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Influence of the sweet red paper extract on the quality and oxidative changes in fats of sausages

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Abstract. *The purpose of this paper is to examine the impact of red sweet peppers extract on the chemical, microbiological and sensory oxidative changes presented through the parameters, acid level and properties, hydrolytic and peroxide number, during the ripening and keeping of sausage type Kulen. For this purpose, five groups of kulen sausages were made (control group I without addition of the extract, group II – 0.1 g/kg, group III – 0.2 g/kg, group IV – 0.3 /kg and group V – 0.4 g/kg extract of sweet peppers). The groups were made according to the rulebook for quality of minced meat, meat preparations and meat products no. 63 from 2013 in Macedonia. After drying and ripening sausages were stored at 4°C. At the beginning, the average initial values of free fatty acids are relatively low (from 0.20 to 0.50%), but during the production (40 day) the content of free fatty acids increased (from 2.20 to 2.50%). During the keeping and storage from 40 to day 75 in group V has the lowest increase in the content of free fatty acids and the highest in group I (2.50% 40 day to 3.78% 75 day). During the ripening, drying and storing of the sausage from day to day 70 in group V the increase in oxidative changes was the lowest (0.58 to 0.72 and from 0.72 on day 40 to 1.12 on day 75) and the highest in group I (0.65 to 1.20 and from 1.20 on day 40 to 2.20 on day75). Although there are no statistically significant differences among the tested samples during the production process and during storage of the sausage (p>0.05) the obtained lower values compared to the oxidative changes in group V are probably the result of antioxidative effect of the extract. Groups IV and V had a good cross section color of the final product compared to the other groups. During the ripening and drying, the extract of sweet pepper has no influence on pH, chemical composition and microbiological status of the final product.*

Keywords: Kulen of sausage type, sweet red pepper extract, sustainability, sensory characteristics

Introduction

One of the most important changes that may occur during the production and preservation of permanent sausage is fat oxidation. Fat oxidation is one of the common causes for spoilage of meat products which is accompanied by a change in the smell, taste, appearance and causes discoloration on the end product (Bakalivanova et al., 2007). Fats interact with the oxygen in the air or as a reaction to earlier absorbed oxygen (Ognjanovich et al., 1985). Oxygen can react with radicals of fatty acids and produces toxic compounds (Gray et al., 1986; Hureli and Nielsen, 1987; Pearson and Gillett, 1999). The basic group of toxic compounds derived from the oxidation of fat are peroxides, aldehydes, ketones, alcohols, polymerized products, cyclic compounds, conjugated fatty acids (dienes and triene), some of which can have cancerous effects (Dragoev, 2004). Oxidative changes begin on phospholipids on cell membranes containing mostly unsaturated fatty acids. During mincing, freezing and other technological operations with meat, phospholipids are able to interact with the oxygen, enzymes of hem – pigments and metal ions. In fresh meat, metmyoglobin – hydrogen peroxide is the primary initiator of lipid oxidation and iron released from metmyoglobin under the influence of hydrogen peroxide is the most important catalyst for the oxidation of lipids (Rhe et al., 1987). In order to prevent oxidation of fats in meat and meat products, different antioxidants are used (Dragoev and Balev, 2004). The antioxidative effect of spices and their essential oils have been studied for some time. Schipault et al. (1952) first wrote about the antioxidative effect of rosemary and sage. Besides rosemary and sage, other herbal spices such as basil, oregano and mint can also affect the antioxidant. In fermented sausages clove, pigment and black pepper have the greatest antioxidant effect (Julau et al., 1987). Rosemary and marjoram entertain changes in the color of meat products (Dragoev 2004). After testing of spices for many years, it was found that carriers of the antioxidant properties are usually phenol compounds that bind metal ions and thus decrease their catalytic role and neutralise the effects of free radicals. The component with antioxidant activity in incense is thymol, in oregano – karvakol, in clove - eugenol, in peppers - capsicin, in ginger - gingerol, in garlic - alin (Savich and Danon, 1982; Gashich, 1992). Extracts of rosemary and sage have antioxidant effect similar to butylhydroxytoluene and better than butylhydroxyanisole (Djarmati et al., 1991). Besides the antioxidant effect of some spices such as garlic and sage, they have antibacterial effect in certain conditions. It has been proven that extractive volatile oils of garlic suppress the development of Bacillus Subtilis Cl. Botulinumtip A, E. Coli, Lactobacillus Plantarum, Staphulococus Aureus, fungi such as Aspergilus Flavus, Candida Albicaus. Besides allicin, garlic contains other ingredients with antibacterial effect, too, such as katekol and protokatekol (Dragoev, 2004). Lately, spice extracts obtained by extraction of organic solvents are used more often. They are more concentrated and stronger than natural spices and are bacteriologically clean. Components with antioxidant activity in thyme are thymol in oregano carvacrol, in clove eugenol, capsaicin in the peppers, in gingerol gingerol, in garlic allicin (Savich and Danon, 1982; Gashich, 1992). It was found that extracts of rosemary and sage have antioxidant effect similar to the antioxidant effect of butylhydroxytoluol and better than butilhydroxianizol (Djarmati et al., 1991).

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Red sweet peppers and lately more often red sweet pepper extract, is used in meat industry usually as raw material in ready spice mixtures. Having in mind the data above, the objective of our study was to check the influence of the sweet red pepper extract on the quality and oxidative changes of fats in sausages.

Material and methods

Tests were performed on dry sausage Kulen. The Kulen according to the Rulebook on the requirements in terms of quality of minced meat, meat preparations and meat products (no. 63 of 29.04.2013) in Macedonia belongs to a group of fermented dry sausages that after filling does not undergo heat treatment just maturing and drying under controlled conditions or in chambers for ripening and drying of durable sausages which automatically regulates the relative humidity, temperature and ventilation in the chamber. Kulen together with smoked tea sausage are among the most widely produced fermented dry sausages in industrial conditions in the R. Macedonia (Kuzelov et al., 2012). The Kulen sausage is produced from second category beef meat (32%), second category pork meat (32%), hard fat tissue (26%), functional mix composed of Glukono delta lactone, dextrose and vitamin C, nitrite salt for brine (2.4%) and sucrose (0.3%).

Five groups of Kulen with three samples from the sausage in every group were produced: control group I with no added sweet pepper extract, group II with 0.1 grams sweet pepper extract on 1 kg mixture, group III with 0.2 g/kg, group IV with 0.3 g/kg and group V with 0.4 g/kg.

The sweet pepper extract is from the company Akras, Austria. It is 100% microbiologically clean and was applied in the product which was previously mixed with nitrite salt. The nitrite salt served as the extract carrier for the process of grinding and mixing so it can be properly allocated in the mixture. After the mincing and mixing of the raw material and applying the extract, sausages are stuffed with a vacuum filler in collagen casings with a diameter of 60 mm and pressed at a length of 30 cm. After a brief period of adjustment the Kulen sausage was smoked 3 – 4 times for 8 hours at temperature 15°C. Then it enters the chamber for ripening and drying where the chamber temperature is 18°C and the relative humidity is 90%. At the end of the ripening and drying the chamber temperature is 14°C and the relative humidity – 75%.The entire production process from filling up to completion of ripening and drying takes 40 days. From day 40 to day 75 Kulen is kept at a temperature of +4°C.

Examination of pH values

The examination of the pH was done with pH meter Ebro with a combined electrode. The test was performed by making holes on several places starting from the centre to the periphery of the product and then the medium value is calculated.

Chemical testing

Chemical testing is performed in all samples of sausages immediately after drying and ripening, and the content of peroxide and acid number level was examined at the 1, 10, 14, 20, 25, 30, 35, 40, 55, 65 and 75 day from the production of Kulen. The chemical tests were performed to test the water content in the sausage by the method of ISO 1442/1998, the protein content by the Keldal method, total fat by the ISO 1443/1992 method, mineral substances by the ISO 936/1999 method, the content of salt by the ISO 1841-1/1999 method, the content of nitrite by the ISO 2918 method, the peroxide number has been studied by the method of ISO 3960: 2001, the acidity level is determined by the method of ISO 660: 2000. From each sample were performed three trials and its value is calculated on average.

Microbiological examination

The number of *lactobacilli* is determined at the MRS-agar at 32°C/72 hours in an aerobic environment. The number of *Micrococci* and *Apathogenic Staphylococci* on Baird parker at 37°C/48 hours. The number of *Enterococci* on blood agar at 32°C/48 hours. The number of *Proteolytic bacteria* on nutrient agar which is added calcium casein peptone at 37°C/48-72 hours. The number of *Lipolytic bacteria* on agar tributirin at 32°C/48 hours. For the testing of each sample 20 grams of homogenized material were taken before seeding with 180 ml of sterile distilled water. From each sample were performed three repetitions - plating, and the results are presented as the average of three repetitions of plating. The total number of bacteria was examined on day 40, day 60 and day 75 after production, following the method of ISO 4833-2008. At the end of production (day 40) was examined number of *Lactobacilli, Micrococci* and *Apathogens Staphylococci, Enterococci, Proteolytic* and *Lipolytic bacteria*.

Sensory examination

At the end of production with quantitative test with descriptive scale from 1 to 10 the sensory properties of the tested Kulen are examined, such as external appearance, cross section, acidity, succulence, consistency, smell, taste and overall acceptability. The panel to assess the sensory properties comprised a group of 5 experienced specialists. Water and bread are served for cleansing the mouth between the assessors' examining of samples.

Statistical processing

The obtained results were statistically processed by Excel program. Descriptive statistics and comparison of results among the tested trials were performed with analysis of variance Anova single factor test and two sample assummptions.

Results and discussion

pH value

The pH value of the sausages during drying and ripening has great effect on the quality of raw sausages. Decline of the pH value causes connecting of the mixture particles, separation-letting out water – drying, creating colour and flavor formation. All these changes are in view of ripening and drying of Kulen (Vukovich, 1988). The results from the oscillation of the pH value during ripening and drying of sausages are given in Table 1. There is no big difference in the initial medium values for all five tested groups of Kulen, i.e. these values are almost the same, ranging from 5.82 to 5.88. On the 15th day of drying and ripening, a decline of pH was noticed in all five tested groups (from 5.22 to 5.25). On the 40th day, the pH values are slightly higher, ranging from 5.20 to 5.28. The process of preservation (drying and ripening) is primarily based on the lowering of pH due to fermentation of added sugars in the acid transformation GDL glukono delta-lactone to gluconic acid or activity of starter cultures(Radetich, 1997). While keeping the Kulen 40 to 75 days in all five trials between the 40th and the 65th day a slight decrease in pH occurs and at the 75th day of production, the pH value in all five trials examined is slightly increased. In all tested groups

Table 1. Distribution of pH values as regards the tested samples of Kulen

Day	Control group I Mean±SD	Group II Mean±SD	Group III Mean±SD	Group IV Mean±SD	Group V Mean±SD
1	5.82±0.04	5.83±0.02	5.84±0.05	5.82±0.04	5.88±0.02
15	5.25±0.02	5.15±0.04	5.22±0.05	5.20±0.02	5.22±0.04
40	5.20±0.05	5.18±0.02	5.18±0.02	5.17±0.05	5.18±0.04
55	5.18±0.02	5.15±0.05	5.15±0.08	5.15±0.07	5.15±0.08
65	5.15±0.07	5.12±0.04	5.12±0.02	5.12±0.05	5.10±0.05
75	5.20±0.05	5.18±0.07	5.15±0.05	5.20±0.08	5.22±0.07

there is no statistically significant difference in the decline of pH during ripening and drying of Kulen by the 75th day. It can be concluded that the addition of sweet pepper extract does not significantly affect the pH decline in Kulen. The results in terms of measuring the pH are consistent with the results obtained by Vukovich,1988, Rede et al. 1990.

Chemical testing

Water content in the tested Kulen groups ranged from 29.10 to 29.55% (Table 2). This water content in produced Kulen is just under the allowed water content for this kind of products according to the Rulebook for the requirements in terms of quality of minced meat, meat preparations and meat products (No.63 of 29.04 2013, Republic of Macedonia).The water content which is lower than allowed may be due to the higher fat content in the product and the fact that Kulen is stuffed in with wrappings with smaller diameter, which allows rapid dehydration. The results in terms of water content are correlated to the results obtained by Vukovich et al. (2004).

The protein content in all tested groups of Kulen ranges from 24.25 to 24.92%. Fat content ranges from 37.28 to 38.22% and the mineral matter from 4.10 to 4.22%. Differences in the content of proteins, fat, mineral matters are small and insignificant. There is no statistically significant difference in regard to the content of protein, fat, minerals in all tested Kulen groups. The results we got correlated with the results that were received by Vukovich et al. (1988), Indzev (2003) and Vukovich (2004). In terms of content of salt and residual nitrite, there are no statistically significant differences between the tested groups. Content of salt in the final product matches the amount that is added to the product. As reason for the lower content of residual nitrite we can present the data given by Peg and Shahidi (2000) who note that nitrogen oxide reacts faster with myoglobin in creating the colour of meat products if the pH value of the stuffing is low. Hence, the end product will have less residual nitrites. Our results regarding the content of sodium chloride in the end product are consistent with the results obtained by Operta et al. (2008), who note that the content of sodium chloride in sausages ranges from 3.31 to 8.33%. The products of brined meat must not contain more than 20mg nitrate sodium salts per 100 grams of product. The content of residual nitrites in all three tested samples fulfilled the conditions prescribed with the Rulebook, and was in accordance with the results of Comi et al. (2005) for naturally fermented Italian sausages.

Microbiological tests

The results from the examination of the total number of bacteria in all analysed groups are presented in Tables 3 and 4. From Table 3 at the end of the production process we can see the dominated lactobacilli and enterococci typical of this type of product. Micrococci

Table 2. Chemical analysis of the tested samples of Kulen

Chemical parameters	Control group I Mean±SD	Group II Mean±SD	Group III Mean±SD	Group IV Mean±SD	Group V Mean±SD
Water, %	29.55±0.28	29.28±0.22	29.25±0.42	29.18±0.20	29.10±0.58
Fat, %	38.22±0.52	38.18±0.48	37.50±0.55	37.42±0.52	37.28±0.42
Proteins, %	24.25±1.12	24.45±0.28	24.52±0.85	24.58±0.48	24.92±0.45
Mineral matters, %	4.22±0.88	4.20±0.80	4.18±0.48	4.12±0.42	4.10±0.52
Nitrite, (mg/kg)	0.42±0.42	0.40±0.28	0.44±0.28	0.42±0.52	0.42±0.78
Salt, %	4.25±0.72	4.22±0.40	4.28±0.42	4.18±0.58	4.20±0.78

Table 3. Bacterial flora of tested samples of Kulen, 40th day of the end of production (log.cfu / g)

Type of bacteria	Control group I Mean±SD	Group II Mean±SD	Group III Mean±SD	Group IV Mean±SD	Group V Mean±SD
Lactobacilli	6.72±0.82	6.55±0.42	6.48±0.85	6.40±0.72	6.52±0.89
Entercocci	4.78±0.52	4.70±0.52	4.55±0.82	4.45±0.55	4.48±0.82
Apathogens Staphylococci	3.30±0.52	3.28±0.48(1)*	3.25±0.48	3.20±0.42(1)*	3.18±0.78
Micrococci	4.88±0.28	4.80±0.85(1)	4.72±0.52	4.58±0.58(1)*	4.50±0.85
Proteolytic Bacteria	3.10±0.42	3.15±0.45(1)*	3.05±0.50	3.18±0.28(1)*	3.00±0.48
Lipolytic Bacteria	4.10±0.58	4.05+/-0.40	3.95±0.45	3.88± 0.45	3.92±0.58

* Number of samples in which is isolated bacteria are examined

Table 4. Microbiological image (total number of bacteria) of tested samples of Kulen

Days	Control group I Mean±SD	Group II Mean±SD	Group III Mean±SD	Group IV Mean±SD	Group V Mean±SD
40	7.2±0.18	7.0±0.22	7.5±0.00	7.4±0.40	7.2±0.15
60	6.8±0.42	6.8±0.58	6.7±0.82	6.5±0.40	6.5±0.52
75	6.7±0.48	6.5±0.52	6.5±0.82	6.7±0.42	6.4±0.28

Table 5. Sensory testing in samples of Kulen

Sensory properties	Control group I Mean±SD	Group II Mean±SD	Group III Mean±SD	Group IV Mean±SD	Group V Mean±SD
External appearance	7.28±0.45	7.20±0.18	7.40±0.40	7.25±0.22	7.10±0.52
Cross section colour	6.52±0.12 *	7.54±18 *	8.10±0.22*	8.58±20 *	9.22±0.18*
Coherence	5.82±0.20	6.28±0.52	6.00±0.00	6.25±0.48	6.92±0.20
Acidity	7.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	7.00±0.40
Spiciness	7.20±0.00	7.18±0.12	7.18±0.15	7.15±0.18	7.10±0.25
Tenderness	6.28±0.42	6.15±0.15	6.18±0.25	6.10±0.22	5.80±0.18
Consistency	8.40±0.20	8.52±0.28	8.55±0.18	8.58±0.50	8.75±0.28
Smell	8.00±0.58	8.12±0.12	8.48±0.45	8.52±0.52	8.88±0.52
Taste	8.18±0.42	8.20±0.22	8.25±0.28	8.40±0.12	8.52±0.45
Total acceptability	4.76±0.00*	5.51±0.42	6.45±0.15	6.92±0.20	7.52+/-12*

and Apatogenic Staphylococci which are important for the maturation of Kulen are set in a very small number of the tested groups of Kulen as a consequence of the low pH at which these bacteria are poorly bred and didn't even grow (Lucke 1985). Bacteria that cause proteolysis are also set in a small number of the tested groups of Kulen. In the tested samples of Kulen Coagulase Positive Staphylococci, Sulfate Reducing Bacteria and other pathogenic bacteria are not found either. The table shows that the total number of bacteria in all tested groups of Kulen ranges from 7.2 to 7.5. The results in Tables 3 and 4 show that there are no statistically significant differences between the tested groups of Kulen in relation to the presence of different types of bacteria and total number of bacteria (p>0,05). In other words, the extract of sweet red pepper doesn't have antibacterial effect.The results show that standard raw material is used in the production of Kulen and the hygiene during production is on a high level.

Sensory testing

Regarding the tested sensory features, outer appearance, coherence, acidity, spiciness, tenderness, consistency, smell, taste and total acceptability the differences between samples are small, insignificant and are not statistically significant (Table 5). In terms of cross section colour between the control group I, and groups III, IV and V, there was a statistically significant difference ($p<0.05$). Group V had a cross-section that was very constant to air compared with the other groups.The typical colour of meat products is obtained with the interaction of nitrites and myoglobin and depends on the percentage of myoglobin that will turn into nitrosyl myoglobin (Toldra, 2002). Better average grades in terms of cross section colour were given to group III, IV and V, probably because the red pepper extract gives the product more attractive colour. The weaker grade of the cross section colour of Kulen is due to the lighter colour in the section which can be explained by greater residue of water, i.e. greater

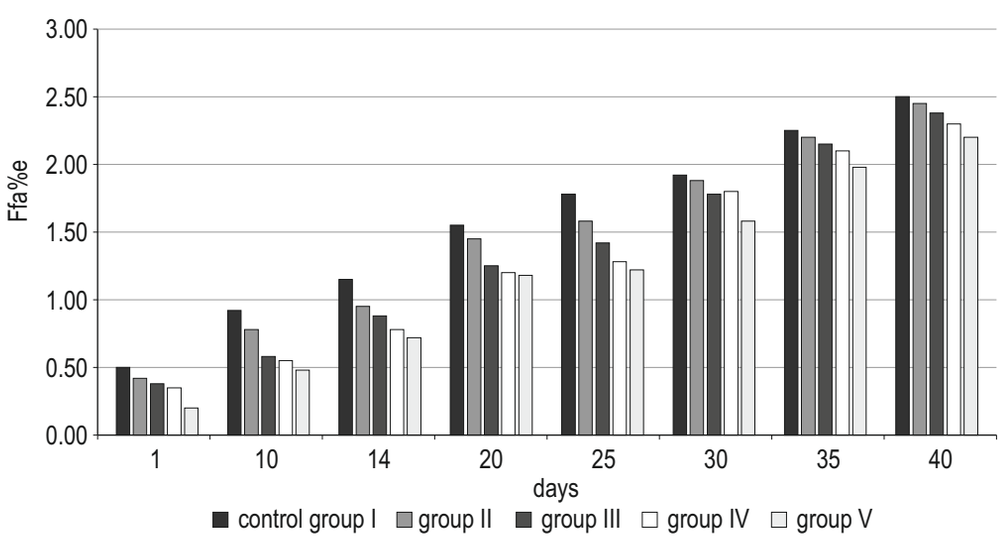

Figure 1. Changing the content of free fatty acids during the drying and maturation of Kulen

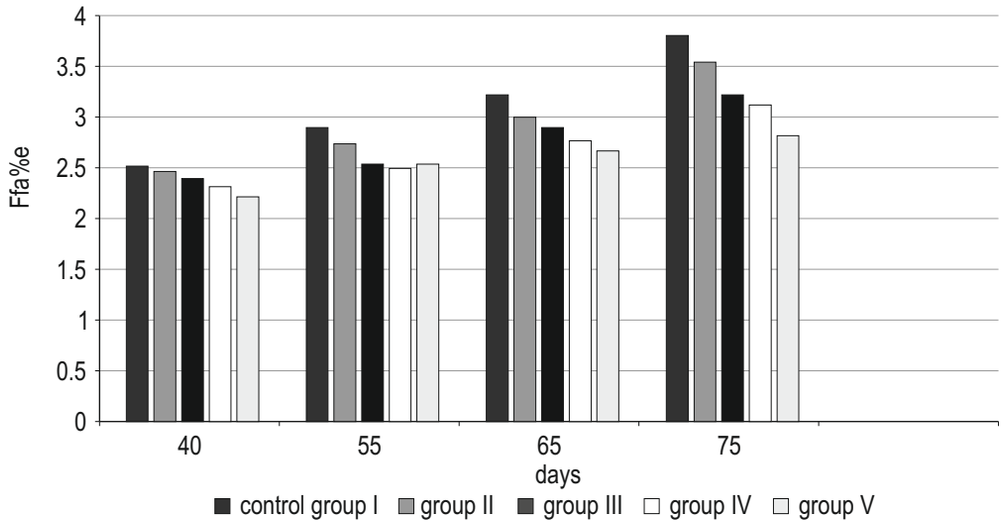

Figure 2. Changes in the content of free fatty acids during the storage of Kulen

value, insufficient cohesion of nitrite and myoglobin and as a result of non-applying of sweet pepper extract. The best score in terms of overall acceptability had rehearsed for group V. The differences in terms of total acceptability between the control group I and group V and the sample with group III and group IV extract are significant or statistically important ($p < 0.05$).

Testing the peroxide number and acid level

The content of free fatty acids in the production of Kulen was almost the same in all tested groups (Figure 1). The average initial values of these acids are relatively low and range from 0.20 to 0.50%. During the production, the content of free fatty acids slightly increases and at the end of the production it ranges from 2.20 to 2.50%. The results are consistent with the data in the literature that suggest that the content of free fatty acids at the end of the fermented sausages ranges from 1 to 5% (Demeyer et al., 1974). Hydrolysis of fats during the maturation of Kulen is being catalyzed by tissue lipases and lipases of the microflora that spawns during ripening Berger et al. (1990). Since no starter cultures were used, the insignificant differences of the free fatty acids content can be explained by the presence of different microorganisms and different lipolytic activity of the present microflora (Selgas et al., 1993; Talon et al., 1996). During storage of Kulen at temperature of 4°C from day 40 to day 75 (Figure 2) in the control groups the largest is the increase in the content of free fatty acids ranging from 2.50 on day 40 to 3.78 on day 75 . Group III had content of free fatty acids from 2.38 (day 40) to 3.10 (day 75), in group V the content of fatty acids was 2.20 (day 40) to 2.80 (day 75 of production). The continuous increase in the content of free fatty acids after 14 days of production when the pH was lower than 5.4 is likely due to the activity of extracellular Lipases of Micrococci the hydrolysis of triglycerides which takes place spontaneously at low pH values (Yasosky et al., 1984; Wagner,1987; Selgas et al., 1993;). During maturation and drying of Kulen (Figure 3) the lowest oxidative changes are found in group (0.58 to 0.72) and the highest in the control group I (0.65 to 1.2). Values obtained at the end of ripening and drying of kulen primarily indicate changes of lipid antioxidant which usually occur during the ripening and drying of Kulen. During the storage of Kulen (from day 40 to day 75) at a temperature of 4°C (Figure 4) an increase of oxidative changes has been determined. The largest

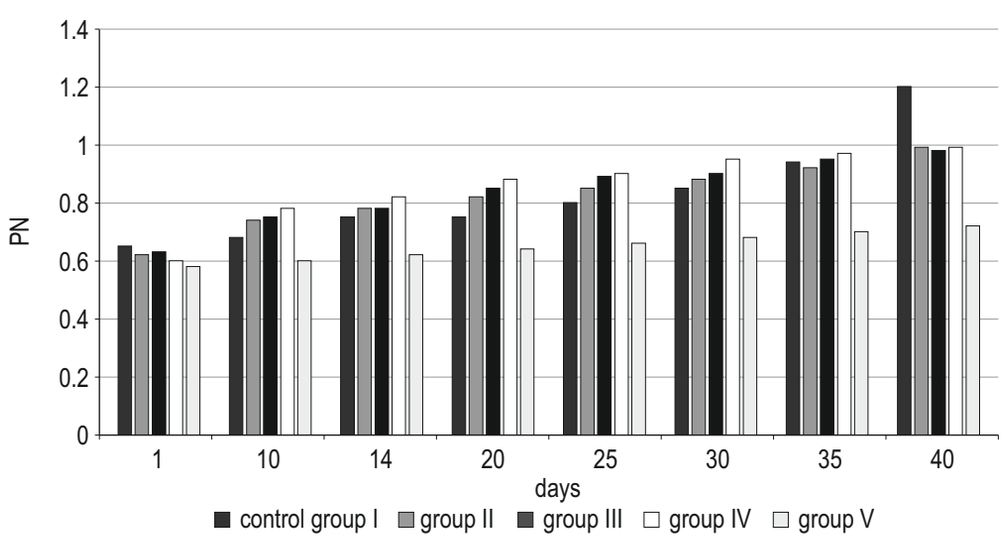

Figure 3. Changes of peroxide number during ripening and drying of Kulen

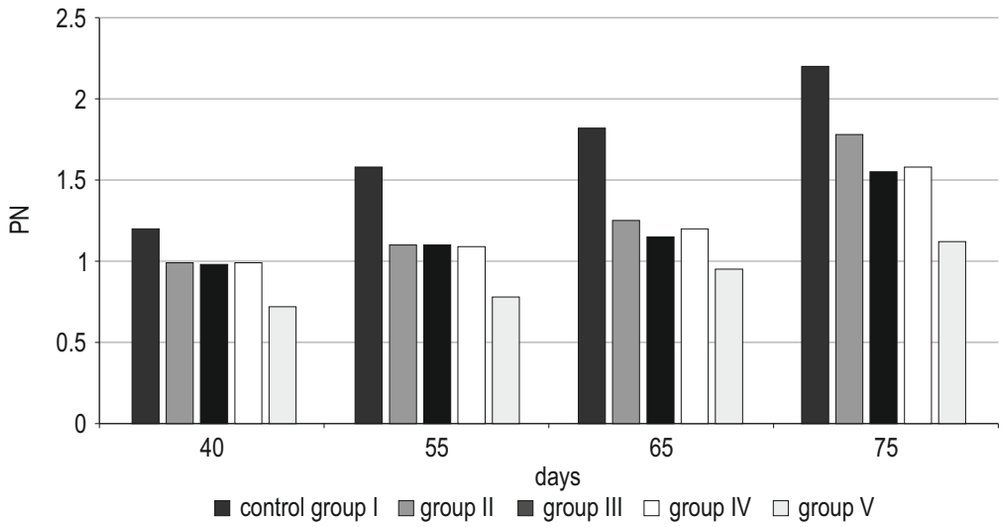

Figure 4. Change number of peroxide during storage of Kulen

increase in oxidative changes was observed in the control group I from 1.20 (day 40) to 2.20 (day 75) and the smallest increase was in group V from 0.72 (day 40) to 1.12 (day 75). Although there are no statistically significant differences between the examined groups in relation to oxidative changes in Kulen during the manufacturing process and during storage ($p>0.05$), it can be concluded that the lower values obtained in terms of oxidative changes in group V are likely a result of the antioxidative effect of the extract. The sweet red pepper extract contains kapsacinoidi, ascorbic acid, tocopherols and carotenoid compounds known for their antioxidant properties.

Conclusion

The use of sweet pepper extract does not affect the pH oscillation during ripening and drying of Kulen, the chemical composition and microbiological image of the end product. When using 0.4 grams of sweet pepper extract on 1 kg of mixture, lower degree of oxidative changes in fats is found during drying and ripening of Kulen in comparison to the use of 0.1, 0.2 and 0.3 grams of extract in 1kg mixture. The use of sweet pepper extract at the amount of 0.2, 0.3 and 0.4 grams on 1kg mixture favourably affects the colour of the end product, but has no effect on other sensory properties.

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