

The Influence of Rosehip Polyphenols on the Quality of Smoked Pork Sausages, Compared to Classic Additives

VALENTIN NICORESCU, CAMELIA PAPUC, CORINA PREDESCU*, IULIANA GAJAILA, CARMEN PETCU, GEORGETA STEFAN
University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, 011464, Bucharest, Romania

In this study, pork sausages added with rosehip polyphenols, sodium nitrite, butylated hydroxyanisole (BHA) and tetrasodium pyrophosphate (TSPP) in different concentrations and combinations were prepared, smoked and refrigerated for 20 days. To evaluate the quality of sausages, chemical (peroxide value, thiobarbituric acid reactive substances, and protein patterns), microbiological (total plate count), and sensory (colour, odour, flavour, texture, and taste) analysis were performed. The results showed that the treatment with rosehip polyphenols (0.005%) and sodium nitrite (0.01%) protected sausages against lipid peroxidation as effective as the sodium nitrite (0.01%), BHA (0.005%) and TSPP (0.3%) mixture. Protein pattern of sausages treated only with rosehip polyphenols (0.04%) was similar to that of the sausages treated with sodium nitrite, BHA and TSPP mixture. Microbiological analysis showed that rosehip polyphenols and sodium nitrite combination exhibited a superior antimicrobial activity compared to the one of sodium nitrite, TSPP and BHA mixture. The scores for all sensory attributes continuously decreased with storage time, regardless of the sample type. For all types of sausages, between the refrigeration time, chemical, microbiological, and sensory parameters, statistically significant correlations were found. These findings suggest that rosehip polyphenols could be used as natural additives with multiple preservative functions for smoked sausages.

Key words: rosehip polyphenols, pork sausages, lipid peroxidation, total plate count, protein patterns, sensory analysis

Nowadays, totally or partially replacement of chemical compounds with natural ones as food additives is an ongoing concern of the food industry and health experts. In recent years, consumers' interest in organic foods increased due to their perception towards chemical preservatives, antibiotics, genetic modification, etc. Because consumers want organic foods, food industry is looking for new processing technologies and new ingredient systems in order to replace chemical compounds with natural ones with low risks for consumers' health, although these solutions generate expensive foods.

The most disputed chemicals used for meat products are nitrites, synthetic antioxidants and phosphates. In meat products, nitrites inhibit bacterial growth [1, 2], inhibit the oxidation of fats, reducing the development of oxidative rancidity [3], contribute to the development of flavour in cured meat products and are responsible for the formation of characteristic pink/red colour in cured and smoked products [4, 5]. After it was recognized that salt contaminated with saltpetre (KNO_3) preserves meat and fish against spoilage better than salt alone, saltpetre has begun to be used for the preparation of meat products. After it was confirmed that nitrite was the agent producing the red colour of meat and its heat stability (early 19th century), in only few years, nitrite was introduced in meat products manufacturing. In the 1970s, a discussion started in USA about the formation of nitrosamines in cured meat products, especially fried bacon [6]. The studies undertaken showed that in stomach [7], and in heated meat products [6, 8], nitrite forms with secondary amines carcinogenic nitrosamine. Although the nitrites use is regulated, given the high number of foods in which they are added and the accumulation of residual nitrites from ingested foods, solutions are being sought to avoid or reduce these preservatives.

Antioxidants are added in meat products to retard oxidative rancidity and to protect the flavour. Synthetic antioxidants butylated hydroxytoluene (BHT), butylated

hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) are added in meat products to prevent oxidative deterioration. BHA and BHT have been used in food products, with some restrictions, since the late 1950s. TBHQ is a more recent addition to the list of antioxidants allowed in foods; in Europe, TBHQ became an accepted antioxidant for food in 2004 [9]. Animal studies have shown that BHT, BHA and TBHQ are able to act as carcinogenic substances [10, 11]; the target organs for BHA and TBHQ are considered to be forestomach and oesophagus, whereas BHT has carcinogenic effects upon the liver of rats and mice [12-14]. Ingestion of large doses of mentioned antioxidants can also cause vasomotor rhinitis, headache, flushing, asthma, conjunctival suffusion, dull retrosternal pain radiating to the back, diaphoresis, or somnolence [9]. Considering that these antioxidants are used in many food products (meat and fish products, oils, sauces, milk powder, snack foods, processed nuts, cake mixes), human body is subjected to ingestion of high doses of BHA, BHT and TBHQ, which can seriously affect the health of consumers.

Phosphates are used to improve water-binding capacity of meat, to solubilize the proteins, to lead to higher yields and stabilized meat emulsions, to act as antioxidants, to improve texture and sensory properties (tenderness, juiciness, colour, flavour), to extend shelf-life, etc. [15-17]. The effect of phosphates on human health is contradictory. The studies undertaken showed that kidneys control the blood phosphorous level and excrete any excess of phosphorous [18]. However, experiments conducted on adult rats showed that excessive intake of dietary phosphate without calcium caused the rise of parathyroid hormone serum concentration and the decrease of bone mineral density [19]. Although, as in the case of nitrites and synthetic antioxidants, the use of phosphates in foods is regulated, the amounts of phosphates ingested by consumers in different foods are high and therefore

* email: durduncorina@yahoo.com; Phone: 0744.867.527,

solutions need to be sought in order to reduce phosphates levels in foods.

The importance of replacing synthetic compounds in meat products with plant materials containing phenolic compounds has been highlighted; some authors reported the effectiveness of plant extracts containing high levels of polyphenols to reduce lipid peroxidation [20-26], colour loss [25, 27], and microbial growth [27, 28] in some types of meats and fish.

Rosehip is the fruit of dog-rose (*Rosa canina* L.) and one of the fruits containing a large variety of important nutritional and medicinal elements. Rosehip is particularly rich in strong antioxidants as vitamin C [29], polyphenols [29] and carotenoids [30] and is used for treating colds and other infections or inflammatory diseases [31 - 33]. In addition, some researchers showed that dog rosehips also have antidiabetic properties [34], and it was suggested that rosehip extracts inhibit lipid accumulation and could be a good solution for preventing obesity [35].

In this study, we performed comparative researches concerning changes in lipid peroxidation, soluble proteins electrophoretic patterns, microbial charge and sensory characteristics of pork smoked sausages treated with neutralised polyphenolic compounds extracted from rosehip, sodium nitrite, BHA and tetrasodium pyrophosphate, in different concentrations and combinations.

Experimental part

Obtaining neutralised solution of rosehip polyphenols

Extraction of rosehip polyphenols

A precisely weight amount of ground air-dried rosehip was extracted with ethanol 60%, in relation of 1:10 (m/V). Extraction was performed at 60°C, on water bath, for 3h. The extracts were filtered through Whatman No. 40 filter paper (Whatman International Ltd., Kent, UK), using a Buchner funnel and then centrifuged for 10 min at 4000 rpm.

Determination of total phenolics

Total phenolic content was estimated by the Folin-Ciocalteu method [36]. 200 µL of diluted sample were added to 1 mL of 1:10 diluted Folin Ciocalteu reagent. After 4 min, 800 µL of sodium carbonate saturated solution (75 g/L) were added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured. Gallic acid (0-500 mg/100 mL) was used for the standard calibration curve. The results were expressed as gallic acid equivalent (GAE)/100 mL extract and calculated as mean value ± SD (n = 5).

Neutralisation of rosehip polyphenols

Following the evaporation of ethanol, rosehip polyphenols were neutralized with 0.01 N NaOH prepared in sterilized water. Neutralised polyphenolic compounds were used for pork sausages preparation.

Preparation of smoked pork sausages

For the preparation of sausages, 20 kg of fresh pork pulp and 5 kg of pork fat were used. After being chopped, meat and fat were minced using a mincer with the hole diameter of 3 mm. Minced pork was then mixed with salt (2.0 %, m/m), paprika (0.3 %, m/m), pepper (0.4%, m/m) and garlic (0.2%, m/m) and divided in five portions. In each portion, rosehip neutralised polyphenols, sodium nitrite, BHA and tetrasodium pyrophosphate were added, in different concentrations and combinations (table 1).

After adding all ingredients, ground meat was mixed and inserted into pork membranes; sausages thus obtained were introduced into the smoking cell, where they were dried up in a hot air flow, pasteurized for 60 min and then smoked at 50°C for 60 min. After smoking, sausages were packed in polyethylene bags and refrigerated at 4°C for 20 days.

Chemical analysis

Peroxide value (PV)

Peroxide value was determined according to a spectrophotometric method [37]. A standard curve was set using cumene hydroperoxide at the concentration range of 0.5-2.0 µg/mL. Peroxide value was expressed as µmol peroxide/kg sample.

Thiobarbituric acid reactive substances (TBARS)

TBARS were determined [38] and the standard curve was set using 1,1,3,3-tetramethoxypropane (TMP) at concentrations ranging from 0 to 10 µg/mL. TBARS were expressed as mg malondialdehyde (MDA) equivalents/kg of sample.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of fresh (day 0) and refrigerated (day 20) smoked pork sausages were assessed by SDS-PAGE [39, 40]. Quantitative analysis of protein band intensity was performed using a Model GS-700 Imaging densitometer (Bio-Rad Laboratories, Hercules, CA, USA) with Molecular Analyst Software version 1.4 (image analysis system).

Microbiological analysis

To assess the effects of the used additives on the microbial development, total plate count (TPC) was determined on day 0 and after 20 days of refrigeration. 10 g from each sample were homogenized (1:9, m/V) with sterile physiological solution, using a stomacher. From this suspension, serial decimal dilutions were made up to 10⁻⁶. From the initial suspension and the subsequent dilutions, 1 mL was used for testing by including in Plate Count Agar (PCA). Plates thus prepared were incubated for 72 h at 30°C. After incubation, the degree of microbial growth was assessed by counting all existing colonies on the plate. To determine TPC, eq. 1 was used:

$$TPC = \frac{\Sigma \text{ of colonies from two successive dilutions}}{(1.1 \times V \times d)} \quad (1)$$

Additive	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Sodium nitrite	-	-	0.01% (m/m)	0.01% (m/m)	0.01% (m/m)
Tetrasodium pyrophosphate	-	-	-	0.3% (m/m)	-
BHA	-	-	-	0.005% (m/m)	-
Rosehip polyphenols	0.04% (m/m)	0.02% (m/m)	-	-	0.005% (m/m)

Table 1
PREPARATION OF PORK SAUSAGES - THE PROPORTION OF ADDED CHEMICALS/ POLYPHENOLS

where V = volume of inoculum (1 mL); d = the first dilution taken into account. The results were expressed as *colony-forming units (CFU)/g sample*.

Sensory analysis

The sensory evaluation was performed by 10 panellists who gave a score for each sample according to their perception on colour, odour, flavour, texture and taste attributes, using a hedonic scale from 1 (the worst) to 10 (the best). The samples were presented to the panellist just after the packagings were opened. Texture was evaluated by panellists during slicing.

Results and discussions

Changes in peroxide value (PV)

Determinations made at intervals of 5 days generally showed a gradual increase of PV for all samples, except for the samples treated with sodium nitrite 0.01% (S3) and samples treated with sodium nitrite 0.01% and polyphenols 0.005% (S5), in which PV decreased after 20 days of storage, compared to day 15. During the 20 days of refrigeration, PV was influenced by the type of treatment and refrigeration period as is shown in figure 1.

After 5 days of refrigeration, PV mean for sausages samples treated with sodium nitrite 0.01% and polyphenols 0.005% (S5) was significantly lower ($p < 0.05$) than PV

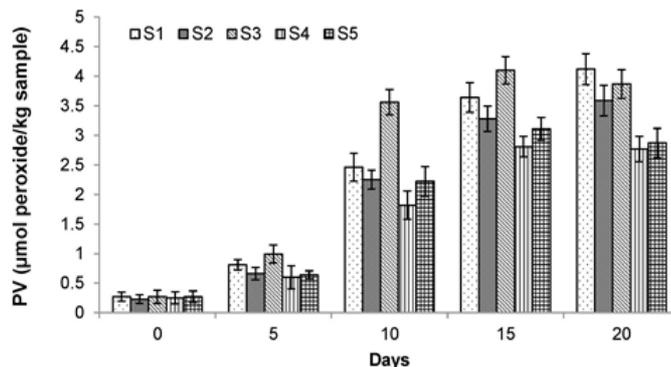


Fig.1. Changes in peroxide value for smoked pork sausages during storage at 4°C for 20 days. S1 -polyphenols 0.04%; S2 -polyphenols 0.02%; S3 -sodium nitrite 0.01%; S4 -sodium nitrite 0.01%, BHA 0.005% and tetrasodium pyrophosphate 0.3%; S5-polyphenols 0.005% and sodium nitrite 0.01%

mean for samples treated with polyphenols 0.04% (S1) and the ones treated only with sodium nitrite 0.01% (S3), while compared to the other samples (S2, S4), the differences were not statistically significant ($p > 0.05$).

After 10 days of refrigeration, PV increased for all samples; PV mean for sausages samples treated with sodium nitrite 0.01% and polyphenols 0.005% (S5) was significantly lower ($p < 0.05$) than PV determined for samples treated with sodium nitrite 0.01% (S3); the differences were not statistically significant ($p > 0.05$) as compared to S1, S2 and S4 samples. A similar pattern was recorded after 15 days of refrigeration.

After 20 days of refrigeration, PV mean for sausages samples treated with sodium nitrite 0.01% and polyphenols 0.005% (S5) was significantly lower ($p < 0.05$) than PV mean for samples treated with polyphenols 0.04% (S1) and the ones treated with sodium nitrite 0.01% (S3).

Overall, the lowest PV was determined for the samples treated with sodium nitrite 0.01%, tetrasodium pyrophosphate 0.3% and BHA 0.005% (S4), but no significant differences were recorded compared with samples treated with sodium nitrite 0.01% and polyphenols 0.005% (S5); these results demonstrate that rosehip

polyphenols in combination with sodium nitrite (S5) are able to protect sausages against lipid peroxidation, for a period of 20 days of refrigeration, almost as effective as the mixture of chemicals (S4).

Changes in TBARS value

TBARS values for all samples increased along with refrigeration time and they are presented in figure 2. During the 20 days of refrigeration, the means of TBARS values for sausages samples treated with sodium nitrite 0.01% and polyphenols 0.005% (S5) were significantly lower ($p < 0.05$) than TBARS values of samples treated only with polyphenols (0.04% - S1 and 0.02% - S2). In case of samples S5, average values of TBARS were significantly lower ($p < 0.05$) compared to TBARS values determined for samples treated with cu sodium nitrite 0.01% (S3) and sodium nitrite 0.01%, tetrasodium pyrophosphate 0.3% and BHA 0.005% (S4), excepting the 10th and the 15th day respectively, when the differences were not statistically significant ($p > 0.05$). TBARS values for sausages samples treated only with rosehip polyphenols (S1, S2) depended on their concentration, the samples with polyphenols 0.04% showing lower TBARS values than samples with polyphenols 0.02%.

Overall, the lowest TBARS values were obtained for samples of pork sausages treated with rosehip polyphenols

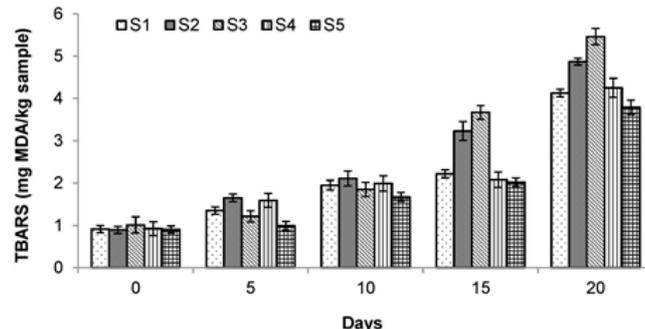


Fig. 2. Changes in TBARS value for smoked pork sausages during storage at 4°C for 20 days. S1-polyphenols 0.04%; S2-polyphenols 0.02%; S3 -sodium nitrite 0.01%; S4 - sodium nitrite 0.01%, BHA 0.005% and tetrasodium pyrophosphate 0.3%; S5 - polyphenols 0.005% and sodium nitrite 0.01%

0.005% and sodium nitrite 0.01% (S5), these results indicating that rosehip polyphenols in combination with sodium nitrite are able to protect lipids against oxidation as effective as sodium nitrite, tetrasodium pyrophosphate and BHA mixture (S4), for a period of 20 days of refrigeration.

Changes in proteins patterns

Protein patterns of fresh (day 0) and refrigerated (day 20) smoked pork sausages treated with rosehip polyphenols (S1 and S2), sodium nitrite (S3), sodium nitrite, tetrasodium pyrophosphate and BHA (S4) and sodium nitrite and rosehip polyphenols (S5) are shown in figure 3. All fresh sausages contain myosin heavy chain (MHC) and actin as major proteins. It was noted that the intensity of the actin band was influenced by the type of treatment applied. Thus, at day 0, for the sample treated with rosehip polyphenols in concentration of 0.04% (S1), actin band intensity was 22% lower compared to the one of the samples treated with rosehip polyphenols in concentration of 0.02% (S2). The differences observed between the intensity of actin bands in the presence of different concentrations of polyphenols indicate that actin forms with rosehip polyphenols polyphenol-protein complexes

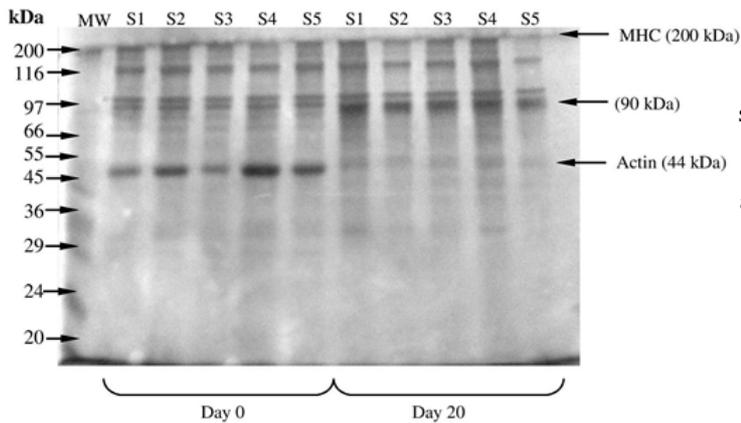


Fig. 3. SDS-PAGE patterns for soluble proteins extracted from smoked pork sausages at day 0 and after 20 days of storage at 4°C. S1 - polyphenols 0.04%; S2 - polyphenols 0.02%; S3 - sodium nitrite 0.01%; S4 - sodium nitrite 0.01%, BHA 0.005% and tetrasodium pyrophosphate 0.3%; S5- polyphenols 0.005% and sodium nitrite 0.01%

with low solubility. Generally, the increase of polyphenols concentration favours the formation of polyphenols-protein insoluble aggregates [41].

At day 0, the strongest intensity of the band corresponding to actin was noted for the sausages treated with sodium nitrite, tetrasodium pyrophosphate and BHA (S4) while the weakest intensity was recorded for sausages treated with sodium nitrite (S3). These observations suggest a more pronounced dissociation of actin-myosin complex in the presence of tetrasodium pyrophosphate, followed by the increase of corresponding proteins solubility, and the presence of some oxidative processes at the protein level, in the absence of an antioxidant, which reduces proteins solubility. Actomyosin dissociates in the presence of pyrophosphate ions and solubility of actin increases [42, 43]. Protein pattern of sausages treated with sodium nitrite and rosehip polyphenols (S5) was similar to that obtained for sausages treated with sodium nitrite, tetrasodium pyrophosphate and BHA (S4), with the difference that actin band intensity was 33% lower. The obtained results demonstrate that rosehip polyphenols reduce the solubility of proteins, especially actin.

After 20 days of refrigeration at 4°C, protein patterns have undergone profound changes. In all sausages samples, the intensity of actin-corresponding band decreased by 60-80%, the strongest decrease being recorded for sausages treated with sodium nitrite (S3). The decrease of actin-corresponding band intensity was associated with the increase of the band corresponding to 90 kDa intensity, suggesting the occurrence of polymerization reactions at the level of some polypeptide chains. After 20 days of refrigeration, protein pattern for sausages treated with rosehip polyphenols in concentration of 0.04% (S1) was similar to that of the sausages treated with sodium nitrite, tetrasodium pyrophosphate and BHA (S4), which means that rosehip polyphenols protected proteins in sausages as efficient as the mixture consisting of sodium nitrite, tetrasodium pyrophosphate and BHA.

Microbiological analysis

Changes in TPC of smoked pork sausages treated with rosehip polyphenols and other substances during 20 days of refrigeration are shown in figure 4. TPC values obtained in day 0 showed a low microbial charge in all samples. After 20 days of refrigeration at 4°C, the microbial charge was significantly different between samples, being strongly influenced by the used additives. TPC values obtained for sausages treated with 0.02% rosehip polyphenols (S2) were significantly lower than the ones obtained for 0.04% concentration (S1) ($p < 0.05$). TPC values obtained for samples treated only with sodium nitrite (S3) were significantly lower than the ones obtained for samples treated with rosehip polyphenols (S1 and S2) ($p < 0.05$),

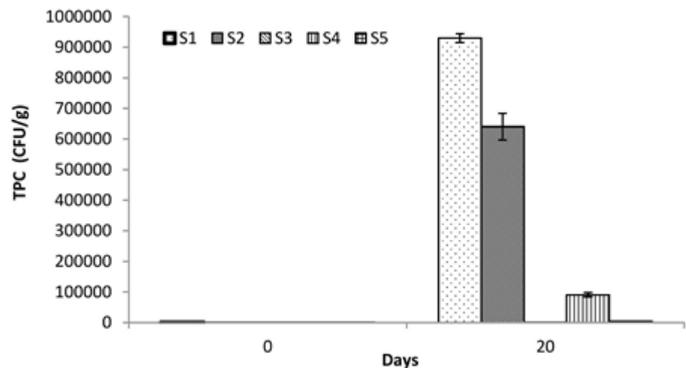


Fig. 4. Changes in total plate count (TPC) in smoked pork sausages during storage at 4°C for 20 days. S1-polyphenols 0.04%; S2 - polyphenols 0.02%; S3-sodium nitrite 0.01%; S4- sodium nitrite 0.01%, BHA 0.005% and tetrasodium pyrophosphate 0.3%; S5 - polyphenols 0.005% and sodium nitrite 0.01%

demonstrating that rosehip polyphenols used alone had a low antimicrobial effect.

TPC values obtained for the samples treated with sodium nitrite, tetrasodium pyrophosphate and BHA (S4) were significantly higher than the ones of the samples treated only with sodium nitrite (S3) ($p < 0.05$). TPC values obtained for the samples treated with rosehip polyphenols and sodium nitrite (S5) were significantly lower than the ones obtained for samples treated with sodium nitrite, tetrasodium pyrophosphate and BHA (S4) ($p < 0.05$). These results demonstrate that the treatment of sausages with rosehip polyphenols in concentration of 0.005% and sodium nitrite 0.01% provides superior antimicrobial activity compared to the treatment with sodium nitrite 0.01%, tetrasodium pyrophosphate 0.3% and BHA 0.005%, but lower than the one of sodium nitrite used alone in concentration of 0.01%.

Sensory analysis

Scores of colour, flavour, odour, texture, and taste of smoked pork sausages treated with rosehip polyphenols (S1 and S2), sodium nitrite (S3), sodium nitrite, BHA and tetrasodium pyrophosphate (S4), and sodium nitrite and rosehip polyphenols (S5) are shown in Figure 5. The scores for all attributes continuously decreased with increasing of storage time.

The colour of sausages was significantly influenced by the used additives and refrigeration period. Between day 0 and day 20, the scores for colour obtained for samples treated with rosehip polyphenols did not differ significantly ($p > 0.05$) depending on the concentration -0.04% (S1) or 0.02% (S2). During storage, the highest scores for colour were obtained for sausages treated with sodium nitrite, BHA and tetrasodium pyrophosphate (S4), while the samples treated only with polyphenols had the lowest scores (the scores for colour in case of S1 and S2 were

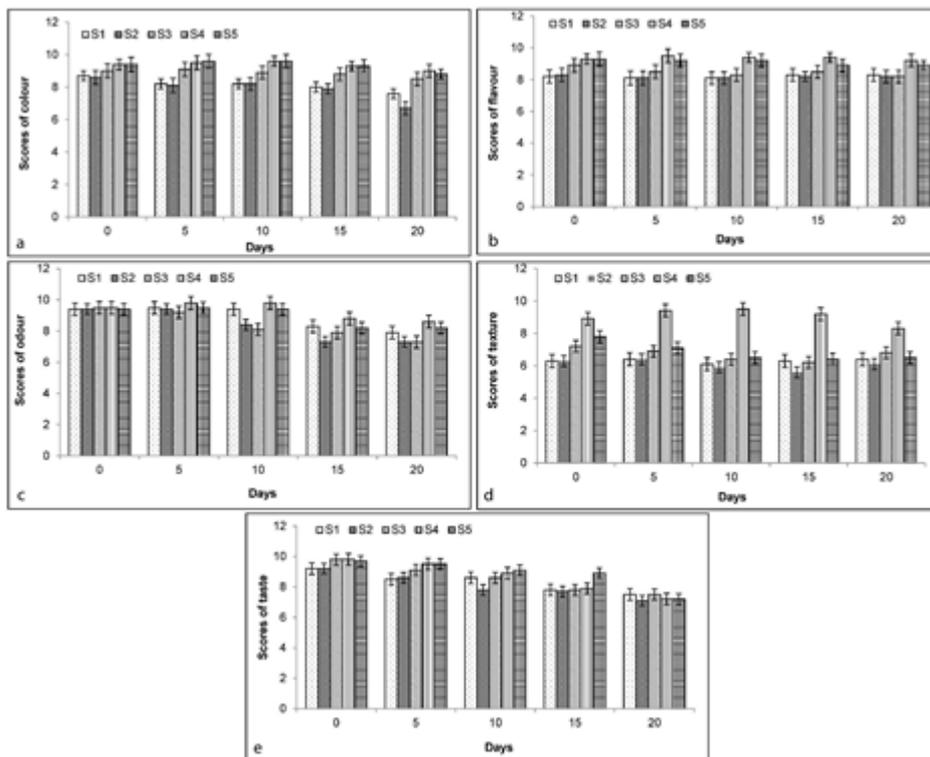


Fig.5. Scores of color (a), flavor (b), odor (c), texture (d), and taste (e) of smoked pork sausages during storage at 4°C for 20 days. S1- polyphenols 0.04%; S2 - polyphenols 0.02%; S3-sodium nitrite 0.01%; S4- sodium nitrite 0.01%, BHA 0.005% and tetrasodium pyrophosphate 0.3%; S5- polyphenols 0.005% and sodium nitrite 0.01%

significantly lower than the ones of S3, S4 and S5; $p < 0.05$). This fact is due to the incapacity of rosehip polyphenols to prevent myoglobin to oxidize to metmyoglobin. Additionally, in the presence of sodium nitrite, myoglobin forms nitric oxide myoglobin, coloured in pink. The scores for colour obtained for samples S3, S4 and S5 were comparable, the differences in mean scores not being statistically significant ($p > 0.05$).

The best scores for flavour were obtained for samples S4 and S5. Average flavour scores for samples treated with polyphenols in concentration of 0.04% (S1) were not significantly different from those of the samples treated with polyphenols in concentration of 0.02% (S2) ($p > 0.05$). Average flavour scores for samples treated with sodium nitrite (S3) were not significantly different from those of the samples treated with polyphenols (S1 and S2) ($p > 0.05$), indicating that, during refrigeration for 20 days, rosehip polyphenols added to smoked pork sausages are able to ensure a flavour similar to the one of sodium nitrite. Average flavour scores of samples S4 were significantly higher than the ones obtained for the samples treated with sodium nitrite (S3) and polyphenols (S1 and S2) ($p < 0.05$).

Rancid odour was detected after 15 days of refrigeration in the case of sausages treated with sodium nitrite (S3) and rosehip polyphenols in concentration of 0.02% (S2), indicating that the use of sodium nitrite in concentration 0.01% alone and rosehip polyphenols in concentration of 0.02% in pork sausages does not sufficiently inhibit the oxidation of lipids, which induces the characteristic rancid odour. For the samples treated with sodium nitrite, BHA and tetrasodium pyrophosphate (S4), the likeness scores were slightly higher than the ones of samples treated with sodium nitrite and rosehip polyphenols (S5). Average scores for odour, on the period 0-20 days, for the samples treated with polyphenols in concentration of 0.04% (S1) were significantly higher than those obtained for the samples treated with 0.02% polyphenols (S2) and for the samples treated with 0.01% sodium nitrite (S3) ($p < 0.05$). The differences between the average odour scores obtained for samples treated with 0.04% polyphenols (S1) and samples treated with sodium nitrite, tetrasodium

pyrophosphate and BHA (S4) were not significant ($p > 0.05$), indicating that the addition of rosehip polyphenols to pork sausages at a concentration of 0.04% ensures an antioxidant activity capable of maintaining an odour similar to that of the samples treated with sodium nitrite, BHA and tetrasodium pyrophosphate. Average odour scores for samples S4 was significantly higher than that of samples S3 and S2 ($p < 0.05$). Average odour scores for the samples S5 was not significantly different from that of the samples S4 ($p > 0.05$), meaning that rosehip polyphenols in concentration of 0.005% along with 0.01% sodium nitrite inhibit lipid oxidation, providing to the sausages an odour similar to that conferred by sodium nitrite, BHA and tetrasodium pyrophosphate mixture. The results obtained for this parameter are consistent with those obtained at TBARS determination and demonstrate that increased TBARS levels above 5 mg MDA/kg give the sausages a rancid odour. On the other hand, the correlation of the results obtained for odour with those obtained for TBARS demonstrates that polyphenols extracted from rosehip in concentration of 0.02% (S2), as well as sodium nitrite at a concentration of 0.01% (S3), are not able to effectively inhibit lipid oxidation in pork sausages during refrigeration for 20 days.

The highest scores for texture were recorded for sausages treated with sodium nitrite, BHA and tetrasodium pyrophosphate (S4). Phosphate-free sausages samples (S1, S2, S3, S5) had a crumbly texture (which increased during refrigeration), with the accumulation of fat in some areas. During the 20 days, average texture scores for the samples treated with 0.04% polyphenols were not significantly different from those of the samples treated with 0.02% polyphenols ($p > 0.05$). Average texture scores for samples treated only with sodium nitrite (S3) were significantly higher than those of the samples treated with polyphenols (S1 and S2) ($p < 0.05$), which means that these concentrations of rosehip polyphenols can not ensure to smoked pork sausages an appropriate texture. Average texture scores for samples treated with 0.005% polyphenols and 0.01% sodium nitrite (S5) were not significantly different from those of the samples treated only with 0.01%

sodium nitrite (S3) ($p > 0.05$). During the 20 days, average texture scores for samples S4 were significantly higher than those of the samples S1, S2, S3 and S5 ($p < 0.05$), which demonstrates that, in the absence of tetrasodium pyrophosphate, rosehip polyphenols can not ensure to smoked pork sausages a texture appreciated by consumers.

Overall, the highest taste scores were obtained for sausages treated with rosehip polyphenols 0.005% and sodium nitrite 0.01% (S5). After 10 days of refrigeration, the samples treated only with polyphenols (S1 and S2) had a slight pungent taste, the differences between their scores not being statistically significant ($p > 0.05$). Average taste scores of the samples treated with 0.01% sodium nitrite (S3) were not significantly different from those of the samples treated only with polyphenols (S1 and S2) ($p > 0.05$). Average taste scores of the samples treated with sodium nitrite, BHA and tetrasodium pyrophosphate (S4) were not significantly different from those of the samples S1, S2 and S3 ($p > 0.05$), which demonstrates that rosehip polyphenols in concentrations of 0.04% and 0.02% do not significantly modify the taste of smoked pork sausages refrigerated for 20 days. Average taste scores for samples S5 were not significantly different from those of the samples S4 ($p > 0.05$), showing that treatment of pork sausages with rosehip polyphenols 0.005% and sodium nitrite 0.01% provide a taste similar to that found in the samples treated with sodium nitrite, BHA and tetrasodium pyrophosphate.

Correlations

For all types of sausages, between the refrigeration time, PV, TBARS, TPC, and sensory parameters, statistically significant correlations were found. Refrigeration period strongly positively correlated with PV ($r = 0.865$), TBARS value ($r = 0.896$) and TPC ($r = 0.788$), and negatively with sausages colour ($r = -0.316$), odour ($r = -0.674$), and taste ($r = -0.790$). Between PV and TBARS value, a significant ($p < 0.05$) strong positive correlation ($r = 0.744$) was observed. Between PV and sensory parameters, significant ($p < 0.05$) negative correlations were found: weak for texture ($r = -0.258$), moderate for colour ($r = -0.342$), strong for odour ($r = -0.699$), and very strong for taste ($r = -0.721$). The results showed that about 7% of the variation of texture, 12% of the variation of colour, 49% of the variation of odour and 52% of the variation of sausages taste were influenced by the relationship of inverse proportionality with PV. Negative correlations were also found between TBARS value and some sensory parameters: moderate for colour ($r = -0.377$), strong for odour ($r = -0.692$), and very strong for taste ($r = -0.758$), which means that about 14% of the variation of colour, 48% of the variation of odour, and 57% of the variation of sausages taste were influenced by the relationship of inverse proportionality with TBARS value. Hundreds of volatile flavour compounds derived from lipid degradation have been found in meat products, including aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids and esters and their concentration increased with storage time [44- 47] and cooking conditions [48 - 50]. The odour and taste of sausages significantly ($p < 0.05$) correlated with TPC: between odour and TPC, a strong negative correlation was found ($r = -0.612$), while between taste and TPC a very strong negative correlation was found ($r = -0.710$), which shows that 37% of the variation of odour and 52% of the variation of sausages taste were influenced by the relationship of inverse proportionality between TPC and odour and taste, respectively. The results obtained correlate with previous

studies on beef stored under different packaging conditions [51].

Conclusions

Polyphenols extracted from rosehip added to pork sausages in a concentration of 0.005% along with 0.01% sodium nitrite are able to retard lipid oxidation, protein degradation, microbial growth and maintain the bright red colour, flavour and taste of smoked pork sausages up to 20 days of refrigeration storage, as effective as the mixture consisting in sodium nitrite 0.01%, BHA 0.005% and tetrasodium pyrophosphate 0.3%. Rosehip polyphenols used alone in concentrations of 0.02 and 0.04% weakly inhibit lipid peroxidation, protein degradation and microbial growth and are unable to maintain colour, flavour and texture of sausages as effectively as associated with sodium nitrite. Rosehip polyphenols could be considered as natural additives with multiple preservative functions in meat and meat products, but their beneficial activity is higher if used along with sodium nitrite.

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