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Combined Effect of Modified Atmosphere Packaging and Addition of Rosemary (*Rosmarinus officinalis*), Ascorbic Acid, Red Beet Root (*Beta vulgaris*), and Sodium Lactate and Their Mixtures on the Stability of Fresh Pork Sausages

LUIS MARTÍNEZ, IRENE CILLA, JOSÉ ANTONIO BELTRÁN, AND PEDRO RONCALÉS*

Department of Animal Production and Food Science, Laboratory of Food Technology, Faculty of Veterinary Science, University of Zaragoza, C/Miguel Servet 177, 50013 Zaragoza, Spain

The effects of rosemary, in combination with ascorbic acid, red beet root, and sodium lactate, as well as their mixtures, on the inhibition of both lipid and pigment oxidation of fresh pork sausages packaged in a modified atmosphere were studied. Sausages (240) were packaged in a 80% O_2 + 20% CO_2 gas mixture and analyzed for CIE a^*, metmyoglobin, TBARS, psychrotrophic aerobes, and sensory discoloration and off-odor throughout 20 days of storage at 2 ± 1 °C. The mixture of rosemary + ascorbic acid + sodium lactate + red beet root extract extended the shelf life of fresh pork sausages from 8 to 16 days. Results demonstrated that all of the components of the mixture contributed to obtaining the maximum delay in color and/or odor decay, due to a combined inhibitory action on both pigment and lipid oxidation, as well as on microbial growth.

KEYWORDS: Fresh sausage; modified atmosphere packaging; rosemary (*Rosmarinus officinalis*); red beet root (*Beta vulgaris*); sodium lactate

INTRODUCTION

The color of fresh meat products is of major importance from a consumer viewpoint (*1*). Hence, color stability during storage and retail display is important to meat processing and the retailers. The widespread use of modified atmosphere packaging (MAP) for fresh meat products has generated problems regarding color stability of fresh ground meat products stored under high concentrations of oxygen during retail display (*2*). Lipid and myoglobin oxidation are closely related (*3*), and they are major factors of meat quality deterioration, affecting color, odor, flavor, texture, and nutritional value (*4*, *5*). Processing operations, such as particle size reduction (*6*), heating, and salting (*7*), can alter the pro-oxidative/antioxidative balance of muscle foods by mixing oxidation catalysts with lipid, oxidizing myoglobin, releasing protein-bound iron, and inactivating antioxidant enzymes (*8*−*10*).

Meat processors are interested in using ingredients that are more acceptable to consumers (*11*). The search of crude extracts of a variety of herbs rich in phenolic compounds is increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional values of food (*12*). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential (*13*). Rosemary (*Rosmarinus officinalis*) is a plant of the Labiatae family, whose major and most active components are carnosol, carnosic acid, and rosmarinic acid (*14*−*18*). Several authors have demonstrated that rosemary, alone or with ascorbic acid (AA), is an important contributor to the oxidative stability of meats, including fresh sausages (*19*−*25*).

However, the use of natural antioxidants alone did not provide a sufficient protection against early discoloration of comminuted meat products such as fresh pork sausages, which showed an improved but limited shelf life in the presence of a rosemary and AA mixture (*25*). Accordingly, interest in natural food colors has increased in the past few years. Red beet root (RBR, *Beta vulgaris*) is a rich source of pigments known as betalains (*26*), which might be envisaged as a natural food colorant despite its poor stability; an interactive effect of light, oxygen, water activity, pH, and temperature on the loss of red pigments has been observed (*27*, *28*). Nevertheless, it has been demonstrated that color shelf life of fresh sausages increased 4 days by the effect of RBR (*29*). Betalains include two classes of compounds: betacyanins, which are red violet, and betaxanthins, which are yellow. The major betalain in red beet is betanin, which is a betanidin 5-O-β-glucoside containing a phenolic and a cyclic amine group (*30*). Purified betanin is a common additive approved for use in meat products (*31*).

Off-odor formation and discoloration are also promoted by microbial spoilage, so growth of psychrotrophic microorganisms results in limiting shelf life (*32*). The use of natural antimicrobials is therefore among the strategies employed to minimize and control undesirable changes in fresh meats. Sodium or

* To whom correspondence should be addressed. Tel: +34-976-761582. Fax: +34-976-761612. E-mail: roncales@unizar.es.

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potassium salts of lactate have been shown to strongly delay the growth of meat spoilage microorganisms (*33*). Particularly, sodium lactate (NaL) is a GRAS ingredient in numerous food products (*34*). The antimicrobial effect of lactates is well-documented; their effect on lipid and pigments oxidation in meats is also well-known (*35*−*37*).

The objective of the present research was to determine the effects of natural antioxidant rosemary, in combination with ascorbic acid, colorant RBR extract, and antimicrobial NaL, as well as their mixtures, on the inhibition of both lipid and pigment oxidations and, consequently, on the extension of the shelf life of fresh pork sausages packaged in a modified atmosphere.

MATERIALS AND METHODS

Materials. Rosemary powder (Flavor Guard P) was kindly supplied from Chr. Hansen GmbH (Holdorf, Germany). AA was obtained from Sigma Chemical Co. (St. Louis, MO). NaL (Acetolac 30) was kindly purchased by Sofral (Strasbourg, France). All reagents and solvents used in this work were of analysis grade and were obtained from Merck (Merck, Darmstadt, Germany) and Sigma (Sigma-Aldrich, Gillinghan-Dorset, England).

RBR Juice Preparation. The crude extract was obtained according to Han et al. (*28*); the roots were washed, dried, and cut into cubes of about $1 \times 1 \times 1$ cm^3 and blanched for 5 min in boiling water. After the water was removed and the beet chunks were rapidly cooled, beet juice was extracted with a standard kitchen food processor (Moulinex). The crude juice was heated to boiling and maintained at 100 °C for 1 min in order to remove coagulating components. After rapid cooling, it was clarified stepwise through a filter (MN 640w, Machinery Nagel GmbH & Co. KG, Düren, Germany). The concentration of betanin was not determined. The clear beet juice was stored under refrigeration until use.

Sample Preparation. Four pork forelegs (initial pH 5.5−5.7) were excised from two pork carcasses 48 h postslaughter from a local supplier (MARBE, Zaragoza, Spain), the external fat was trimmed off, and the pork was ground using an industrial grinder machine (Gesame, S. L., Barcelona, Spain) through a plate with 4 mm holes. Minced lean meat was divided in eight batches, which were mixed with NaCl (sodium chloride) (to a final concentration of 20 g kg^{-1}) and (concentrations as suggested by the providers) with either (i) control (no additions), (ii) rosemary powder (1 g kg^{-1}) + AA (500 mg kg^{-1}), (iii) NaL (30 g kg^{-1}), (iv) rosemary powder (1 g kg^{-1}) + AA (500 mg kg^{-1}) + NaL (30 g kg^{-1}), (v) RBR juice (1 mL kg^{-1}), (vi) RBR juice (1 mL kg^{-1}) + rosemary powder (1 g kg^{-1}) + AA (500 mg kg^{-1}), (vii) RBR juice (1 mL kg^{-1}) + NaL (30 g kg^{-1}), or (viii) RBR juice (1 mL kg^{-1}) + rosemary powder (1 g kg^{-1}) + AA (500 mg kg^{-1}) + NaL (30 g kg^{-1}). To ensure even distribution of the components throughout, they were mixed with NaCl and dissolved in distilled water (50 mL kg^{-1}). The same amount of distilled water was added to the control batch.

Two hundred forty sausages (weight, 80 g; diameter, 17 mm; length, 15 cm) were stuffed into collagen casings Colfan F (Viscofan S. A., Caseda, Spain) to a size of 17 mm. The fresh sausages were placed on polypropylene trays ($22.0 \times 13.5 \times 4.5$ cm^3), introduced in a pouch made of a polyethylene and polyamide (80/20 μm thickness) with a water vapor permeability of 5−7 g/m^2/24 h at 23 °C and an oxygen permeability of 40−50 mL/m^2/24 h at 23 °C (Sidlaw Packaging-Soplaril, Barcelona, Spain), and filled with a 80% O_2 + 20% CO_2 gas mixture, supplied by Abelló Linde S. A. (Barcelona, Spain). The fresh sausages were stored for 20 days at 2 ± 1 °C in the dark, as a first approach to establish the shelf life of the sausages prior to subsequent studies of retail life under lighting. Five packs were opened for subsequent analysis for each treatment every 4 days of storage; two of them were used for microbial sampling alone, while the remaining three were used first for sensory analysis, second for color instrumental analysis, and thereafter for the determination of pH and TBARS.

Color Measurement. A reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan) with D65 illuminant and 10° standard observer position was used to measure color changes in the surface of fresh pork sausages by recording CIE L^* (lightness), a^* (redness), and b^* (yellowness) values. The h^* value (hue angle) and C^* (chroma) were calculated as follows: $h^* = \tan^{-1}(b^*/a^*)$ and $C^* = [(a^*)^2 + (b^*)^2]^{0.5}$ (*38*). Exterior surface measurements were obtained in 10 locations 30 min after package opening. Each value was the mean of 30 determinations, trying to avoid the zones with an excess of fat to achieve the measurements that were representative of the real color of the fresh pork sausages. Because of the large volume of data associated with this study, only a^* values were statistically analyzed and related to other properties. a^* values were considered to be important because they reflect the amount of red color and would be of interest to compare with visual evaluations of color (*39*). Color data were obtained at days 0, 4, 8, 12, 16, and 20 for fresh pork sausages.

Metmyoglobin (Metmb) Percentage. The surface Metmb percentage was estimated spectrophotometrically measuring reflectance at 525 and 572 nm (*40*). The average value of the ratios of $(K/S)_{572}$ (absorption is defined as "K" and scattering is defined as "S") to $(K/S)_{525}$ at the beginning of the experiment was fixed as 0% Metmb. The value of 100% MetMb was obtained after oxidizing a sample in a 1% (w/v) solution of potassium ferricyanid. The average value for each fresh sausage was the mean of 30 determinations.

Lipid Oxidation. It was measured in triplicate by the 2-thiobarbituric acid (TBA) method (*41*). Meat samples of 10 g were taken and mixed with 10% trichloroacetic acid, using an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany). Samples were centrifuged at 2300*g* for 30 min at 5 °C; supernatants were filtered through quantitative paper (MN 640 W, Machinery-Nagel GmbH & Co. KG). Two milliliters of the filtrate was taken and mixed with 2 mL of thiobarbituric acid (20 mM); tube contents were homogenized and incubated at 97 °C for 20 min in boiling water. Absorbance was measured spectrophotometrically (Unicam model 5625 UV/vis, Cambridge, United Kingdom) at 532 nm against a blank that contained all of the reagents minus the sample. The concentration of the samples was calculated using slope and intercept data of the calculated least-squares fit of the curve. Results were reported as TBA reactive substances (TBARS) in mg malonaldehyde/kg sample.

Microbial Sampling and Analysis. Twenty-five grams of the meat mixture was taken from the fresh sausages after opening of the package with a sterile scalpel and tweezers and was mixed with 225 mL of 0.1% sterile peptone water. Each sample was homogenized in a Stomacher Lab Blender (model BA6021; Seward Laboratory UAC House, London) for 1 min. Serial 10-fold dilutions were prepared by diluting 1 mL in 9 mL of 0.1% sterile peptone water. Two plates were prepared from each dilution by pouring 1 mL in plate count agar (PCA) fluid agar (Merck). Duplicate plates were counted after incubation at 7 °C for 10 days (*42*). Counts were expressed as the log of colony-forming units (CFU)/g.

Determination of Meat pH. The pH of meat samples was measured using a micro pH meter model 2001 (Crison Instruments, Barcelona, Spain) after homogenization of 3 g of sample in 27 mL of distilled water for 10 s at 1300 rpm with an Ultra-Turrax T25 (Janke & Kunkel). Each value was the mean of three replicates.

Sensory Evaluation. Samples of fresh sausages were evaluated by a six-member trained panel (four males and two females and ranged in age from 25 to 48 years). They were habitual with meat and taste panels and consisted of graduate students and faculty of the Food Technology Laboratory. Although already skilled in this kind of evaluation, panelists received further training prior to analysis according to the method described by Djenane et al. (*43*). Four open-discussion sessions of approximately 1 h were held to familiarize the individual with the attributes and the scale to use. For rating surface discoloration, samples with about 0, 10, 20, 60, and 100% discoloration were presented. For rating odor, meat samples presenting different off-odor characteristics within the range of the evaluation scale were used. Standard samples were packaged pork sausages either fresh or stored at 4°C for different times for up to 3 weeks to allow discoloration and off-odor formation related to meat deterioration. The samples were presented in polystyrene trays identified with three-digit random numbers. Each panelist received samples of each treatment randomly numbered and served. All samples were evaluated under cool white fluorescent lightning, positioned so that it provided 800 lux at the counter surface. The samples for evaluation were presented at room

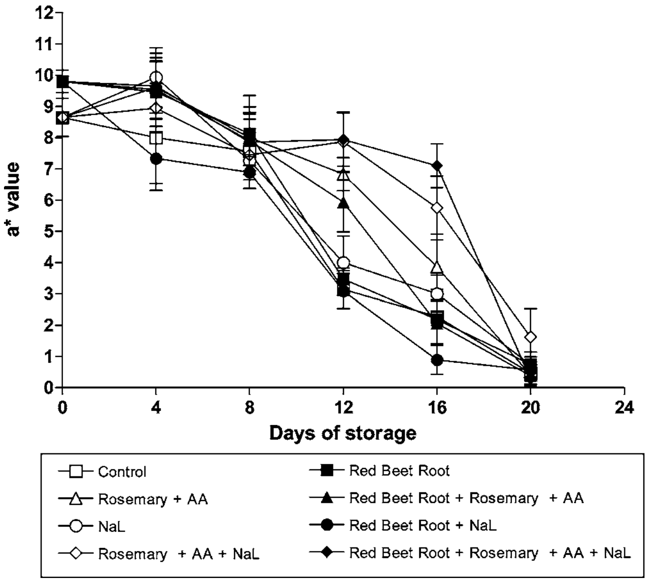

Figure 1. Evolution of *a** values in fresh pork sausages treated with different mixtures of rosemary + AA, RBR, and NaL, packaged under modified atmosphere (80% O_2 + 20% CO_2) and stored at 2 ± 1 °C.

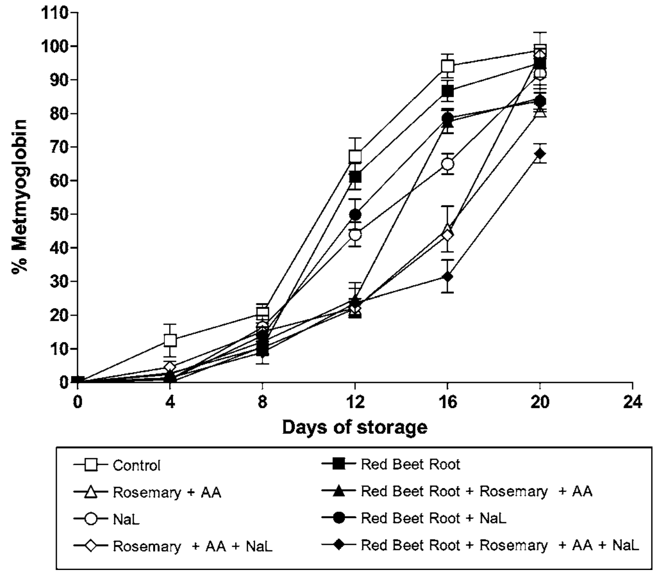

Figure 2. Evolution of Metmb percentage in fresh pork sausages treated with different mixtures of rosemary + AA, RBR, and NaL, packaged under modified atmosphere (80% O_2 + 20% CO_2) and stored at 2 ± 1 °C.

temperature (about 25 °C). Samples of fresh sausages were evaluated 30 min after opening using a five-point scale according to Djenane et al. (*37*): Discoloration scores referred to the percentage of discolored surface: 1, none; 2, 0−10%; 3, 11−20%; 4, 21−60%; and 5, 61−100%. Odor scores referred to the intensity of off-odors associated with meat spoilage: 1, none; 2, slight; 3, small; 4, moderate; and 5, extreme.

Statistical Data Analysis. Analysis of variance (ANOVA) was determined, using the least square difference (LSD) method of the General Linear Model procedure of SPSS (*44*), each day of storage to determine the significance of differences among samples. The statistical significance of differences between mean values was analyzed by using Tukey's test of the general linear model. Differences were considered significant at the $p < 0.05$ level. Pearson's method was used in order to assess correlation between sensory scores (discoloration, off-odor) and instrumental variables (Metmb formation, redness, and TBARS values). The entire experiment was replicated twice.

RESULTS AND DISCUSSION

Color. The evolution of surface redness values (*a**) of fresh pork sausages is shown in **Figure 1**. Sausage red color increased due to the incorporation of colorant RBR from 8.6 to 9.6 ($p < 0.05$). This effect was already observed by Martínez et al. (*29*). However, all of the samples suffered a progressive redness decrease throughout the storage period ($p < 0.05$). At the 12th day, *a** of control sausages drastically fell down to values near 3. All sausages without the antioxidant mixture of rosemary + AA had the same behavior, showing no significant differences with control samples. On the contrary, sausages with the antioxidant mixture had *a** values above 5 at the 8th day of storage, but only those containing antioxidants and antimicrobial NaL showed values above 5 at the 16th day of storage. Sausages also including RBR reached the highest values of all samples at that time ($p < 0.05$).

Similar results were found by Martínez et al. (*25*) regarding the use of a mix of rosemary and AA. McCarthy et al. (*20*) observed that inclusion of rosemary increased significantly the *a** value on pork meat at the 9th day of storage. A synergic effect of rosemary and AA was reported by Sanchez-Escalante et al. (*21*, *24*) and Djenane et al. (*22*). Concerning the effect of lactate, Tan and Shelef (*32*) found that red color (*a** value) was enhanced by lactates immediately after its addition to the meat. However, *a** values declined rapidly after 4 days at 2 °C and were 50−70% lower than the initial values after 8 days. Jensen et al. (*33*) found that the use of 2% NaL in pork chops preserved the red color the first days of storage, but meat suffered a pronounced decrease of *a** thereafter. Brewer et al. (*45*) reported a decrease in *a** values with an increase in NaL in fresh ground pork with initial cell numbers higher than 5 log CFU/g refrigerated for 14 days. The addition of NaL, alone or in combination with sodium propionate, was reported to improve lean meat pigment in vacuum-packaged ground beef (*46*). On the other hand, Bloukas et al. (*47*) found that 2.0% lactate added to vacuum-packed, low-fat frankfurters did not affect color stability during 6 weeks of chill storage. Our results did not show any protecting effect of lactate on red color.

Most noticeable was the fact that RBR did not protect sausages from discoloration, despite it increased redness. It only was effective when it was present within a complete mixture with antioxidants and antimicrobials. This behavior might be explained because betalaines, like other natural pigments, are subject to rapid degradation by oxygen and light. Therefore, products containing those colorants must be protected against oxidation. Furthermore, pH values of sausages with RBR were the lowest of all samples (about 5.6; $p < 0.05$; results not shown). Nevertheless, Wettasinghe et al. (*48*) have reported that high-pigment red phenotypes of beet root are most capable of inhibiting Metmb H_2O_2-mediated oxidation.

Metmb Formation. Figure 2 shows the values of surface Metmb percentage. Results were in very good agreement with those of CIE *a**, although in the contrary sense; so, all of the comments for **Figure 1** apply to **Figure 2**. According to the results shown, a Metmb percentage of 40% corresponded approximately to an *a** value of 5. In fact, Djenane et al. (*22*) reported that about 30−40% Metmb was needed to cause relevant meat discoloration. A similar behavior was observed in a previous work with fresh pork sausages (*2*).

Lipid Oxidation. TBARS values are depicted in **Figure 3**. All sausages started with very low values of about 0.105 mg malonaldehyde/kg sample, indicative of a low lipid oxidation. All samples without antioxidant added suffered a rapid increase of TBARS values, reaching numbers close to 1.5 or above at the 8th day of storage. At days 12−16, all of them had values of about 3 or above, with the exception of sausages with NaL,

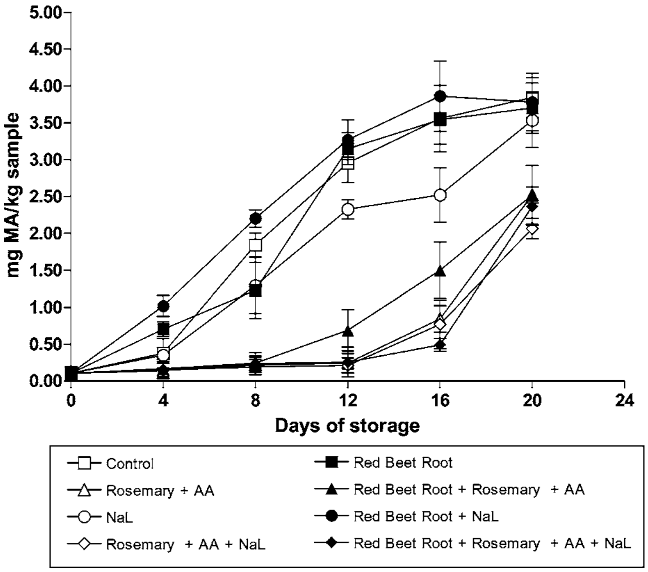

Figure 3. Evolution of lipid oxidation (TBARS) values in fresh pork sausages treated with different mixtures of rosemary + AA, RBR, and NaL, packaged under modified atmosphere (80% O_2 + 20% CO_2) and stored at 2 ± 1 °C.

which had significantly lower TBARS numbers ($p < 0.05$). The presence of a high concentration of oxygen within the package favored lipid oxidation (*2*). Lipid oxidation was strongly inhibited in all sausages containing the antioxidant mixture of rosemary and AA; TBARS values were significantly lower ($p < 0.05$) from the 8th day of storage onward. They needed 16−20 days of storage to reach numbers above 1.5. A similar behavior was already reported by Martínez et al. (*25*).

According to our results, neither lactate nor RBR exerted any antioxidant effect. Studies on the effect of NaL in nitrite-cured meat products showed that 2.0% lactate did not affect lipid oxidation in vacuum-packed low-fat frankfurters during 6 weeks of chill storage (*47*) or in cured cooked mutton during 4 days of aerobic chill storage (*49*). Regarding the lack of effect of RBR, Kahkonen et al. (*12*) found high antioxidant activities in beetroot peel extracts, examined by autoxidation of methyl-linoleate, due to its content of phenolic compounds, while they did not find those compounds in the pulp extracts. However, previous studies have shown a broad range of antioxidant activities in crude extracts of beet root tissues (*50*) and betalain pigments have specifically been shown to possess various antioxidant functions (*30*, *51*, *52*). In any case, a possible interference of the red color of RBR on the TBARS pigment formation should not be discarded. Therefore, further research appears to be needed to deepen the effect of beet root extracts on oxidation.

Large analogies were evident in **Figures 1−3**, indicating coupling between pigment oxidation and lipid oxidation through a mechanism in which the catalytic species (i.e., free radicals) involved in the initiation of lipid oxidation are generated through myoglobin oxidation, as recently discussed (*53*). Indeed, color changes occurred more rapidly than lipid oxidation, as seen from the comparison between development of a^* (**Figure 1**) and TBARS values (**Figure 3**) as a function of time. Because oxidation of myoglobin is specifically acid-catalyzed, the lower pH of sausages with RBR might explain the decreased color stability and increased lipid oxidation in those sausages. To our knowledge, no information is available from the scientific literature on the effects of beetroot extracts on fresh meat lipid oxidation.

Microbial Counts. Counts of psychrotrophic aerobes on sausages throughout storage are shown in **Figure 4**. All samples started with high microbial counts of near 5 log CFU/g, which are common in fresh meat products and should be referred to intense manipulation during sausage preparation, grinding, and stuffing. There were no significant differences among the different samples at the 4th day of storage ($p > 0.05$). On the contrary, large significant differences ($p < 0.05$) were evident after 12 days of storage; sausages formulated without NaL had rapidly growing counts, which reached 7 log CFU/g already at 12th day ($p < 0.05$) and about 8 log CFU/g at the end of the storage period. All samples containing NaL had significantly lower counts ($p < 0.05$) throughout storage; they did not reach 7 log CFU/g even after 16 days of storage. These results clearly demonstrated the inhibitory effect of NaL on microbial growth by extending the lag phase, in accordance with previous reports of Brewer et al. (*54*), who reported that microbial deterioration was delayed by 7−10 days in fresh pork sausage containing 2

Table 1. Influence of Different Mixtures of Rosemary + AA, RBR, and NaL on Sensory Panel Scores (Mean ± SD) of Fresh Pork Sausages Packaged in Modified Atmosphere (80% O_2 + 20% CO_2) at 2 ± 1 °C[a]

		days of storage					
attribute	treatment	0	4	8	12	16	20
discoloration[b]	control	1.0 ± 0.0 av	1.0 ± 0.0 av	2.4 ± 0.3 bw	4.3 ± 0.2 cx	5.0 ± 0.0 cy	5.0 ± 0.0 ay
	rosemary + AA	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	3.3 ± 0.2 bw	3.6 ± 0.5 bw	5.0 ± 0.0 ax
	NaL	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	3.2 ± 0.2 bw	3.3 ± 0.3 bw	5.0 ± 0.0 ax
	rosemary + AA + NaL	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	2.4 ± 0.1 aw	3.3 ± 0.2 bx	5.0 ± 0.0 ay
	RBR	1.0 ± 0.0 av	1.0 ± 0.0 av	2.2 ± 0.4 bw	4.1 ± 0.2 cx	5.0 ± 0.0 cy	5.0 ± 0.0 ax
	RBR + rosemary + AA	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	3.1 ± 0.3 bw	5.0 ± 0.0 cx	5.0 ± 0.0 ax
	RBR + NaL	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	4.1 ± 0.2 cw	5.0 ± 0.0 cx	5.0 ± 0.0 ax
	RBR + rosemary + AA + NaL	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	2.2 ± 0.4 aw	2.6 ± 0.4 aw	5.0 ± 0.0 ax
off odor[c]	control	1.0 ± 0.0 av	1.0 ± 0.0 av	2.5 ± 0.3 bw	4.5 ± 0.2 dx	5.0 ± 0.0 dy	5.0 ± 0.0 by
	rosemary + AA	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	3.0 ± 0.2 bw	4.3 ± 0.3 ax
	NaL	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	3.3 ± 0.3 cw	4.4 ± 0.2 cx	5.0 ± 0.0 by
	rosemary + AA + NaL	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	2.1 ± 0.3 aw	4.0 ± 0.2 ax
	RBR	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	5.0 ± 0.0 ew	5.0 ± 0.0 dw	5.0 ± 0.0 bw
	RBR + rosemary + AA	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	2.2 ± 0.2 bw	4.2 ± 0.3 cx	4.3 ± 0.4 ax
	RBR + NaL	1.0 ± 0.0 av	1.0 ± 0.0 av	2.3 ± 0.3 bw	4.2 ± 0.2 dx	5.0 ± 0.0 dy	5.0 ± 0.0 by
	RBR + rosemary + AA + NaL	1.0 ± 0.0 av	1.0 ± 0.0 av	1.0 ± 0.0 av	2.3 ± 0.0 bw	2.2 ± 0.3 aw	4.4 ± 0.4 ax

[a] Values in the same column with the same letter (a–e) are not significantly different at the 5% level. Values in the same row with the same letter (v–y) are not significantly different at the 5% level. [b] Discoloration: 1, none; 2, 0–10%; 3, 11–20%; 4, 21–60%; and 5, 61–100%. [c] Off odor: 1, none; 2, slight; 3, small; 4, moderate; and 5, extreme.

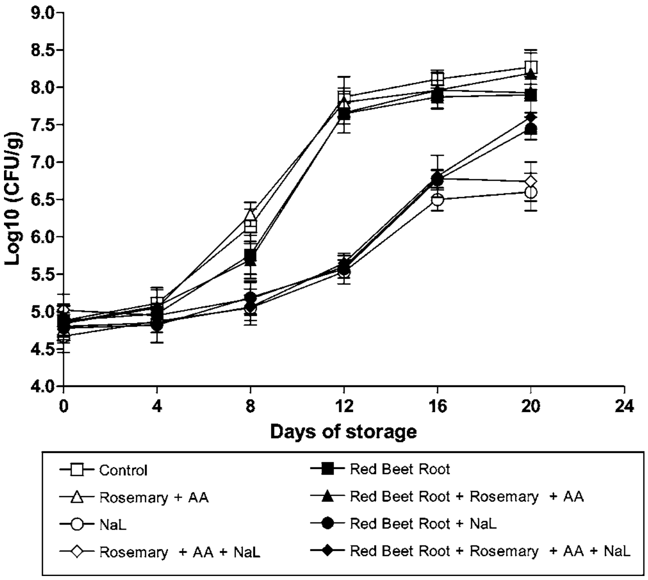

Figure 4. Evolution of psychrotropic aerobes (PCA) in fresh pork sausages treated with different mixtures of rosemary + AA, RBR, and NaL, packaged under modified atmosphere (80% O_2 + 20% CO_2) and stored at 2 ± 1 °C.

or 3% NaL, and Lamkey et al. (*55*), who showed that NaL was effective in maintaining low microbial numbers for extended times in fresh pork sausage chubs stored at 4 °C. Addition of 1−2% NaL to vacuum-packaged fresh pork sausage increased the shelf life by a minimum of 2 weeks, extended the lag phase of aerobic bacteria, altered the growth rate of anaerobic bacteria, and inhibited the growth of anaerobic lactic acid-producing bacteria (*56*).

Sensory Evaluation. Sensory scores for discoloration and off-odor are summarized in **Table 1**. Discoloration of fresh sausages markedly increased since the 8th day and throughout storage following all treatments. Control, RBR sausages, and RBR sausages + NaL were given scores below 3, corresponding to 11−20% discoloration, until the 8th day of storage. Nevertheless, it is worth emphasizing that control samples had scores significantly higher ($p < 0.05$) at that time than any other sausage samples, except those with RBR alone. Sausages containing rosemary + AA + NaL were given the lowest scores, 2.2−2.4 at the 12th of storage; those containing RBR as well did not reach a score of 3 even at the 16th day of storage. Therefore, they would be considered acceptable according to their color (*57*), while the rest of samples would be considered unacceptable. These results appeared to be in close agreement with those of Metmb formation and *a** index. In fact, the correlation coefficients (R^2) of the regression lines were 0.93 and 0.91, respectively.

Regarding off-odor, scores increased throughout storage for all samples, too. At the 12th day of storage, all sausages without rosemary + AA added were given scores above 3. Those containing the antioxidant mixture had scores below 3, a limit of acceptance according to Martínez et al. (*57*), at the same time; furthermore, samples with antioxidant and antimicrobial agents reached the 16th day of storage with scores below 3, independently of the presence of RBR. Sensory results of odor kept a very close relationship with those of lipid oxidation; higher TBARS values corresponded to more intense off-odor, showing a correlation coefficient (R^2) of 0.86.

It might be concluded from these results that the mixture of rosemary + AA + NaL + RBR extract extended the shelf life of fresh pork sausages from 8 to 16 days, showing only small differences with the mixture excluding RBR. Results also demonstrated that all of the components of the mixture contributed to obtaining the maximum delay in color and/or odor decay, due to a combined inhibitory action on both pigment and lipid oxidation, as well as on microbial growth.

ABBREVIATIONS USED

MAP, modified atmosphere packaging; RBR, red beet root; NaL, sodium lactate; NaCl, sodium chloride; TBARS, thiobarbituric acid reactive substances; *L**, lightness; *a**, redness; *b**, yellowness, *h**, hue angle; *C**, chroma; Metmb, metmyoglobin; TBA, 2-thiobarbituric acid; CFU, colony-forming units; PCA, plate count agar; LSD, least-squares difference; AA, ascorbic acid; MA, malonaldehyde.

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