoilage, the L* lighting parameter, has a significant decrease in both vacuum and non-vacuum samples ($P<0.05$). The amount of L* in the first day of storage at refrigerator temperatures was 31.7 in both vacuum and non-vacuum meats. The statistically decrease in the rate of L* for non-vacuum meat was observed from the first day and hits 21.61 in the twelfth day. But this significant decrease began four days later for vacuumed meat, and finally reaches to 24.9 in the last day ($P<0.05$). The initial values of the a* redness index in the vacuum and non-vacuum meat, were20.72 and 19.74, respectively. These values significantly decreased simultaneously with the declining in the L* level and durability time with a tendency towards negative values. The amount of redness parameter of the non-vacuum and vacuum meat in the last day was observed 5.70 and 3.7, respectively which represents a significant reduction in the amount of both meats ($P<0.05$).Deviation in the yellowness parameter (b*) as the two other L* and a* parameters showed a significant decrease towards negative values in both samples ($P<0.05$). Reduce speed of b* in non-vacuum sample was much higher than the vacuum sample which the highest rate was observed in the first four days. The initial amount of this index in non-vacuum meat was 1.48.After four days it reaches to-0.66 which was an outstanding reduction but in next days no significant changes were maintained in the storage period ($P<0.05$).In vacuum meat a significant statistically decrease was observed in all storage days ($P<0.05$).

Evaluation of the coefficients of correlation between the colorimetric parameters and amounts of total volatile base nitrogen is shown in Table 2.According to the presented table, the highest correlation between a* of the vacuum meat and total volatile base nitrogen was observed in the same meat which showed a significant negative correlation ($P<0.05$).

The results of the analyzing the regression relation between the rate of change of total volatile base nitrogen and colorimetric parameters in non-vacuum and vacuum respectively indicated the below equations: TVB-N(N-VAC) = 132.97 -1.54 a* - 1.15 b* -2.63 L* ($R^2_{adjusted}$ = 91.6 $P<0.05$) : TVB-N(VAC) = 71.87 -1.57 a* - 1.06 b* -0.60 L* ($R^2_{adjusted}$ = 98.2 $P<0.05$)

Sensory evaluation

Sensorial color tests concluded to identify the changes in the color and its acceptability. Acceptable test results (Figure 5) shows that acceptance rate decreased with increasing storage time which this decrease in non-vacuum meat is faster than vacuum meat. Also colorimetric test results shown in Figure 6 indicate that the red color of meat changes to dark red during the storage period.

The Maximum speed of this change was observed between the fourth to eighth days for the two samples. Deviations conducted from the two tests were all statistically significant (P<0.05).

Table 1 - Table used in the sensory test

PART A	PART B
1 = Extremely bright cherry-red	**7 = Like very much**
2 = Bright cherry-red	**6 = Like moderately**
3 = Moderately bright cherry-red	**5 = Like slightly**
4 = Slightly bright cherry-red	**4 = Neutral**
5 = Slightly dark cherry-red	**3 = Dislike slightly**
6 = Moderately dark red	**2 = Dislike moderately**
7 = Dark red	**1 = Dislike very much**
8 = Extremely dark red	******************************

Table 2 –Important correlation coefficients between the levels of total volatile base nitrogen and colorimetric parameters

		L*		a*		b*	
		Non-Vacuum	Vacuum	Non-Vacuum	Vacuum	Non-Vacuum	Vacuum
TVB-N	Non-Vacuum	-94.86**	-67.139**	-94.46**	-92.24**	-63.81**	**-95.02****
	Vacuum	-84.82**	-78.26**	-78.36**	-98.63**	-50.71*	**-82.39****

*Indicates significant difference at the 5% level.
* *Indicates significant difference at the 1% level.

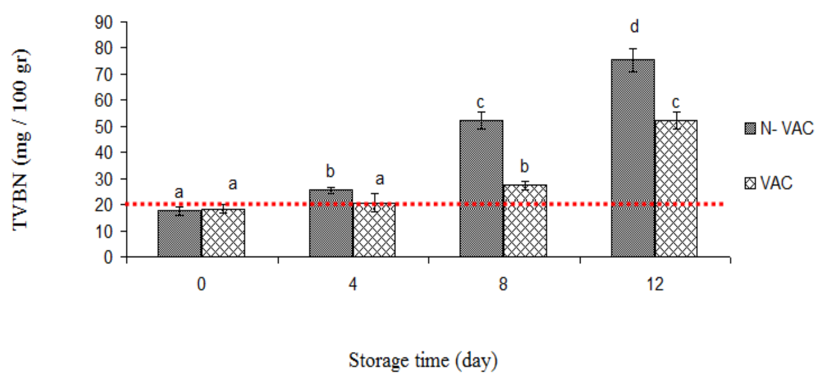

Figure 1 - Mean (± SD) total volatile base nitrogen (in mg N per 100 g of meat) for stored beef at different storage days at refrigerator temperature (n=3)

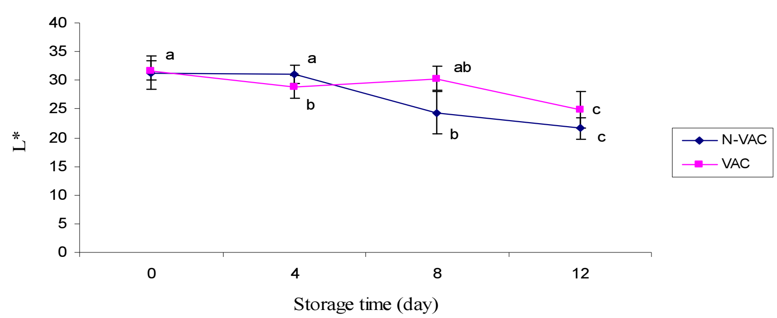

Figure 2 - Mean (± SD) change rate of the L* in the beef stored at different storage days at refrigerator temperature (n=3)

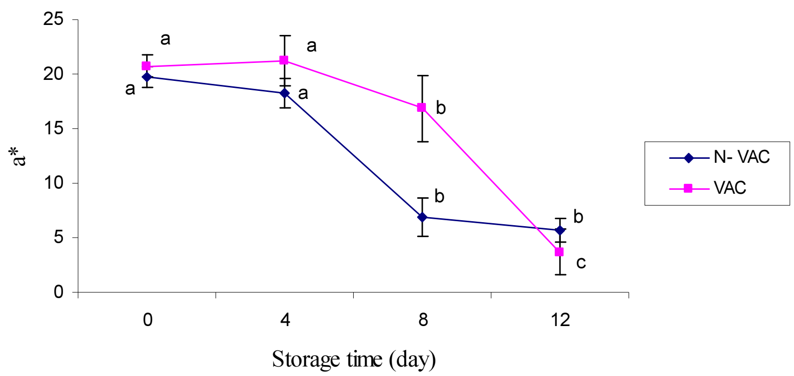

Figure 3- Mean (± SD) change rate of the a* in the beef stored at different storage days at refrigerator temperature (n=3)

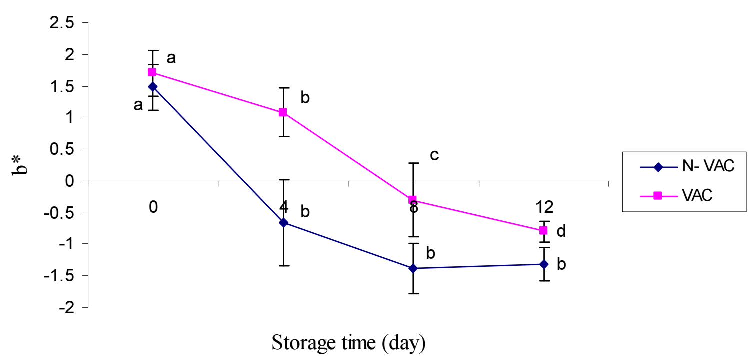

Figure 4 - Mean (± SD) change rate of the b* in the beef stored at different storage days at refrigerator temperature (n=3)

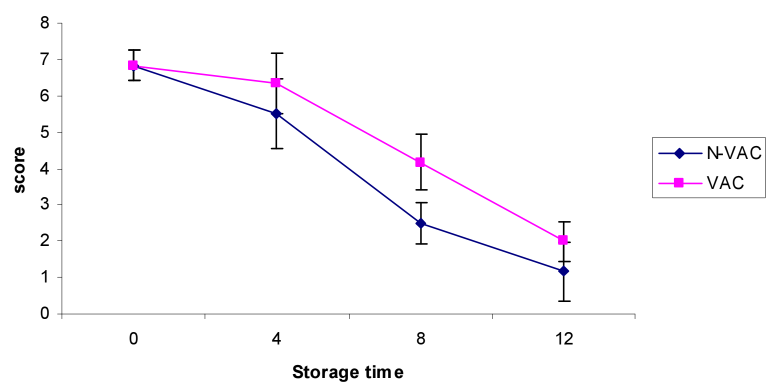

Figure 5 – The average of colour score (from acceptance prospect) for beef stored over different storage days at refrigerator temperature (n=3)

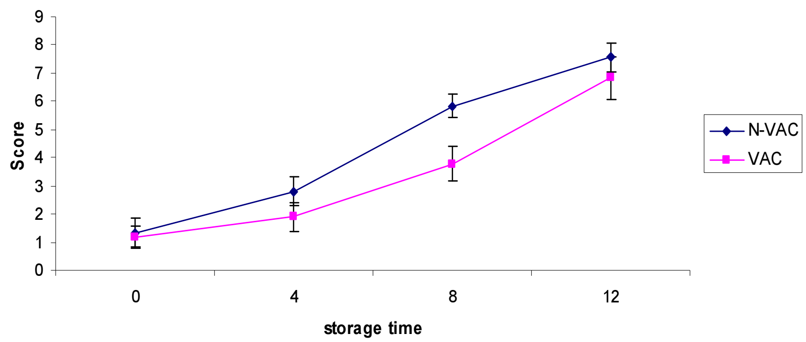

Figure 6 - The average of changes in the colour score for beef stored over different storage days at refrigerator temperature (n=3)

DISCUSSION

Total volatile base nitrogen

The total volatile base nitrogen includes compounds such as ammonia, dimethyl amine, tri methyl amine and volatile nitrogen compounds which are formed by decomposition of other ingredients and uses as an index to assess the degree of tissue destruction and corruption during the early storage days [2]. According to figure 1 a significant increase in the levels of total volatile base nitrogen was observed, for both vacuum and non-vacuum beef. The level of total volatile base nitrogen in the first day of storage at refrigerator temperature in vacuum and non-vacuum meat was 18.33 and 17.5 mg, respectively. Different values for the initial level of total volatile base nitrogen is reported which among them 18.3 mg for beef [22] and 7 mg for minced Beef cattle [2] could be noted.

With increasing the storage time, the levels of total volatile base nitrogen in both groups increased. This speed up on production could be due to increase in proteolysis process induced by enzyme activities and bacterial decomposition of the meat in maintaining time [23, 24]. The Increase in the growth rate of total volatile base nitrogen was more in non-vacuum meat. It is due to the effective role of hypoxia to inhibition of important aerobic bacteria which destroys the meat including "Gram-negative psychotropic *Pseudomonas* species" [25]. This notion suggests a close relation between the amount of total volatile base nitrogen to bacterial load and reducing the survival time. The proposed acceptable limit is declared to be 20 milligrams per 100 grams of beef which based on these standard, non-vacuum samples of meat in the second day and vacuum samples in the fourth day were over the limit. Short shelf-life of these samples shows the inappropriate initial quality of raw meat bought from the store. However, in some resources, the beef contains 30 mg of total volatile base nitrogen will not consider inappropriate. Which based on these resources, the shelf-life of this study samples could be significantly increased.

Studying the relations between the amount of total volatile base nitrogen and storage time at refrigerator temperature, indicates a linear relation (R^2_{adjusted} = 94.1; $P < 0.05$) in the non-vacuum samples which could be used to estimate the amount of total volatile base nitrogen of beef. But the nonlinear relation between total volatile base nitrogen and holding time was not statistically meaningful (R^2_{adjusted}= 95.91; $P<0.05$).

Increasing trend of determination of total volatile base nitrogen was consistent with the results of other investigators [2]. So that the amount of this index in beef kept in the refrigerator increased from7.37 to 20.35 mg nitrogen per hundred grams of meat for blank sample within two days of receipt.

Colorimetric parameters

In recent decades, the use of computer vision system enhanced to explore some meat quality parameters, especially in the measurement of the meat color which is due to its advantages including high speed and non-destructive properties [26]. The color of fresh meat if affected by various factors such as animal genetics, consumed dietary and growing conditions. The final color of fresh meat is made by the main pigment of the meat, myoglobin. Differences in the levels of this pigment would cause a change in the redness of the meat [21]. Colorimetric analysis of used meat indicated the amount of L*, a* and b* for the non-vacuum meat, respectively, 31.7, 19.74,1.48. These values for the vacuum meat are31.7,20.72 and 1.7, respectively. These numbers represents the color of fresh meat used in this study. Concurrent with the increase in the level of total volatile base nitrogen,the brightness parameter L* had a significant gradual decline and on the twelfth day, reached to the lowest level in both groups.

In the early days of storage, the level of L* in the vacuum sample had fewer changes relative to non-vacuum sample which indicates the effect of presence of oxygen on the stability of meat's surface brightness during storage. The reduction of L* interprets that the meat is getting darker. With the increase in total volatile base nitrogen and decrease in the L* during the twelve storage days, a* index also has a significant decreased. But the change in the redness of non-vacuum red-meat was more severe, and greatly reduced its rate. In the vacuum sample due to lower levels of corruption a rising from the presence of the amine and the absence of oxygen, the amount of tissue damage and discoloration of meat was fewer and the rate of redness decrease has reduced. Similar to the L* and a*, b* values showed an inverse relation with the amount of total volatile base nitrogen which represents a decrease in yellowish of the meat during the maintaining time. According to the results of the colorimetric analysis of samples, the color of red meat gradually tends to less brightness, Yellowness and redness during storage in the refrigerator. As the factors of this color change the protein denaturation of meat could be pointed. This causes loss of muscle water and changes in the color of meat's surface [27].

Decreasing rate of L* observed in this study is not in consistent with results of other studies carried on red meat. So that the brightness of frozen semimembranosus beef that had been stored for 7 days at refrigerator temperatures declined from40.86 to 40.68 which was not statistically significant [28].

Studies on a* parameter for beef stored at refrigerator temperature for 7 days showed an increasing trend similar to this the Investigation [29]. The observed reduction trend in b* values was consistent with the results of the measurement of this parameters on (ground beef) stored at 4 +. So the value of this parameter in the control sample dropped from 23.7 to 17.20 within 7 days [30].

Meat color change during storage is affected by factors which are caused by changes after death such as changes in the pH, fatty acids, proteins and pigments, the level of unbound water and physical structure of the meat [21, 31]. The color change caused by corruption could not be limited to specific factor, and a set of physical, chemical and biological factors, contribute to this change. Also correlation coefficient showed that the a* parameter of vacuum meat has a higher correlation with the amount of its total volatile base nitrogen. With studying on regression relations between the amino index of corruption and colorimetric parameters, multivariable predictive models were obtained. The results of regression equations presented in this study indicate that the amount of total volatile base nitrogen can be estimated by measuring the colorimetric parameters.

According to obtained definition of coefficient, this method could be used as a new, rapid, and low-cost method comparing to other routine laboratory procedures to estimate the quality of beef.

Sensory evaluation

According to the results shown in Figure 5, the rating of desired factor in the sensorial evaluation has a significant decrease during storage ($P<0.05$). According to figure 6, the initial cherry-red color of fresh meat gradually tends toward darkness and redness reduction. The results observed by Panelists were in full compliance with the results measured by colorimetric parameters.

Because according to panelists observations during the storage time, the meat redness tends to intense darkness. It can be due to an increase in total volatile base nitrogen resulting from the corruption or the production of dark meat myoglobin from cherry color oxy-myoglobin during the oxidation process [8].

Conclusion

In this study, the amount of total volatile base nitrogen of beef stored at a temperature of +4, maintained for 0, 4, 8 and 12 days were measured by image processing technique. Thus the amount of this index measured by conventional laboratory methods and then compared with colorimetric parameters obtained from image processing system. The results indicated that the beef lost its brightness due to amino corruption and tends to more darkness. The decay rate and the intensity of the color change in the vacuum meat were slower than non-vacuum meat which indicates the absence of oxygen in the reduction of spoilage. The parameter a* was the one with the most observed changes in colorimetric parameters, which its level within twelve days in vacuum and non-vacuum meat declined, 17.02 and 14.04 units respectively. Examining the regression equation between total volatile base nitrogen and colorimetric parameters of vacuum meat, logistic regression model of TVB-N(VAC) = 71.87 -1.57 a* - 1.06 b* -0.60 L* (R^2_{adjusted} = 98.2 $P<0.05$) was obtained. Due to the mentioned advantages, such as the independency from laboratory materials and equipment, it is quick and easy procedure and the applicability of the results of image processing of samples, this technique and obtained predictive models can be used as an adjunct method to estimate the incidence of beef corruption.

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