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Research Article

Antioxidative effect of dry distilled rose petals extract in traditional Bulgarian dry fermented sausages with reduced nitrate content

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Abstract
The aim of study was to explore the dry distilled rose petals extract (DDRPE) addition to Bulgarian lukanka type dry fermented sausages with half-reduced nitrate content. There was studied the antioxidative effect of DDRPE in lukanka filling mass on color characteristics, pH, acid value (AV), peroxide value (POV), TBARS and sensory characteristics. Four samples: C_{100} - 0.500 g/kg nitrate without DDRPE, C_{50} - 0.250 g/kg nitrate without DDRPE, R_1 - 0.250 g/kg nitrate and 1.140 g/kg DDRPE and R_2 - 0.250 g/kg nitrate and 2.280 g/kg DDRPE were examined dynamically on the 1^{-st}, the 10^{-th} and 18^{-th} d during the sausage processing. The color brightness (L*) at the end of the experiment was the highest in C_{100} and R_1 while the redness (a*) and yellowness (b*) values were higher in R_1 samples only. The pH was the lowest in R_2 and R_1. An increase in AV during the studied period was evidence for an outgoing lipolysis in all samples. In comparison to using 2.280 g/kg DDRPE (R_2), the addition of 1.140 g/kg DDRPE (R_1) demonstrates a better inhibitory effect on lipid peroxidation (POV, TBARS) of traditional Bulgarian dry fermented sausages. C_{100} had the highest sensory scores followed by R_2.

Keywords
dry fermented sausages, nitrate reduction, *Rosa damascena* Mill, oxidative stability, sensory characteristics

Abbreviations
AV – acid value; DDRPE – dry distilled rose (*Rosa damascene* Mill.) petals extract; pH – the negative log of the hydrogen ion concentration; POV – peroxide value; TBARS – 2-thiobarbituric acid reactive substances

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Introduction

For the past few years much attention has been paid to developing new meat products which may prevent the risk of diseases (Zhang et al. 2010) such as the so-called healthy food. Different innovations in the meat industry were targeted at reducing the content of unhealthy substances (Toldrå 2011) i.e., to replace or reduce the nitrite content in meat products (Balev et al. 2014; Bulambaeva et al. 2014; Vlahova-Vangelova et al. 2014). Nitrates are used as an auxiliary material in the coloring of meat products i.e., they were meant to give their characteristic pinkish red colour (Zhang et al. 2007). Nitrite also has preservative properties, inhibiting the growth of some microorganisms and *Clostridium botulinum* causing some of the worst food poisonings (Davidson and Harrison 2002). Despite the benefits of nitrite incorporation residuals have a negative effect on the quality of meat products because of their ability to form nitrosamines substances with a strong carcinogenic effect (Sebranek and Bacus 2007).

Our research has shown that dry distilled rose petals extracts (DDRPE) can be used for the production of cooked sausages with 50% reduced nitrate content (Vlahova-Vangelova et al. 2014). The DDRPE up to 0.05% does not change the sensory properties and stabilizes the colour of the crosscutting surface of sausages with 50% reduced nitrate content (Balev et al. 2014).

An interesting by-product which is a waste in the production of the Bulgarian rose (*Rosa damascena* Mill) oil and rose water is the distillate rose petals. It is a typical raw material that has been established to contain a wide range of phenolic antioxidant components - flavonoids with synergistic effect (Shikov et al. 2008) with proven antioxidant and antibacterial properties (Özkan et al. 2004). The DDRPE are good source of polyphenols (Kammerer et al. 2005) as well.

The high antioxidant activity of the phenolic compounds is due to the activity of free radicals and the chelate activity of the transition metals (Mukai et al. 2005; Andjelkovié et al. 2006). The antioxidant effect of phenolic compounds in complex biological systems such as meat is difficult to predict because it depends on their ability to remove free radicals from the system by interacting with transition metal ions (Dragoev 2009). All the above examples of the use of dry distillate rose petals when it is incorporated into meat products show the significant potential of this by-product for its incorporation in functional meat products with potential health effects.

Last but not least, the recovery of this by-product would help solve major environmental problems related to environmental pollution. By utilizing the by-products from the rose oil production in the perfumery industry it will contribute to the development of the waste-free technologies and thus it could deliver significant economic, environmental, social and health benefits. Therefore, the aim of this study was to explore the DDRPE addition to Bulgarian Lukanka type dry fermented sausages with half-reduced nitrate content produced according to the traditional for the region technology during 18 days period of drying.

Materials and Methods

Meat raw materials. In this experiment were used chilled to 0 - 40°C beef topside (pH = 6.60) and pork chest (pH = 6.55). The beef and pork meat were supplied by the company Kartevi brothers' Ltd, village of Benkovski, district of Plovdiv, Bulgaria.

Ingredients and additives. The sodium chloride (salt), sugar, spices and sodium nitrate (E251) were bought from the local market. The dry distilled rose (*Rosa damascena* Mill) petals extract was produced in the Department of Food Preservation and Refrigeration Technology, Technological Faculty, University of Food Technology, Plovdiv, Bulgaria.

Sample preparation. Samples of the Bulgarian lukanka type dry fermented sausages were produced according to the traditional for the region technology, following the recipe: beef round with fat up to 10% - 600 g/kg, pork shoulder with fat up to 5% - 200 g/kg, pork belly with fat up to 50% - 200 g/kg, cooking salt - 23 g/kg, sugar - 3 g/kg, black pepper (*Piper nigrum*) - 3 g/kg, cumin (*Cuminum cyminum*) - 3 g/kg. The four different samples were prepared by adding: control samples C_{100} – 0.500 g/kg sodium nitrate without any addition of DDRPE; control samples C_{50} – 0.250 g/kg sodium nitrate without DDRPE; experimental samples R_1 – 0.250 g/kg sodium nitrate and addition of 1.140 g/kg DDRPE and experimental samples R_2 - 0.250 g/kg sodium nitrate and addition of 2.280 g/kg DDRPE.The effects of DDRPE supplementation have been studied previously and a

potentially positive effect has been identified at concentrations not higher than 2.280 g/kg (Balev et al., 2014). Therefore, we used the indicated concentrations as the upper limit of the additive, which we reduced by half in two other experimental samples, 0.250 sodium nitrite and 1.140 g/kg DDRPE, respectively. For comparison, a sample of sausage C50 with the addition of 0.250 g/kg sodium nitrate without DDRPE was produced. The experiments were made dynamically on 1^{-st}, 10^{-th} and 18^{-th} d of the sausage processing.

Colour characteristics. Colorimeter Konica Minolta model CR-410 (Konica Minolta Holding, Sending, Inc., Tokyo, Japan) was used to evaluate the CIE L* a*, b* colour properties of the sausages (Hunt et al. 2012) on 1^{st}, 10^{th} and 18^{th} d of the processing at 8 - 15°C.

pH value. The pH value of the sausage samples was determined by pH-meter MS 2004 equipped with pH combination recorder S 450 CD (Sensorex pH Electrode Station, USA) (Young et al. 2004).

Lipid extraction. Total lipids were extracted from the sausage filling mass by the Bligh and Dyer (1959) method and after evaporation of the eluent they were immediately used for subsequent lipid analyses.

Acid value. As a standard of the rate of lipolysis the AV of the extracted lipids was measured following EN ISO 660:2020 procedure (Dragoev et al. 2016).

Peroxide value. The presence of primary lipid oxidation products was expressed by the POV. It was measured by using the procedure described by Shantha and Decker (1994). The double beam UV-VIS spectrophotometer Camspec model M550 (Camspec Ltd, Cambridge, UK) was used.

2-Thiobarbituric acid reactive substances. TBARS were determined by the method described by Botsoglou et al. (1994). The double beam UV-VIS spectrophotometer Camspec model M550 (Camspec Ltd, Cambridge, UK) was used.

Sensory analysis. The sensory characteristics of the samples were determined after opening the packages. A panel consisting of five members with proven tasting abilities (Meilgaard et al. 1999) was used. The samples were assessed within the range of 1 to 5 scores.

Statistical analysis. The data of the different samples were analyzed independently by SAS software (SAS Institute, Inc. 1990). The Student-Newman-Keuls post hoc test was used to compare the differences among the means. The mean values and standard errors of the mean were reported. The significance of differences was defined at $p < 0.05$.

Results and Discussion

Colour characteristics. At the end of the experiment (18^{th} d) the colour brightness (L*) was the highest ($p < 0.05$) in samples C_{100} and R_1 (Table 1) while the colour redness (a*) and yellowness (b*) values were higher ($p < 0.05$) in samples R_1 only (Table 1).

At the beginning of the experiment (1^{-st} d) the highest colour brightness (L*) was found in dry fermented sausages with 100% nitrate content (samples C_{100}). In comparison, the colour brightness (L*) in experimental samples R_1 and R_2 were with 5.7% and 5.4% lower ($p < 0.05$) (Table l). Different trend was found at the 10^{-th} d of sausage production. Compared to samples C_{50}, with the addition of 1.14 g/kg DDRPE to dry fermented sausages with half-reduced nitrate content (samples R_1) the colour brightness (L*) increases by 8.9% ($p < 0.05$). In experimental samples R_2 with the addition of 2.28 g/kg DDRPE the established colour brightness (L*) was closest to those of samples C_{100}. At the end of the experiment (18^{-th} d) the colour brightness (L*) in two experimental samples (R_1 and R_2) decreased by 11.64 % and 6.07 % respectively compared to the 10^{-th} d (Table 1). On the 18^{-th} d the colour brightness (L*) was the lowest in samples C_{50} and R_2 followed by samples R_1 which was closer to sausages with 100% nitrate content (control samples C_{100}). In conclusion, for the processing period of 18 days? the colour brightness (L*) was most stable in dry fermented sausages with half-reduced nitrate content with addition of 1.14 g/kg DDRPE (samples R_1) with a total decrease of 8.2% (Table 1).

On the first day of the experiment few deviations of the colour redness (a*) were established. No significant difference ($p \geq 0.05$) in colour redness (a*) of the samples with half-reduced nitrate content (samples C_{50}, R_1 and R_2) was found. The only exception was samples C_{100} which had 7.4% higher colour redness (a*) compared to samples C_{50} ($p < 0.05$). After the 10^{-th} d of processing the colour

redness (a*) decreased in all studied samples. On the 10-th d the lowest colour redness (a*) was established in dry fermented sausages with half-reduced nitrate content with addition of 1.14 g/kg DDRPE (samples R_1) followed by samples C_{50}. In comparison, the colour redness (a*) in control samples C_{100} was 24% higher than those in samples R_1 ($p < 0.05$) (Table 1). A different trend was observed at the end of the experiment (18-th d). In all studied samples the colour redness (a*) increased except for control samples C_{100}. The addition of 1.14 g/kg DDRPE (samples R_1) again showed a stabilizing effect with a slightest change in the colour redness (a*) for the entire experimental period (Table 1).

Table 1. Color characteristics of the sausages for the period of 18 days of processing

Samples		**C_{100}**	**C_{50}**	**R_1**	**R_2**
Brightness (L*)	1 d	45.06±0.10 ^{c,z}	43.59±0.16 ^{c,y}	42.65±0.20 ^{b,x}	42.76±0.23 ^{c,x}
	10 d	39.51±0.10 ^{a,w}	40.92±0.31 ^{b,y}	44.58±0.58 ^{c,z}	40.36±0.15 ^{b,x}
	18 d	40.38±0.29 ^{b,y}	37.58±0.22 ^{a,w}	39.39±0.28 ^{a,x}	37.91±0.26 ^{a,w}
Redness (a*)	1 d	19.57±0.25 ^{b,z}	18.22±0.03 ^{c,w}	18.33±0.03 ^{c,x}	18.52±0.17 ^{c,y}
	10 d	16.50±0.02 ^{a,z}	15.70±0.29 ^{a,x}	13.30±0.16 ^{a,x}	16.17±0.13 ^{a,y}
	18 d	16.45±0.10 ^{a,w}	17.27±0.08 ^{b,y}	17.69±0.08 ^{b,z}	16.57±0.01 ^{b,x}
Yellowness (b*)	1 d	9.79±0.15 ^{c,x}	9.05±0.05 ^{c,w}	9.05±0.05 ^{c,w}	9.07±0.10 ^{c,w}
	10 d	4.47±0.03 ^{a,w}	4.94±0.04 ^{b,y}	4.77±0.22 ^{a,x}	5.04±0.03 ^{b,z}
	18 d	5.53±0.27 ^{b,y}	4.46±0.09 ^{a,w}	5.55±0.03 ^{b,y}	4.76±0.06 ^{a,x}

$^{a, b, c}$ – Means in the same column with different superscript letters differ significantly ($p < 0.05$).
$^{w, x, y, z}$ – Means in the same row with different superscript letters differ significantly ($p < 0.05$).
SEM – standard error of the mean.

On the 1-st d of experiment (in the filling mass of sausages) no significant differences were established ($p \geq 0.05$) in the colour yellowness (b*) of samples with half-reduced nitrate content (C_{50}, R_1 and R_2). The only exception was found in control samples C_{100} which had 8.2% higher colour yellowness (b*) compared to samples C_{50} ($P < 0.05$) (Table 1). After the 10-th d of processing a significant ($p < 0.05$) decrease of colour yellowness (b*) was found in all studied samples. On the 10-th d the colour yellowness (b*) in dry fermented sausages with 100% nitrate content (control samples C_{100}) was found to be the lowest. The highest colour yellowness (b*) for the same studied period was established in sausages with half-reduced nitrate content (samples C_{50}) and in sausages with addition of 2.28 g/kg DDRPE (samples R_2). At the end of the experiment (18-th d) a different trend was observed. In two of the studied samples controls C_{100} and R_1 the colour yellowness (b*) increased ($p < 0.05$) while in samples C_{50} and R_2 significantly ($p < 0.05$) decreased compared to 10-th d (Table 1). During the studied period (18-th d) the most stable colour yellowness (b*) was found in the dry fermented sausages with addition of 1.14 g/kg DDRPE (samples R_1) (Table 1). The observed changes in the colour characteristics of dry fermented sausages with reduced nitrate content and addition of DDRPE can be explained by the antioxidant and antibacterial properties of the used flavonoids (Özkan et al. 2004) contained in rose petals. Exactly like antioxidants the flavonoids can stabilize the

cross-section surface color of the sausages. They can improve the colour stability by fortification of sausages with polyphenol co-pigments naturally occurring in rose petals (Mollov et al. 2007).

On the other hand, their antibacterial activity suppresses growth of putrefactive microflora preventing the myoglobin oxidation process and transformation of red pigments to gray brown colour metmyoglobin.

pH value. At the start of the experiment (the 1^{-st} d), the pH of the four studied samples was not significantly ($p \geq 0.05$) different (Fig. 1). After the 10^{-th} d the pH in all studied samples (C_{100}, C_{50}, R_1 and R_2) decreased.

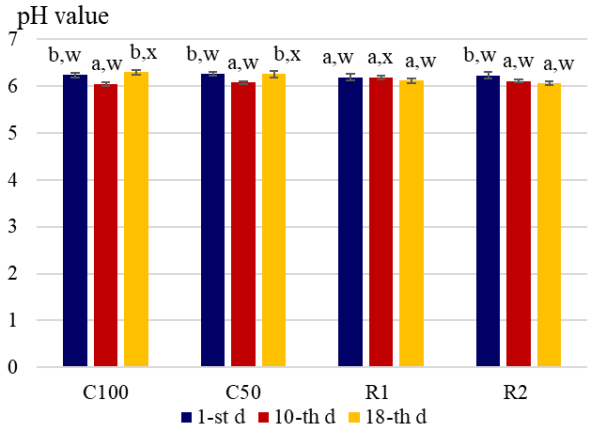


Figure 1. pH values of the four studied samples of traditional Bulgarian dry fermented sausages type lukanka on the 1^{-st}, the 10^{-th} and the 18^{-th} d of processing at 8 - 15°C. pH values marked with

$^{a, b}$ – Means in the same column with different superscript letters differ significantly ($p < 0.05$).
^{w} – Means in the same row with superscript letters ^{w} are not differ significantly ($p < 0.05$).

Compared to the first day the significant ($P < 0.05$) decrease of 3% of pH was found in the dry fermented sausages with 100% nitrate (control samples C_{100}) and with 50% reduced nitrate content (samples C_{50}). Only the pH of the dry fermented sausages with half-reduced nitrate content and addition of 1.14 g/kg DDRPE (samples R_1) did not significantly ($p \geq 0.05$) differ between the 1st and the 10^{-th} d of the experiment (Fig. 1). On the 18^{-th} d the pH values of control samples C_{100} and half-reduced nitrate content (samples C_{50}) increased. On the opposite, a decrease of pH values in the dry fermented sausages with addition of 1.14 g/kg DDRPE (samples R_1) and 2.28 g/kg DDRPE (samples R_2) was established (Fig. 1). Compared to control samples C_{100}, the addition of 1.14 g/kg DDRPE (samples R_1) stabilizes the pH of dry fermented sausages. In the end product the lowest pH values were determined in samples R_2 and R_1. The extracts of dry distilled rose petals exhibit a slightly acidic reaction. 22 kaempferol and quercetin glycosides contained in DDRPE are possibly responsible for it (Schieber et al. 2005).

Lipolysis. The increase of AV during the studied period (18^{-th} d) was an evidence for an outgoing lipolysis in all studied samples (C_{100}, C_{50}, R_1 and R_2). The highest free fatty acid content was found on the 10^{-th} d in control samples C_{100} followed by the dry fermented sausages with addition of 2.28 g/kg DDRPE (samples R_2) and 1.14 g/kg DDRPE (samples R_1). At the end of the experiment the total increase in AV was 1.30 times for control samples C_{100}, 1.46 times for samples C_{50}, 1.54 times for samples R_1 and 1.72 times for samples R_2. The comparison between AV of samples C_{50} (with a half-reduced nitrate content only) and dry fermented sausages with addition of 1.14 g/kg DDRPE (samples R_1) shows that the differences were not significant ($p \geq 0.05$) (Table 2). The addition of 2.28 g/kg DDRPE (samples R_2) affects negatively the lipolytic changes in dry fermented sausages and on the 18^{-th} d of the experiment the AV was 1.28 times as high as those in control samples C_{100} (Table 2). It was concluded that during the 18-day study period lipolysis in all samples (C_{100}, C_{50}, R_1 and R_2) developed and AV increased. This trend is intensified when the nitrate content of sausages is halved. In this case the results show the addition of 1.14 or 2.28 g/kg DDRPE stimulates the lipolytic changes during the 18-day period of processing of traditional Bulgarian lukanka type dry fermented sausages.

Changes in primary and secondary products of lipid peroxidation. The results for any changes of POV in all samples show the typical induction period with a peak on the 10^{-th} d. At the end of the experiment (18^{-th} d) a decrease of primary lipid oxidation products was found. The 1.44, 2.47, 2.80-

and 2.18-times reductions of POV in samples C_{100}, C_{50}, R_1 and R_2 , respectively, were established. On the 18-th day of the experiment significantly ($P < 0.05$) a lower POV was observed in Bulgarian lukanka type dry fermented sausages with addition of 1.14 g/kg DDRPE (samples R_1). The POV of control samples C_{100} and samples R_2 (sausages with addition of 2.28 g/kg DDRPE) did not significantly (P 0.05) differ at the end of the experiment (18-th d) (Table 2). The result allows us to conclude that the addition of a low concentration (1.14 g/kg) DDRPE (samples R_1) demonstrates an ability to inhibit hydroperoxides formation and shows antioxidative action against accumulation of primary lipid oxidation products in traditional Bulgarian lukanka type dry fermented sausages under the conditions of this experiment. Thus, the established phenomenon can be explained by the synergistic effect and the antioxidant activity of the phenolic compounds of the DDRPE (Shikov et al. 2008) in terms of evacuating of free radicals from the system in the presence of transition metals as chelating agents (Mukai et al. 2005; Andjelkovié et al. 2006).

In all examined samples the secondary product of lipid peroxidation (expressed by TBARS) increased during the first ten days of the processing of traditional Bulgarian lukanka type dry fermented sausages. Until the 10-th d the malonaldehyde formation (TBARS) was highest in samples C_{50} with half-reduced nitrate content only and in samples R_2 (dry fermented sausages with addition of 2.28 g/kg DDRPE) and it was significantly ($P < 0.05$) lower in control samples C_{100}. At the end of the experiment the TBARS of control samples C_{100} and samples C_{50} stabilize around 0.78 - 0.88 mg MDA/kg (Table 2). In comparison to those levels the TBARS of the samples R_1 were a little bit higher ($P < 0.05$) (Table 2) and they were the highest (1.41 mg MDA/kg) in samples R_2. The results show that the addition of 1.14 and especially 2.28 g/kg DDRPE had not an inhibitory effect on the secondary products of the lipid oxidation in the examined dry fermented sausages and it even stimulated the oxidation process. In previous researches Balev et al. (2014) and Vlahova-Vangelova et al. (2014) established the positive effect of DDRPE addition in cooked sausages but in traditional Bulgarian lukanka type dry fermented sausages this effect cannot be confirmed. The conclusion was made that in comparison to 2.280 g/kg DDRPE (samples R_2) supplementation the addition of 1.140 g/kg DDRPE (samples R_1) demonstrated a better inhibition effect on the lipid oxidative changes (POV and TBARS) in the traditional Bulgarian lukanka type dry fermented sausages. In conclusion, neither the addition of 2.280 g/kg DDRPE (samples R_2), nor the one of 1.140 g/kg DDRPE (samples R_1) have an inhibitory effect on the accumulation of the secondary lipid oxidation products (expressed by TBARS) during the 18-day period of processing of traditional Bulgarian lukanka type dry fermented sausages.

Table 2. Lipolitical anf lipid peroxidation changes of the traditional Bulgarian lukanka type dry fermented sausages during the 18-day period of their processing

Samples		**C_{100}**	**C_{50}**	**R_1**	**R_2**
AV, mg KOH/g	1 d	0.72 ±0.01 ^{a,z}	0.70 ±0.03 ^{a,z}	0.72 ±0.01 ^{a,z}	0.71 ±0.01 ^{a,z}
	10 d	0.98 ±0.02 ^{b,z}	0.76 ±0.02 ^{b,x}	0.91 ±0.01 ^{b,y}	0.92 ±0.01 ^{b,y}
	18 d	0.94 ±0.03 ^{b,x}	1.02 ±0.05 ^{c,y}	1.11 ±0.05 ^{c,y}	1.21 ±0.01 ^{c,z}
POV, meq O_2/kg	1 d	2.00 ±0.22 ^{b,x}	3.27 ±0.03 ^{b,z}	1.87 ±0.18 ^{b,x}	2.78 ±0.21 ^{b,y}
	10 d	3.48 ±0.07 ^{c,y}	3.02 ±0.36 ^{b,x}	2.95 ±0.44 ^{c,x}	3.27 ±0.15 ^{c,xy}
	18 d	1.38 ±0.28 ^{a,y}	1.32 ±0.22 ^{a,y}	0.67 ±0.11 ^{a,x}	1.27 ±0.06 ^{a,y}
TBARS, mg MDA/kg	1 d	0.60 ±0.03 ^{a,y}	0.54 ±0.04 ^{a,x}	0.55 ±0.02 ^{a,x}	0.52 ±0.02 ^{a,x}
	10 d	0.75 ±0.02 ^{b,x}	1.10 ±0.08 ^{c,z}	0.97 ±0.02 ^{b,y}	1.10 ±0.03 ^{b,z}
	18 d	0.78 ±0.05 ^{b,x}	0.88 ±0.05 ^{b,x}	0.95 ±0.05 ^{b,y}	1.41 ±0.02 ^{c,z}

$^{a, b, c}$ – Means in the same column with different superscript letters differ significantly ($p < 0.05$).
$^{x, y, z}$ – Means in the same row with different superscript letters differ significantly ($p < 0.05$).
SEM – standard error of the mean.

Sensory quality. The results from the sensory analysis indicated that the control samples C_{100} were rated with the highest sensory scores followed by the sensory scores of samples R_2 and R_1 respectively (Fig. 2). The differences between the means of the sensory scores of samples R_2 and R_1 from one hand and control samples C_{100} from the other were significant ($P < 0.05$) but not so substantial and deviated around 0.25 units except in the texture scores where there were found no differences (Fig. 2). Those results allow us to conclude that the half-reduction of nitrate content in dry fermented sausages is the main factor affecting the decrease of the sensory quality of traditional Bulgarian lukanka. The addition of 1.14 or 2.28 g/kg DDRPE to the sausage filling mass to some extent helps preserve the sensory characteristics. This is not enough to compensate for the 50% reduction of nitrate in the Bulgarian lukanka formulation probably because that DDRPE flavonols, despite structural similarity, have different antioxidant effects (Wang et al. 2006).

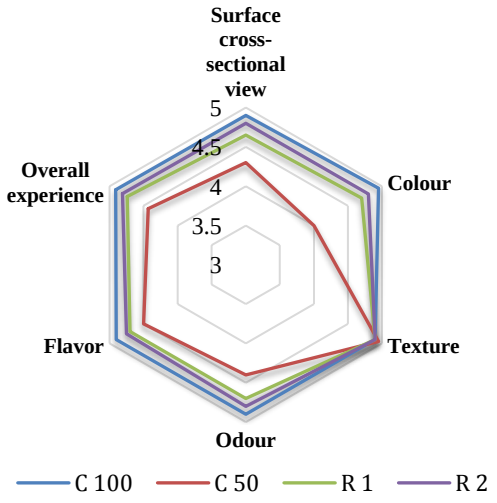


Figure 2. Sensory scores of the dry fermented sausages on the 18-th d of the sausages processing

Conclusions

The analysis of the obtained results shows that the addition of 1.140 g/kg and 2.280 g/kg of DDRPE can weakly inhibit the processes of lipid and pigment oxidation in Bulgarian lukanka type dry fermented sausages with half-reduced nitrate content and have stabilizing effect on the final pH of the end product at about 6.05 - 6.15. A dose of 1.140 g/kg DDRPE gives slightly better results. It can be recommended that the study of the addition of DDRPE to dry fermented sausages with a half-reduced nitrate content be further developed by testing combinations with other natural antioxidants or with packaging under a modified atmosphere.

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