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THE RELATIONSHIP BETWEEN NITRITE CONCENTRATION AND COLOUR PARAMETERS DURING STORAGE OF MEAT PRODUCT - *SUMMER SAUSAGES*

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Abstract: *The purpose of this paper is to assess the meat product quality (especially summer sausage type) during storage based on relationship between nitrite concentration and colour parameters. Nitrites are used for conservation of meat products, being responsible for colour characteristics, formation of distinct flavours which allows the differentiation from nitrite-free products. It also prevents and controls the oxidation of lipids and serves as antimicrobial ingredient composition by it self or through synergy with other ingredients. Nitrite reacts with myoglobin through nitrogen monoxide resulting pink-red nitroso-myoglobin compound associated by customers with a tasty and nutritious food.*
Concentrations of nitrites were quantified by molecular absorption spectrometry method using Griess reagents. The colour reflectance of meat products was focused on determination of L^*, $a^*$and b^*. Also, pH was measured by applying the potentiometric method and moisture content of the meat product was determined by drying in the oven, as they can influence both the colour and nitrite concentration.
*Our results show that during storage nitrate concentrations did not differ greatly, and the colour does not have a major influence because it remains pink – red. Measurements of colour parameters showed that during storage components L * and b * registered slight decreases and chromatic component a * a significant difference respectively.*

Keywords: *Griess method, colour reflectance, quality of meat products.*

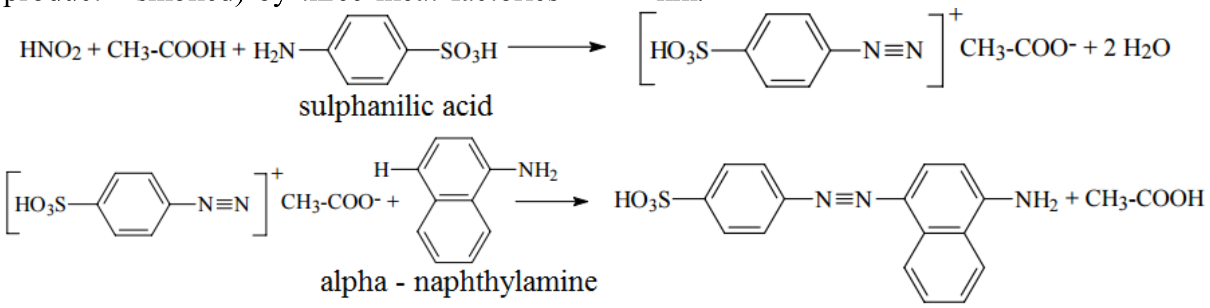
1. Introduction

Nitrites and nitrates are added to meat products for technological and antibacterial reasons: (1) act as fixers of color and prevents meat browning [1], (2) help to development flavor [2, 3], (3) have antioxidant properties, which hampers oxidation of lipids responsible for the rancidity [4 – 7] and (4) protection against the spreading of *Clostridium botulinum* [8, 9].
Salting mix which include nitrites is added in meat products for maturing red obtained after a number of reactions, until *NO* - myoglobin (Fe^{2+}) is formed [10]. The red system of cycle *NO* - porphyrin exists in meat products heated to 120°C, although by heating *NO* - myoglobin of moiety the protein is denatured. An advantage for the customer is that the thermally stable red color will be changed to alter bacteria, therefore, the consumer will recognize alteration by changing the color [11].
As an unintended consequence of maturation nitrite formation is discussed much in literature *N* - nitrosamines (NA), which could be carcinogens [12 - 21] and therefore the controlling and checking of the added dosage is compulsory. In order to secure the meat products quality and safety for customers the addition of nitrates

and especially that of nitrites is strictly monitored. The maximum admitted quantity of these additives is determined by the law [22, 23].
The purpose of this paper is to assess the quality of the meat product - *summer sausage* type during storage by analyzing the relationship between nitrite concentration and colour parameters.

2. Matherials and Methods

They were analyzed samples of meat products (*sausages summer* - cooked product - smoked) by three meat factories in Suceava. The analyzes of the meat products have been made on the 1st day, 7 days and 15 days using the product provided by the local manufacturers.
Determinations of nitrites content were performed by molecular absorption spectrometry method using Jenway spectrophotometer, model 6400. Nitrites were determined based on their diazotization reactions, in acid medium, with sulphanilic acid and subsequent coupling with alpha - naphthylamine, which results in a complex with red color, which measure the absorbance at λ =520 nm.



Nitrite content was calculated from the standard curve prepared with standard sodium nitrite solution (1 ml working standard solution = 0.001 mg NO_2^-), as the standard method (Griess). The curve equation obtained is shown in equation 1:

$$y = 0.0668x – 0.0521, R^2 = 0.9985. \quad (1)$$

Proteins were eliminated from samples by heating at temperatures of 60ºC - 70ºC for 20 minutes and using solutions of potassium ferrous cyanide 10%, zinc acetate 22%, considered to be the most efficient.
In order to determine the moisture content of summer sausage samples we used the drying method oven until a constant weight [24], and the loss of weight is calculated with equation 2:

$$water\ (\%) = \frac{G_1 - G_2}{G_1 - G} x100 \quad (2)$$

where: G – vial mass (g), G_1 – vial sample weight before drying (g) şi G_2 – vial sample mass after drying (g).

The color is measured using reflection spectrum of a sample and comparison with a standard illuminant. The amount of light energy from the sample reflects is manipulated and reduced to tristimulus values X, Y and Z. These values correspond to the physiological response of the three types of color receptors in the human eye. X, Y and Z values are combined into the uniform color space values: L^*, a^* and b^* [25]. The *Chroma*, considered a quantitative attribute of coloring, is used to determine the difference degree of the hue angle (h^*) in comparison with a grey color of the same luminosity. The description of a color using the hue angle attribute refers to how humans perceive an object's color - red, orange, green, blue, etc. The 0º or 360º angle represents the red hue, while angles of 90º, 180º and 270º represent yellow, green and blue hues [26, 27]. Within the study, the HR 4000 CG-UV-NIR spectrometer (Ocean Optics Inc., Dunedin,

FL) which is based on the reflection property of samples was used. In this respect, a scanning with 0.025 nm resolution has been carried out for the entire wavelength spectrum ranging from 200 up to 1100 nm. A tungsten halogen light source (UV-VIS-NIR Light Source DH-2000, Mikropack) was used. The experimental data was displayed and stored using the operating software OOI Base32 including OOIColor from Ocean Optics. The light from Light Source DH-2000 was focused to the sample through an array of optical fibres (QR400-7-UV/BX, Ocean Optics Inc., Dunedin, FL). Reflectivity was measured as against a reference standard – WS- Diffuse Reflectance Standard [25].
All the equipment used in this work belongs to the Food Safety Research Centre of the Faculty of Food Engineering, Ștefan cel Mare University of Suceava, Romania.

3. Results and Discussion

Nitrates and nitrites are used in meat curing process in order to maintain its red. Myoglobin - meat pigment and hemoglobin - the blood pigment that reacts with nitrogen oxide which is the degradation product of nitrite and nitrate, forming nitrozomioglobina (*NO-Mb*) and nitrozohemoglobina (*NO-Hb*), two pigments salting. The pigments have a high stability over time and under the action of heat treatment are formed nitroso-chromogens (nitroso-miochromogen and nitroso-hemochromogen) by denaturing the protein part of the pigments, ie globin.

The formation mechanism of nitrosopigment is [28]:

$NaNO_3 + 2H \rightarrow NaNO_2 + H_2O$

- reduction bacterial

- reduction due to their muscle enzymes

$NaNO_2 \xrightarrow{pH\ acid} HNO_2 + NaR$

$2HNO_2 \rightarrow NO + NO_2 + H_2O$

- reductants meat

- or added acidic pH, reducing bacteria

$NO + Mb\ (Hb) \rightarrow NO\text{-}Mb$ *sau* $NO\text{-}Hb$

From the reactions results that nitrate has no direct effect coloring meat, but it serves as a source of nitrite. Denitrifying bacteria secrete nitrate reductase and thereby the conversion of nitrate to nitrite at pH values greater than 5.8. It can be considered and action reductases own muscle tissue of the meat on the conversion of nitrate into nitrite [29].
Following the test results obtained on the first day it was found that the three producers have complied with the provisions of the legislation which stipulates a maximum of 7 mg/kg sodium nitrite and 45 - 50% humidity. During storage we have changed organoleptic characteristics, the product dries significantly and so was recorded and a change in the content of sodium nitrite. As can be observed from *Figure 1* it is evident that the sodium nitrite quantity increased in all three samples analyzed during storage, with the decrease in moisture content and modification of the organoleptic characteristics. The results obtained in this study are consistent with those in the field, similar results were obtained by [30].

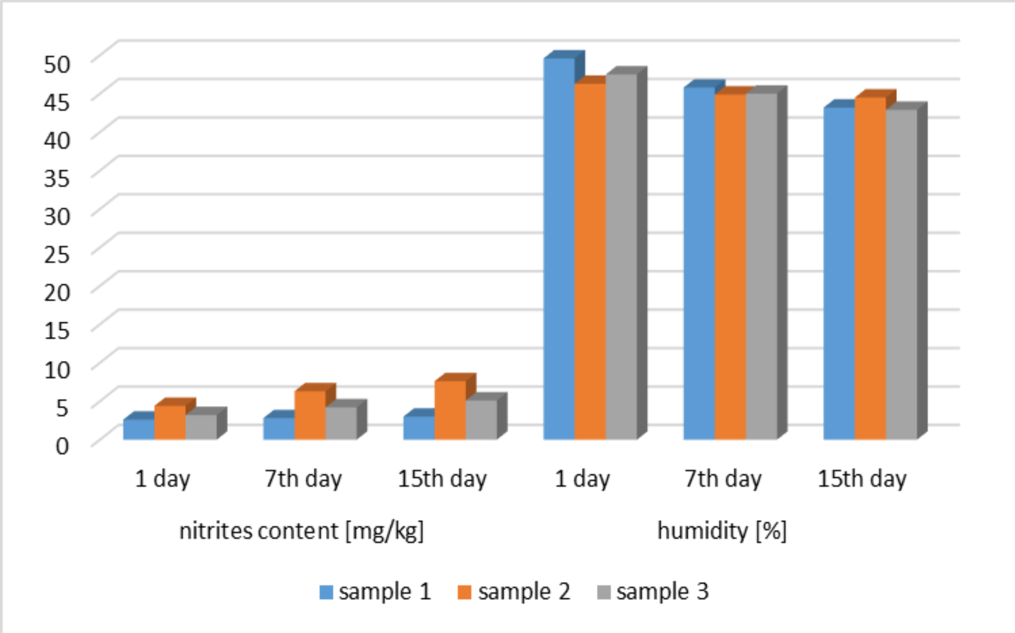


Fig. 1. Variation content of nitrites and humidity in three samples during storage

Immediately after salting nitrate it was observed that at the surface, the meat has bright red color, while in depth the meat color is red purple. An occurrence of nitrite and salting pigment that forms in the air oxidizes and becomes a gray color. In a more advanced stage meat is red and has an acid reaction. Nitrite disappears immediately and has no effective action on the formation of color [29].
Color evaluation is more than a numerical expression. Color is an important quality attribute in food industry and it influences the consumers' choice and preferences. The food color measurement can be used as indirect quality indicator of other attributes such as: flavor and pigment content [27, 31]. The aspect is one of the most important sensory attributes of fresh and processed food. The color of food is the first quality parameter appraised by consumers and it is momentous for product acceptance. Food aspect, greatly determined by color, is the first sensation that the consumer perceives and uses as a tool to accept or reject a certain type of food. Food visual aspect has a strong influence on consumer's opinion on food quality. Color can be correlated with other quality attributes such as: sensory, nutritional and visual or non-visual defects and helps control the products' quality. Color is a perceptual phenomenon which depends on the observer and on the color observing conditions. L^*, a^*, b^*, *hue angle* (h^*) and *Chroma* parameters for the samples subjected to the assay analysis were measured.
Lightness, *Figure 2*, all three samples showed values similar to the analysis of the first day, while after seven days of storage under refrigeration have made some changes in color parameters. Thus was observed a slight decrease in lightness, color becomes darker sausages summer samples, and 15 days after the change was very visible parameters degradation products due to changes. L^* parameter recorded the lowest values observed since the closure sharp color products.

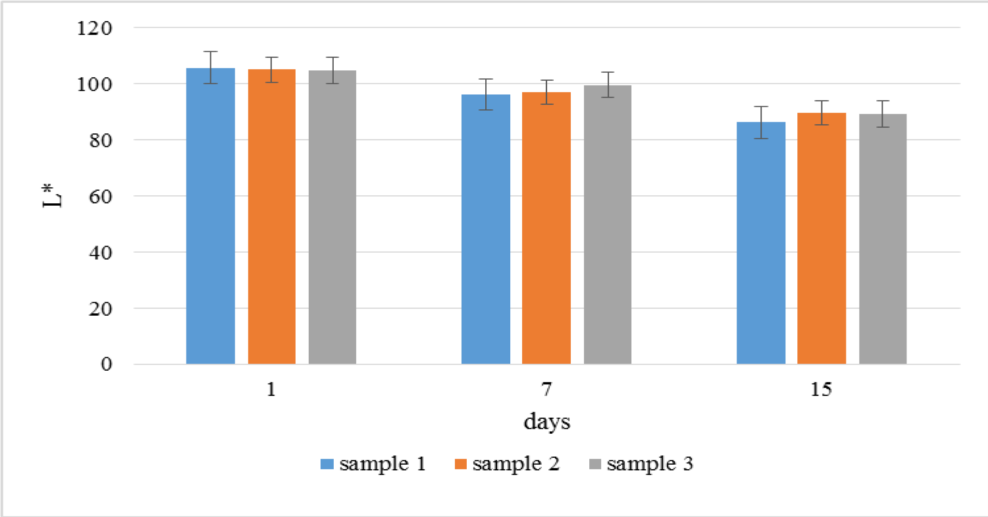


Fig. 2. Variance based on the retention period of lightness

In the case of a^* parameter in the first day the values obtained from the three samples were negative, the lowest value obtained for sample 1 and sample 2 at the most, as shown in *Figure 3*. Darkening, which was observed after 7 days of storage, it was also reflected in the change of the parameter values a^*. For all the evidence is on the rise and so when determining all values were positive, the most visible change is observed in sample 3. After 15 days of storage in a refrigerator, sample 1 and sample 2 had similar values to a^* and the increase being higher in sample 1, while for sample 2, the value in the seventh day of storage the difference was small. Thus, during storage organoleptic characteristics of the products have changed, the most visible change being the darkening pink initial samples of the product, sausages summer, and after expiry acquiring a dark red.

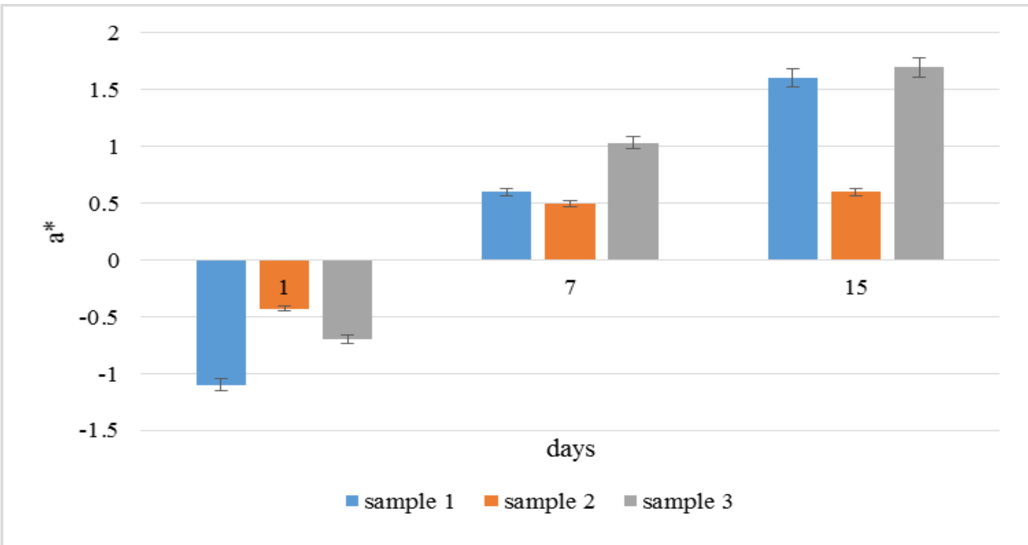


Fig. 3. Variance based on the retention period of a^* parameter

Another parameter which describes the b^* color of a product whose variation in time is shown in *Figure 4*. The values of b^* were positive for the first two samples analyzes from day one, but with significant differences, because for the first sample value is much higher, while the value for the third sample is slightly negative with a value of - 0.4. The b^* value has dropped for all samples after seven days of storage; the most significant decrease observed for the samples 1 and 3, resulting in a negative value. Following the intensification of degradation processes for all 3 samples b^* parameter has a significant decline, all values are negative.

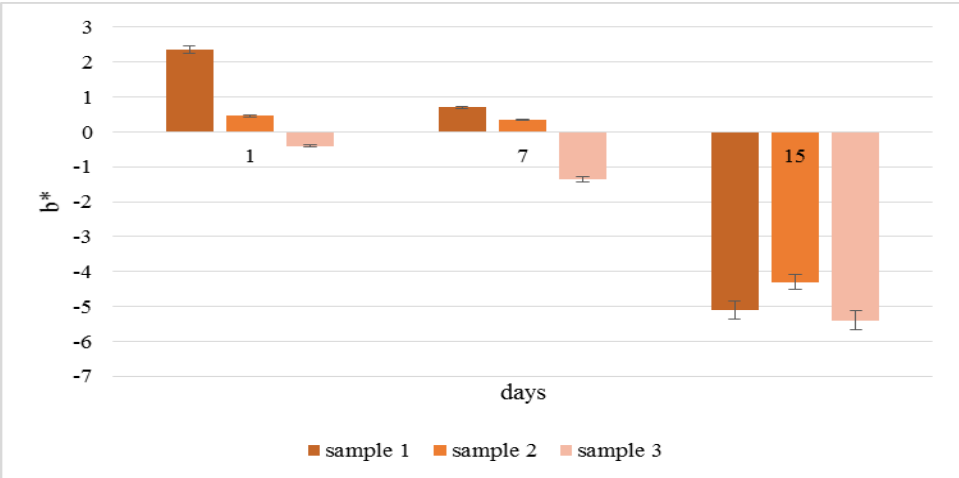


Fig. 4. Variance based on the retention period of b^* parameter

The variation of quality of the color attribute, *Hue angle*, is represented in *Figure 5*. A larger angle indicates a less yellow in the sample. Sample 1 on the first day of storage had the largest angle; indeed not visible yellowish hue. At the opposite pole sample 3 yellowish hue is observable with the naked eye. After seven days the angle register with a fall in the first two tests, the most visible being the sample 1. For sample 3 is a slight increase angle. After 15 days of storage yellowish nuances come into sharper focus, registering approximately equal to three samples. While decreasing the angle may connote a rancid fat [32] which causes the yellow color.

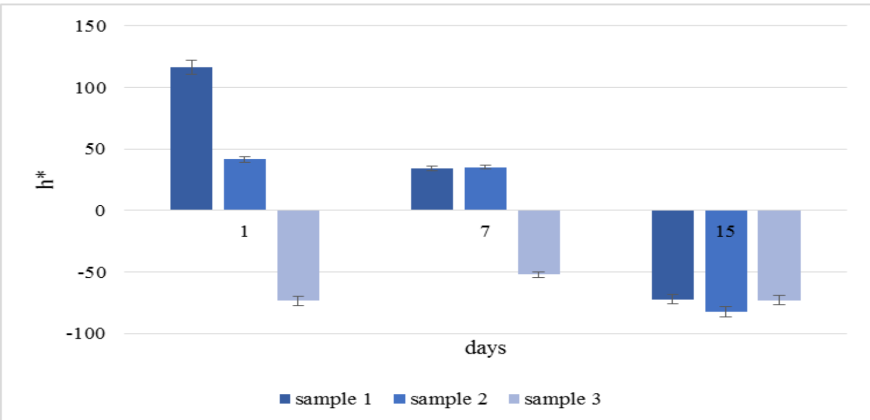


Fig. 5. Variance based on the retention period of *hue angle* parameter

The parameter variation of *Chroma* obtained are shown in *Figure 6*. Regarding *Chroma* parameter after the first day of storage, it seems that the color intensity of sample 1 is most perceived by the human eye, with the highest value. At the opposite pole is the color intensity of the sample 3. For sample 1 *Chroma* parameter decreased after 7 days of storage, while the sample 2 and sample 3 was a slight increase. *Chroma* parameter has registered the highest values after a 15-day shelf life, color intensity is easily perceived by the human eye due to the formation brown color.

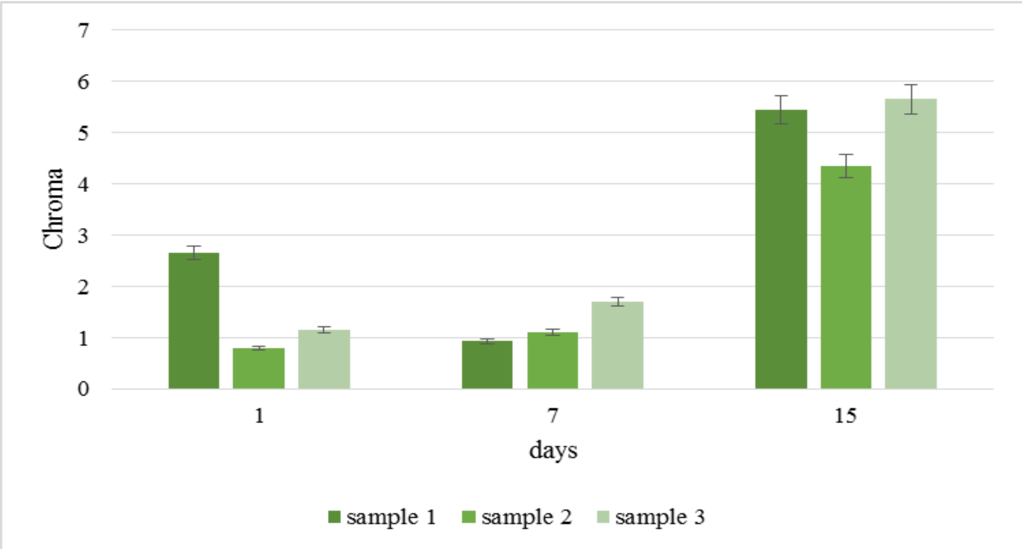


Fig. 6. *Chroma* parameter variation depending on storage period

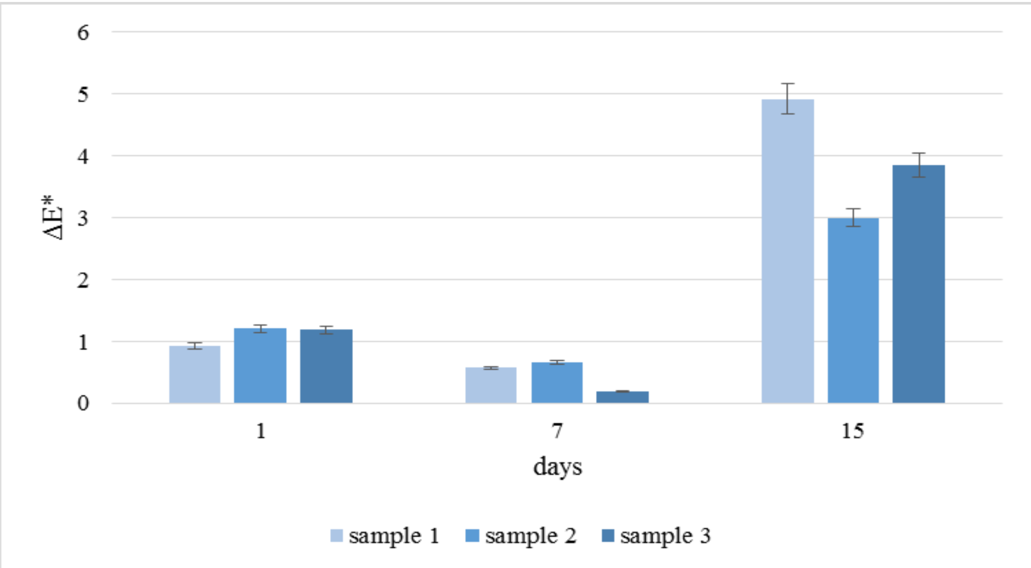


Fig. 7. The difference in color during storage period

ΔE^* – represents the color difference magnitude between the value of the initial color and the value of the final color and gives the distance between the points representing the color, being calculated according to the equation [27]:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

Perceptible color differences can be classified analytically as very distinct (ΔE^* >3), distinct (1.5 < ΔE^* < 3) and less distinct (1.5 < ΔE^*) [27]. In some cases the total color difference and chromaticity are considered the most sensitive parameters for measuring color degradation. As can be seen from *Figure 7*, in the fifteenth day color difference is very noticeable, and the highest value is recorded sample 1, although all samples inregistrated color difference great on the fifteenth day storage.

4. Conclusion

The residual amount of nitrite increased after 15 days of storage in all the three samples of the sausages summer products. For the second sample an amount slightly higher than the permitted quantity was obtained. The increase in residual nitrite after storage is closely correlated with dehydration products, which is visible and determines the concentration of sodium nitrite. During the 15 days of products storage the organoleptic characteristics have significantly changed, the color is easily observed that goes from pale pink to deep red and after the 15th day of storage becomes dark red-brown. Therefore, the lightness decreased during storage due to darkening. Also, by decreasing the content of water and increasing the amount of residual nitrite the a^* value increased in time, while the parameter b^* decreased due to degradative processes. *Chroma* parameter, responsible for perceiving color intensity to the human eye, has grown and presented elevated values after 15 days of storage of the product. Products with more pronounced tinged pink, with shades of yellow were observed *Hue angle* parameter values are greater, while the sample with a higher fat content were obtained lower values of this parameter. Also, after 15 days of storage of the product in all samples was registred a decrease in the parameter *Hue angle* and thus the appearance of yellow shades.

So it is obvious that there is a close correlation between the amount of sodium nitrite in samples sausages summer humidity and color parameters changes during storage.

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