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Effect of the addition of different forms of rosemary (*Rosmarinus officinalis* L.) on the quality of vacuum-packed minced pork

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Abstract

Introduction: Apart from their antioxidant activity, plant-derived bioactive compounds can also positively affect the quality of meat and meat products by improving their sensory and microbiological properties and preventing discolouration. The aim of this study was to determine how the addition of different forms of rosemary improved the quality of pork. **Material and Methods:** Minced pork samples were divided into a control sample without additives (C) and three experimental samples with certified additives (15 mg/kg of meat each), *i.e.* rosemary oleoresin (ROL), extract (REX) and essential oil (REO). Each was further divided into three subsamples; the first was evaluated before storage, and the second and third were evaluated after respective 7- and 14-day vacuum-packed storage at 2°C. The TBARS value was expressed as mg of malondialdehyde (MDA) per kg of meat. Colour was determined based on the values of the standard colour space values of L* (lightness), a* (redness) and b* (yellowness) as well as C* (chroma) and h° (hue angle). Sensory attributes of the samples were evaluated on a nine-point scale. The pour-plating procedure was used for the enumeration of *Pseudomonas*, mesophilic lactic acid bacteria, psychrotrophic bacteria and rods of the *Enterobacteriaceae* family. Within each bacterial group, the most common colonies were identified by matrix-assisted laser desorption and ionization (MALDI). **Results:** Lipid oxidation was most effectively inhibited by REO. The addition of ROL and REO to pork lightened its colour. Meat with REO had stronger redness, whereas meat with ROL had stronger yellowness. The addition of REX affected the sensory properties of pork most beneficially. Neither *Enterobacteriaceae* nor *Pseudomonas* spp. were detected in REO pork, which also contained lower counts of lactic acid bacteria than group C pork. **Conclusion:** The results of this study indicate that rosemary has antioxidant and antimicrobial properties, and may improve the colour and sensory attributes of pork. The effect exerted by rosemary on meat quality may vary depending on the physical form of the additive.

Keywords: *Rosmarinus officinalis* L., antioxidant, antimicrobial, pork quality, meat colour.

Introduction

Preservatives and antioxidants prolong the shelf-life of meat and meat products, mostly by inhibiting fat rancidification and microbiological processes (9). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) limit lipid oxidation, but they may also exert adverse effects on human health (35). Consumers are increasingly likely to make informed food choices and avoid meat products containing synthetic additives. In turn, food producers often replace synthetic additives with natural substances to meet customer expectations (12). Research has shown that natural antioxidants are often more effective than synthetic additives in preventing adverse changes in the quality of meat and meat products (6). Recent years have witnessed a growing interest in plant-based preparations because of their natural origin and health-promoting properties. These substances exhibit anticancer and anti-inflammatory activities, reduce the risk of atherosclerosis, diabetes and heart attack, decrease blood cholesterol levels and blood pressure, and boost the immune system (18). Apart from their antioxidant activity, plant-derived bioactive compounds can also positively affect the quality of meat and meat products by improving their sensory and microbiological properties and preventing discolouration (28).

Herbs and spices, including rosemary (*Rosmarinus officinalis*), can be a rich source of natural antioxidants for use in the meat industry (22). Rosemary is a popular culinary herb that contains antioxidant and antimicrobial substances such as rosmanol, rosmariquinone, rosmaridiphenol and carnosol (24). It can be used as a flavouring in the forms of fresh or dried leaves, essential oil or extracts (including oleoresin). Rosemary oil is a colourless or pale-yellow liquid that turns brown during prolonged storage. Similarly to dried leaves, rosemary oil has a pungent, spicy flavour and a strong, herbal aroma. It is distilled from rosemary leaves and flowers. Rosemary extract is obtained from leaves by extraction. Oleoresins are semi-solid extracts that contain essential oils; their taste is much stronger than that of the corresponding herbs (31).

The antioxidant and antimicrobial activities of rosemary have been described in the literature (4, 17), and they have been attributed to the presence of phenolic acids (caffeic, ferulic and rosmarinic acids) and phenolic diterpenes (carnosic acid and carnosol). However, the relative efficacies of different forms of rosemary have been insufficiently investigated (14, 34). The optimal amounts and forms of the natural plant-based additives used in the meat industry should be established, and their effectiveness in preserving various types of fresh and processed meat should be evaluated. Therefore, the aim of this study was to determine if the quality of vacuum-packaged and cold-stored minced pork was significantly affected by the addition of rosemary and to what different extents it was affected by different forms (oleoresin – ROL, extract – REX and essential oil – REO). The analysed parameters were the rate of lipid oxidation, pH, colour, microbiological quality and sensory properties, which were evaluated during refrigerated storage.

Material and Methods

Samples. The experimental materials comprised 12 samples of *musculus longissimus dorsi* (LD muscle) from the carcasses of pigs raised in Poland categorised as class E in the EUROP classification system. The meat was purchased in a processing plant in north-eastern Poland. During and after carcass chilling at 2°C for 24 h in the meat-processing plant, the pH of the LD muscle was measured at the 15-h and 24-h points between the 1st and the 2nd lumbar vertebrae to eliminate meat with quality defects such as being pale, soft and exudative or dark, firm and dry. Next, during half-carcass dressing, the LD muscles were cut between the 4th and the 5th thoracic vertebrae and along the flank edge anterior to the hip bone, leaving cartilage at the loin.

The muscle samples were processed in an electric meat mincer to pass through a 2.7 mm mesh screen and divided into four portions. The first, the control portion (C) contained no additives. Natural food additives approved for use and available in retailers were added at 15 mg/kg of meat to the remaining portions. Rosemary oleoresin (ROL) was added to the second portion, rosemary extract (REX) was added to the third and rosemary essential oil (REO) to the fourth. The final amounts of additives were determined based on the results of a preliminary laboratory study. A sensory analysis was performed by a consumer assessment conducted by university staff members. Samples were evaluated before storage (ST0) and after 7 (ST7) and 14 (ST14) days of storage in a refrigerator (2°C). The weight of each sample was approximately 200 g (10 samples per group). The ST7 and ST14 samples were vacuum packaged in polyamide/polyethylene bags with enhanced gas barrier performance (permeability: O_2 = 27 cm^3/m^2/24 h/0.1 Mpa; CO_2 = 86 cm^3/m^2/24 h/0.1 Mpa; N_2 = 8 cm^3/m^2/24 h/0.1 Mpa; moisture vapour < 3.5 g/m^2/24 h) with the use of a PP15 (MGO) Tepro Vacu Tronic 2000 vacuum packaging machine (Tepro, Koszalin, Poland). The vacuum-packaging procedure achieved a vacuum of 99%, equal to 1.3 kPa. Samples for assessment at ST0 were not packaged.

Evaluation of pH. The pH of the investigated meat was measured in homogenates (meat-to-distilled water ratio of 1 : 1) with a Polilyte Lab combination electrode (Hamilton Bonaduz, Bonaduz, Switzerland) and a 340i pH-meter equipped with a TFK 325 temperature sensor (WTW Wissenschaftlich-Technische Werkstätten, Weilheim, Germany).

Lipid oxidation. The rate of lipid oxidation was determined in a thiobarbituric acid reactive substance (TBARS) assay. Absorbance was measured with a Specord 40 spectrophotometer (Analytik Jena, Jena, Germany) at a wavelength of 532 nm. The TBARS value was expressed as mg of malondialdehyde (MDA) per kg of meat (25) using the following formula:

$$TBARS = 5.5 \times A$$

where A is the absorbance value of the tested sample and 5.5 is the conversion factor calculated from the standard curve and the dilutions used.

Colour. Colour was determined based on the standard colour space values of L* (lightness), a* (redness) and b* (yellowness) as well as C* (chroma) and h° (hue angle). The L*, a* and b* parameters were measured by the reflectance method using a HunterLab MiniScan XE Plus spectrocolorimeter (Hunter Associates Laboratory, Reston, VA, USA) with the standard D65 illuminant, a 10° standard observer angle and a 2.54-cm-diameter aperture. The values of L*, a* and b* were the means of three replicate measurements performed at random sites across the muscle before and after storage, on unpackaged samples. The apparatus was standardised using black and white standard plates. The values of C* were calculated from the following formula: $C^* = (a^{*2} + b^{*2})^{1/2}$. The values of h° were calculated from the following formula: $h° = \tan^{-1}(b \div a)$.

Sensory analysis. A sensory analysis was performed by six panellists in individual compartments illuminated with white light (500 lx) and maintained at a temperature of 2°C. The colour, taste and aroma of the meat were assessed after heating to 96°C in a 0.6% NaCl solution for 90 min. Between evaluations, the panellists were offered still mineral water with a neutral taste and aroma for cleansing the palate. The samples were spherical in shape and had a weight of around 20 g. The samples were evaluated by the panellists immediately after heat treatment. All sensory attributes of the samples were evaluated during a single session on a nine-point scale: 9 – liked extremely, 8 – liked very much, 7 – liked moderately, 6 – liked slightly, 5 – neither liked nor dislike, 4 – disliked slightly, 3 – disliked moderately, 2 – disliked very much, 1 – disliked extremely.

Microbiological analysis. The preparation of samples for microbiological analysis, identification of bacterial colonies and counting of the identified bacteria (*Pseudomonas* spp., lactic acid bacteria (LAB), psychrotrophic bacteria and *Enterobacteriaceae*) were carried out with the method given by Śmiecińska *et al.* (32).

Statistical analysis. The results were processed statistically using the Statistica version 13.3 (TIBCO Software Inc., Palo Alto, CA, USA). The normality of data distribution was checked by the Shapiro–Wilk test. The effects of experimental factors – additive (C, ROL, REX or REO) and storage time (ST0, ST7 or ST14) – on the analysed parameters of meat and their interactions were determined by two-way analysis of variance. Since interactions between the experimental factors were not found (P-value > 0.05), the significance of differences between group means was estimated by Tukey's test and the significance of differences between group means was determined at P-value ≤ 0.05. Variability was expressed as the standard error of the mean.

Results

Lipid oxidation and pH. The effect of different forms of rosemary and of ST on the pH and TBARS values (mg MDA/kg of meat) of the minced pork is presented in Table 1.

Pork samples to which ROL had been added were characterised by higher pH values than the control samples (C) and samples with the addition of REX and REO (P-value < 0.001). The pH of the vacuum-packaged pork was also influenced by ST, and was higher at ST14 than at ST0 and ST7 (P-value < 0.001).

Storage time had no impact on oxidative changes in minced pork (P-value = 0.146). Rosemary essential oil was most effective in preventing lipid oxidation (P-value < 0.001), whereas no significant differences in TBARS values were observed between C, ROL and REX samples (P-value > 0.05).

Colour parameters. The colour parameters (L*, a*, b*, C* and h°) of minced pork are presented in Table 2.

The addition of ROL and REO caused an increase in the values of L* relative to these values where REX was added and where nothing was added (C) (P-value < 0.001). Meat samples with the addition of REO had stronger redness (a*), and meat samples with the addition of ROL displayed more yellowness (b*), compared with the remaining samples (P-value < 0.001).

Table 1. Mean thiobarbituric acid reactive substance (TBARS) values as mg malondialdehyde/kg meat and pH values of minced pork without and with one of three forms of rosemary

	Additive				Storage time (ST)			SEM	P-value	
	C n = 30	ROL n = 30	REX n = 30	REO n = 30	ST0 n = 40	ST7 n = 40	ST14 n = 40		Additive	ST
pH	5.68 ^{b}	5.72 ^{a}	5.68 ^{b}	5.70 ^{b}	5.68 ^{b}	5.69 ^{b}	5.72 ^{a}	0.003	<0.001	<0.001
TBARS	0.49 ^{a}	0.39 ^{a}	0.41 ^{a}	0.13 ^{b}	0.36	0.34	0.32	0.013	<0.001	0.146

$^{a, b}$ – superscript letters indicate significant difference within an experimental factor (P-value ≤ 0.05); SEM – standard error of the mean; C – control (meat without additives); ROL – meat with rosemary oleoresin; REX – meat with rosemary extract; REO – meat with rosemary essential oil; ST0 – before storage; ST7 – after 7 days of storage; ST14 – after 14 days of storage

Table 2. Mean colour parameters (lightness (L*), redness (a*), yellowness (b*), chroma (C*) and hue angle (h°)) of minced pork without and with one of three forms of rosemary

	Additive				Storage time (ST)			SEM	P-value	
	C n = 30	ROL n = 30	REX n = 30	REO n = 30	ST0 n = 40	ST7 n = 40	ST14 n = 40		Additive	ST
L*	58.55 $^{b, c}$	59.61 ^{a}	57.74 ^{b}	60.24 ^{a}	59.03	59.03	59.04	0.142	<0.001	0.964
a*	9.45 ^{b}	8.31 $^{b, c}$	9.72 ^{b}	10.66 ^{a}	9.36	9.55	9.70	0.127	<0.001	0.183
b*	17.56 $^{a, b, c}$	22.34 ^{a}	21.12 ^{b}	19.00 ^{c}	19.96	20.01	20.05	0.215	<0.001	0.824
C*	19.96 $^{b, c}$	23.84 ^{a}	23.25 ^{a}	21.79 ^{b}	22.10	22.19	22.32	0.187	<0.001	0.546
h°	61.79 ^{b}	69.62 ^{a}	65.29 $^{b, c}$	60.74 ^{b}	64.74	63.22	63.98	0.419	<0.001	0.371

$^{a, b, c}$ – superscript letters indicate significant difference within an experimental factor (P-value ≤ 0.05); SEM – standard error of the mean; C – control (meat without additives); ROL – meat with rosemary oleoresin; REX – meat with rosemary extract; REO – meat with rosemary essential oil; ST0 – before storage; ST7 – after 7 days of storage; ST14 – after 14 days of storage

Table 3. Mean scores for sensory properties of minced pork without and with one of three forms of rosemary, as assessed by six panellists

	Additive				Storage time (ST)			SEM	P-value	
	C n = 30	ROL n = 30	REX n = 30	REO n = 30	ST0 n = 40	ST7 n = 40	ST14 n = 40		Additive	ST
Aroma	7.02 [b, c]	7.95 [a]	8.15 [a]	7.40 [b]	7.53	7.65	7.72	0.069	<0.001	0.177
Colour	7.25 [b]	7.30 [b]	7.75 [a]	7.60 [a]	7.22 [b]	7.68 [a]	7.72 [a]	0.048	<0.001	<0.001
Taste	7.30 [b, c]	8.20 [a]	8.45 [a]	6.65 [b]	7.52	7.68	7.77	0.087	<0.001	0.153

9 – liked extremely; 8 – liked very much; 7 – liked moderately; 6 – liked slightly; 5 – neither liked nor dislike; 4 – disliked slightly; 3 – disliked moderately; 2 – disliked very much; 1 – disliked extremely
[a, b, c] – superscript letters indicate significant difference within an experimental factor ($P \leq 0.05$); SEM – standard error of the mean; C – control (meat without additives); ROL – meat with rosemary oleoresin; REX – meat with rosemary extract; REO – meat with rosemary essential oil; ST0 – before storage; ST7 – after 7 days of storage; ST14 – after 14 days of storage

Table 4. Microbiological quality as mean $\log_{10}$ colony-forming units (CFU)/g of minced pork without or with one of three forms of rosemary

	Additive				Storage time (ST)			SEM	P-value	
	C n = 30	ROL n = 30	REX n = 30	REO n = 30	ST0 n = 40	ST7 n = 40	ST14 n = 40		Additive	ST
Enterobacteriaceae	1.76	2.24	2.23	ND	ND	1.12 [b]	3.11 [a]	0.339	0.054	<0.001
Pseudomonas spp.	0.98	2.07	1.74	ND	ND	0.98 [b]	2.40 [a]	0.284	0.059	<0.001
Lactic acid bacteria	3.54 [a]	3.38	3.41	2.86 [b]	3.26	3.29	3.33	0.093	0.048	0.709
Total aerobic psychrotrophic bacteria	2.20	2.46	2.52	2.05	ND	2.45 [b]	4.62 [a]	0.378	0.971	<0.001

[a, b] – superscript letters indicate significant difference within an experimental factor (P-value ≤ 0.05); SEM – standard error of the mean; C – control (meat without additives); ROL – meat with rosemary oleoresin; REX – meat with rosemary extract; REO – meat with rosemary essential oil; ST0 – before storage; ST7 – after 7 days of storage; ST14 – after 14 days of storage; ND – not detected (below the detection limit and $<1 \times 10^1$ CFU/g)

Pork seasoned with ROL and REX was characterised by higher chroma (C*) values than C and REO samples (P-value < 0.001), and the hue angles (h°) were higher in pork containing ROL than in the remaining samples (P-value < 0.001). All colour parameters remained highly stable during storage (P-value > 0.05).

Sensory properties. The panellists scored the heated minced pork samples as shown in Table 3.

Rosemary extract exerted the most beneficial influence on the aroma, taste and colour of minced pork (P-value < 0.001). Samples containing ROL also received high scores for aroma and taste, and samples with the addition of REO scored highly for colour. Control and REO samples were characterised by the lowest aroma and taste desirability (P-value < 0.001). Only colour was significantly affected by ST, and it received higher scores at ST7 and ST14 than at ST0 (P-value < 0.001).

Microbiological quality. Bacteria of the *Enterobacteriaceae* family and the *Pseudomonas* genus were not detected in meat samples to which REO had been added: bacterial counts in the analysed samples were $<1 \times 10^1$ colony-forming units (CFU)/g. Minced pork with the addition of REO had lower LAB counts than the control samples (P-value = 0.048) and lower counts of psychrotrophic bacteria than the remaining samples (P-value > 0.05) (Table 4).

The counts of all identified bacteria increased during storage (P-value < 0.001), and only the increase in the abundance of LAB was not significant.

Discussion

The pH value of meat is an important parameter that affects various quality attributes such as colour, texture, water-retention capacity, flavour and the stability of its microbial population (7). Meat should have the lowest possible pH during storage to ensure shelf-life stability. Vergara *et al*. (34) added different forms of rosemary (dried powdered, extract, essential oil and oleoresin) to vacuum-packaged lamb meat burgers and found that pH values decreased in all such samples relative to the control group (P-value < 0.001), and that the samples containing oleoresin had the lowest pH (<5.5). These findings differ from the results of this study and from those reported earlier also by Vergara *et al.* (33), when lamb meat burgers seasoned with oregano oleoresin were characterised by the highest pH values (P-value < 0.001). According to them, the effect caused by the format of a herb depends on the herb itself, and a decrease in the pH of vacuum-packaged meat may be due to the growth of LAB that produce lactic acid and the dissolution of CO_2 in the meat aqueous phase.

Amariei *et al.* (5) evaluated the effect of three essential oils (rosemary, thyme and oregano) at different concentrations (0%, 0.5%, 1% and 1.5%) on the pH of minced beef and pork mixed 1 : 1 and found that it was highest in the control samples stored for 4 days at 4°C in closed plastic containers. In that study, all essential oils (regardless of their concentration) effectively prevented any increase in meat pH during storage (P-value < 0.001). Essential oils and other plant-based additives used as natural food preservatives minimise pH changes because they contain antimicrobial compounds such as the isomeric phenols thymol and carvacrol; their precursors α-terpinene, p-cymene and α-pinene; and the alcohols α-terpineol and linalool (23). Rashidaie Abandansarie *et al.* (27) also demonstrated that the lower pH of samples containing REX than of the control samples resulted from the antibacterial activity of the additive (P-value < 0.05). According to the cited authors, the main reason for an increase in pH was the production of volatile amino acids by some spoilage microorganisms such as *Pseudomonas*. Prolonged ST contributes to an increase in the amounts of secondary metabolites produced by microorganisms and to protein deamination; amino acids are broken down and ammonia is produced and accumulates in meat, which increases its pH.

Lipids may undergo changes in response to biochemical (enzymes) and chemical factors (presence of molecular oxygen and exposure to light, increased temperature or water). Lipid oxidation leads to the formation of various compounds such as aldehydes and ketones, which may induce undesirable changes in the colour, aroma, taste, texture and nutritional value of meat and meat products (4). Adverse changes in lipids can be limited provided that meat is stored under conditions that suppress enzyme activity, *i.e.* at low temperatures and with the use of intelligent packaging systems and antioxidants. Recent research has focused on replacing synthetic antioxidants with their natural alternatives (11).

According to Borella *et al.* (10), meat products are considered suitable for consumption if their TBARS values are below 3 mg MDA/kg of meat. In the current study, the TBARS values were much lower in all samples. Vergara *et al.* (34) found that rosemary was effective in preventing rancidity in lamb meat burgers, but that the antioxidant effectiveness of this herb depended on the form in which it was added. In the cited study, ROL was a more potent antioxidant than REO and REX, for which the TBARS values were close to 2 mg MDA/kg at ST14 – a level which compromised consumer acceptability (6). In the present study, TBARS values were considerably below 1 mg MDA/kg of meat, *i.e.* the threshold value for the development of off-odours and discolouration associated with oxidative rancidity (34). Hać-Szymańczuk *et al.* (14) demonstrated that out of REX, dried rosemary and REO, REX exhibited the highest antioxidant capacity in chicken meatballs stored for 14 days and held TBARS values to below 1 mg MDA/kg of meat in all experimental groups, similarly to this experiment. Borella *et al.* (10) also noted low TBARS values in mixed-meat hamburgers (beef, chicken and turkey) with the addition of REX at different concentrations, most likely because the quality of meat was high before processing and the lipid content of the samples was low.

Hać-Szymańczuk *et al.* (14) found that during prolonged storage, lipid oxidation progressed in all meatballs made from vacuum-packaged chicken. However, similarly to the present study, the increase in TBARS values was not significant (P-value > 0.05) in samples protected by any of the rosemary antioxidants. Vergara *et al.* (34) noted an increase (P-value < 0.001) in the TBARS values of vacuum-packaged lamb burgers up to 7 days of storage in the control group samples and in those seasoned with rosemary powder, REO and ROL, and noted this increase up to 10 days of storage only in burgers with the addition of REX; TBARS values did not increase between 10 and 14 days of storage (P-value > 0.05). Olivas-Méndez *et al.* (20) reported that REO, garlic essential oil and chipotle pepper oleoresin (CPO), applied alone or in combination, inhibited lipid oxidation in beef hamburgers packed in polyethylene bags and stored aerobically at 4°C. The rate of lipid oxidation was lower in treatments with CPO and CPO combined with REO (P-value < 0.05). Both additives contain antioxidants; therefore, their combination could produce a synergistic effect. The present study and previous research show that the rate of lipid oxidation may vary depending on the type and form of antioxidant as well as the type of meat and its content of fat and unsaturated fatty acids. In addition, lipid autoxidation is delayed by vacuum packaging because the rate of oxidative change is affected by oxygen concentration. Özyürek *et al.* (21) found that vacuum-packaged and wrapped samples of both fresh lean and chuck beef maintained significantly lower TBARS values than unpackaged samples during all storage periods (P-value < 0.05). However, it should be stressed that oxygen cannot be entirely removed from vacuum or oxygen-free packaging. Even small amounts of residual oxygen can induce lipid autoxidation, in particular in meat with high concentrations of unsaturated fatty acids that are susceptible to oxidative processes.

Piruz and Khani (26) observed a minor influence of ST on the colour of vacuum-packaged and refrigerated chicken breast muscles to which REO or thyme essential oil had been added, which is consistent with the present findings. According to Abdelfattah *et al.* (1) and Borella *et al.* (10), antioxidants present in rosemary delay the oxidation of haem pigments, thus inhibiting the undesirable dark-brown discolouration caused by the formation of metmyoglobin. The rate of oxidation and hence of colour change in meat and meat products is also determined by the storage method (19). In a study by Karpińska-Tymoszczyk (16), the values of b* increased in products packaged in a normal atmosphere as compared with those packaged under vacuum, and differences in the values of this parameter were observed during storage, from the beginning of the oxidation process when yellowness tended to increase because of rancidity.

In the current study, minced pork containing REO was characterised by the highest values of L* and a*. Semenova *et al.* (30) investigated the effects of peppermint, orange, rosemary and cinnamon essential oils on the colour stability of minced pork after five days of chilled storage and noted a significant increase (P-value < 0.05) in redness (a*) in samples containing REO and cinnamon essential oil relative to the control group. However, none of the tested oils significantly (P-value > 0.05) affected the stability of the yellow component (b*). Vergara *et al.* (34) analysed the effects exerted by different forms of rosemary on colour parameters in lamb meat burgers stored for 14 days and found that the values of the L* parameter remained highly stable in all samples. Redness (a*) increased in samples with powdered rosemary (P-value < 0.05), and yellowness (b*) decreased in samples with powdered rosemary and ROL (P-value < 0.01 and P-value < 0.001, respectively). In the cited study, ST affected the colour parameters of rosemary-seasoned meat (P-value < 0.05), which was not observed in this experiment. The high stability of colour coordinates noted in meat samples in this experiment and in the cited study can probably be attributed to the addition of rosemary (18), which is a potent antioxidant, and to the vacuum packaging (7). The differences between the results of the present study and previous research findings could be due to the differences in the tested additives (their type, concentration and composition), and in lipid oxidation patterns, muscle type and light intensity (26).

The less desirable aroma of the control samples could be caused by higher LAB counts (3.54 log CFU/g). According to Kaur *et al.* (17), LAB constitute a significant part of natural microbiota in meat and the dominant spoilage microbiota in meat packaged under vacuum or in a modified atmosphere, and they form slime and off-odours when 7 log CFU/g meat is reached. Herbal extracts, including REX, prevent oxidative changes in meat and contain volatile flavour components that improve the aroma of meat and meat products (13). According to Al-Hijazeen (2), the antioxidant activity of REX extends the shelf life of meat by retarding the formation of aldehydes, sulphur compounds and hydrocarbons, which are responsible for meat rancidity and the development of off-odour volatiles.

Hać-Szymańczuk *et al.* (14) found that the sensory acceptability of REO was lower than that of dried rosemary and REX (P-value < 0.05) because of the dominant aroma of the former. In the present experiment, REO also scored lower for taste and aroma. In the cited study, the appearance and colour of chicken meatballs with the addition of dried rosemary and REX were as acceptable as those of the control samples throughout the storage period. Al-Hijazeen (2) reported that the odour of minced chicken meat supplemented with oregano essential oil and REX at different concentrations was more intense and received higher scores (P-value < 0.05) than control and BHA samples. A positive effect of REX and oregano essential oil on the sensory attributes of poultry meat was also observed by Al-Hijazeen and Al-Rawashdeh (3). They concluded that both natural additives could be effective substitutes for synthetic BHA since they have superior flavour characteristics, exert anti-deterioration effects and are better accepted by consumers.

Borella *et al.* (10) added two commercial rosemary extracts in different concentrations to hamburgers which were made from a mixture of beef, chicken and turkey meat, and refrigerated and stored them for 120 days. They reported that the control product had a residual bitter taste, whereas the formulations with REX liquid condiment had a fresher meaty flavour. A positive impact of REX on the sensory attributes of meat was also confirmed in this study, when the samples containing this form of rosemary scored highest in the sensory analysis.

Piruz and Khani (26) compared the efficacy of thyme essential oil and REO added to vacuum-packaged and refrigerated chicken breast muscles and found that both additives improved the colour of experimental samples relative to the control samples, but that the taste and odour of meat were not desirable at higher additive concentrations. A similar influence of REO was also observed in this experiment, most likely because REO emits a strong aroma composed of camphor (14.5%), cineol (12%), borneol (10.5%), pinene (8.5%) and camphene (7%) (8). Therefore, the concentration of REO should be properly adjusted to different types of meat and meat products.

According to Kompelly *et al.* (18), the antimicrobial action of rosemary results from the effects of phenolic constituents such as rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol, which disintegrate bacterial cell membranes and increase their permeability, thus inhibiting microbial growth and development. Gram-positive bacteria are particularly susceptible to the active ingredients of rosemary. Moreover, rosemary effectively inhibits the proliferation of *Salmonella* and *Listeria* bacteria, *Yersinia enterocolitica*, *Staphylococcus aureus* and *Bacillus cereus*, as well as yeasts and moulds (24). This positive effect, also observed in the current study, is usually associated with an improvement in meat quality, including in its sensory properties and colour stability, which was also reported by other authors (5).

Similarly to this experiment, the study by Hać-Szymańczuk *et al.* (15) revealed a positive effect of REO on the microbial quality of mechanically deboned poultry meat stored at −18°C for four months. In that study, REO was more effective in inhibiting the growth of psychrotrophic bacteria than dried rosemary and REX (P-value ≤ 0.05). The present study and previous research (29) indicate that the bacteriostatic activity of natural additives may vary depending on their physical form and concentration, the type of meat to which they are added and the nature of the meat's processing. Differences in effect on microbial growth also manifest from the differences in properties between natural spices and seasonings added to minced meat (13).

Al-Hijazeen (2) compared the bacteriostatic activity of oregano essential oil and REX with that of a BHA/BHT mixture in minced chicken and found that a combination of oregano essential oil and REX exerted the highest antibacterial effect, noting the effect to be significantly stronger (P-value > 0.05). However, the antibacterial effect of synthetic BHA/BHT was comparable to that of the natural additives when the latter were added separately. According to Vergara *et al.* (33, 34), rosemary in any of its forms is more effective than oregano in inhibiting microbial growth, although the powdered form is least effective. Based on the counts of the particular microbes determined in their studies and the contents of those microbes considered safe, the authors concluded that the shelf life was 10 days for lamb hamburgers seasoned with powdered rosemary and the control samples and 14 days for samples containing REX, REO and ROL.

In the investigation by Vergara *et al.* (34), *Enterobacteriaceae* counts in vacuum-packaged lamb burgers stored for 14 days were similar to those noted in this experiment, although the initial abundance of these microorganisms in the cited study was considerably higher (close to 2 log CFU/g). *Enterobacteriaceae* counts increased during storage (P-value < 0.001) in both the present and the cited experiments, but the analysed forms of rosemary exhibited different antimicrobial activities. Vergara *et al.* (34) demonstrated that the growth of *Enterobacteriaceae* was most effectively inhibited by REX, followed by REO and ROL (which were equally effective), and powdered rosemary was the least effective. The counts of *Pseudomonas* spp. in lamb burgers stored for 14 days were much higher than those noted in this experiment; they reached nearly 7 log CFU/g, which is considered the limit for slime development and undesirable colour changes (17). Moreover, the LAB counts at ST14 determined by Vergara *et al*. (34) were also higher than in this study (close to 5.5 log CFU/g). The efficacy of rosemary was in the following descending order by form: REX (lowest LAB counts), REO (intermediate values) and powdered rosemary and ROL (highest LAB counts). Hać-Szymańczuk *et al.* (14) analysed the influence of different rosemary preparations on the microbiological quality of poultry meat and found that, similarly to this experiment, the counts of psychrotrophic bacteria increased during storage (P-value > 0.05) and the abundance of these microorganisms was not significantly affected by the addition of dried rosemary, REX or REO. The differences between groups noted in this experiment and in the cited study may suggest that the antimicrobial effectiveness of rosemary in inhibiting microbial growth depends on the format added. According to the available literature, the antimicrobial activity of plant-based additives may be determined by their concentrations, storage conditions and substrate composition (7).

Conclusion

The results of this study indicate that rosemary has antioxidant and antimicrobial properties, and that this additive may positively affect the colour and sensory attributes of pork, in particular when combined with vacuum packaging. However, the effect exerted by rosemary on meat quality may vary depending on the physical form of the herb. Lipid oxidation was most effectively inhibited by rosemary essential oil. The tested rosemary-based additives and, probably, the vacuum packaging, delayed lipid oxidation during storage, which significantly extended the shelf life of the meat. Moreover, rosemary, in particular essential oil, had a beneficial influence on the colour of minced pork. The values of all colour parameters remained highly stable during storage. Microbial growth was suppressed only by essential oil, whereas the addition of rosemary extract exerted the most beneficial effect on the aroma, colour and taste of minced pork. An analysis of the impact of storage time on the examined quality attributes of pork revealed that refrigerated storage could be prolonged beyond 14 days with the use of the tested additives and vacuum packaging.

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