COMBINED EFFECT OF MODIFIED ATMOSPHERE PACKAGING AND ADDITION OF ROSEMARY, ASCORBIC ACID, RED BEET ROOT AND SODIUM LACTATE AND THEIR MIXTURES ON THE STABILITY OF FRESH PORK SAUSAGES

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Introduction

The colour of fresh meat products is of major importance from consumer viewpoint. The widespread of modified atmosphere packaging (MAP) for fresh meat products has generated problems regarding colour stability of fresh ground meat products stored under high concentrations of oxygen during retail display (Martínez et al., 2005). In addition, the use of natural colorants has been investigated with respect to colour stability of fresh meat products (Martínez et al, 2004). Lipid and myoglobin oxidation are closely related (Faustman and Cassens, 1989). Currently, the search of crude extracts of fruits, herbs, vegetables, cereal, and other plant material rich in phenolics are increasingly of interest in the food industry since they retard oxidative degradation of lipids and thereby improve the quality and nutritional values of food (Kahkonen et al., 1999). Rosemary (*Rosmarinus officinalis*) is a natural plant of the *Labiatae* family; its major and most active components are carnosol, carnosic acid and rosmarinic acid. Several authors had demonstrated that rosemary, alone or with ascorbic acid (AA), effectively contributes to oxidative stability of meat products (Djenane et al., 2003; Sánchez- Escalante et al., 2003). Red beet root (*Beta vulgaris*) is a rich source of the betalain pigments group betacyanins (red) and betaxanthins (yellow). Red beet root powder has been used to simulate cured meat colour in cooked, smoked, semidry and fermented sausages. Besides oxidative processes, growth of psychrotrophic microorganisms results in microbial spoilage and limited shelf life. Sodium or potassium salts of lactic acid have been shown to delay growth of meat spoilage microorganisms (Jensen et al., 2002).

Objectives

The objectives of the present research was to determine the effects of natural antioxidant rosemary in combination with ascorbic acid, natural colorant red beet root and sodium lactate, and their mixtures, on the inhibition of both lipid and pigment oxidations, and consequently, on the extension of quality characteristics of fresh pork sausages packaged in modified atmosphere.

Methodology

Preparation of samples. Four pork forelegs (initial pH 5.5-5.7) were excised from two pork carcasses 48 h post-slaughter from a local supplier (MARBE, Zaragoza, Spain), trimmed external fat off, and ground using an industrial grinder machine (Gesame, S. L., Barcelona, Spain) through a plate with 4 mm holes. Minced meat was divided in eight batches, which were mixed with NaCl (to a final concentration of 2%) and with either: 1) Control (no additions), 2) rosemary powder (1000 ppm) + ascorbic acid (500 ppm), 3) sodium lactate (3%), 4) rosemary powder (1000 ppm) + ascorbic acid (500 ppm) + sodium lactate (3%), 5) red beet root (1 ml kg^{-1}) , 6) red beet root (1 ml kg^{-1}) + rosemary powder (1000 ppm) + ascorbic acid (500 ppm), 7) red beet root (1 ml kg^{-1}) + sodium lactate (3%), 8) red beet root (1 ml kg^{-1}) + rosemary powder (1000 ppm) + ascorbic acid (500 ppm) + sodium lactate (3%). The fresh sausages were stuffed into collagen casings, Colfan F (Viscofan S.A., Caseda, Spain), placed on polypropylene trays, introduced in a pouch made of a polyethylene and polyamide and filled with a 80% O_2 + 20% CO_2 gas mixture. Sausages were stored for 20 days at 2 ± 1°C in the dark.

Red beet juice preparation. Fresh red beet roots (*Beta vulgaris*) were purchased from a commercial store. Samples were washed, dried and cut into cubes of about 1cm x 1cm, which were boiled for 5 min for blanching (Han et al, 1998). After rapid cooling, beet juice was extracted with a standard kitchen food processor. The crude juice was boiled at 100ºC for 1 min. After rapid cooling, it was filtered stepwise (MN 640w, Machinery Nagel GmbH & Co. KG, Düren, Alemania). The clear beet juice was stored at refrigeration until use.

Meat colour and metmyoglobin analysis. Meat colour was measured at the surface of fresh sausages using a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan), 30 min after packaging opening. CIE L*, a*, b* parameters were recorded. The metmyoglobin percentage at the surface was estimated spectrophotometrically, according to Stewart et al. (1965), by measuring reflectance at 525 and 572 nm. The average value for each fresh sausage was the mean of 30 determinations.

Lipid oxidation analysis. Lipid oxidation was measured by the 2-thiobarbituric acid (TBA) method of Pfalzgraf (1995). TBARS values were expressed as mg malonaldehyde/kg sample.

Microbial analysis. Counts of aerobic psychrotrophic flora were determined in Plate Count Agar (PCA; Merck; Darmstadt, Germany) after incubation at 10°C for 7 days (Elliott et al., 1983). Counts were expressed as the $\log_{10}$ of colony forming units (CFU)/g.

Sensory evaluation: Six-member trained panel evaluated fresh pork sausages for discolouration and off-odour according to procedures of Djenane et al. (2001). Discoloration; scores referred to percentage of discolored surface: 1 = none, 2 = 0-10%, 3 = 11-20%, 4 = 21-60%, and 5 = 61-100%; Odour scores referred to the intensity of odours associated to meat spoilage: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme. Results were expressed as the predominant score given by panellists.

Statistical analyses. The significance of differences among samples at each day of storage was determined by analysis of variance using the least square difference method of the General Linear Model procedure of SPSS (SPSS 11.5, 2002).

Results & Discussion

Colour (a* values; Fig. 1). Samples with red beet root (RBR) added had higher a* values ($p<0.05$) than control sausages (Martínez et al., 2004). Later they were lower (about 7.5), similar to control samples at the 8th day. After that they suffered a loss of redness with the exception of rosemary + AA + NaL samples with or without RBR, which maintained its colour (8) until the 12th day ($p<0.05$) and 16th day, respectively. Tan and Shelef (2002) found that red colour was enhanced by lactates immediately after their addition to the meat. On the other hand, Jensen et al. (2003) found in pork chops that the use of 2% NaL preserved red colour the first days of storage, after that meat suffered a pronounced decrease of their a* values.

Metmyoblogin percentage (Fig. 2). The amount of metmyoglobin was lower ($p<0.05$) in samples containing sodium lactate and natural antioxidants which maintained a high level of acceptability (below 30%) after 12 days of storage. Djenane et al. (2002) reported that about 30-40% metmyoglobin was needed for causing relevant meat discoloration. Only control samples, RBR and RBR + NaL exceeded this level of acceptability after 12 days of storage ($p<0.05$). The best effect was shown by RBR + rosemary + AA + NaL, these samples had values near 30% after 16 days ($p<0.05$).

Lipid oxidation (Fig. 3). Lipid oxidation was low in sausages packaged with antioxidant alone or with NaL added throughout all the storage time ($p<0.05$), TBARS values after 16 days of the storage were just below 1 mg/kg in all samples with rosemary + AA added, with the exception of RBR with antioxidants, that increased their values to near 1.5 larger than the other sausages packaged with rosemary + AA ($p<0.05$). Results did not show an effective antioxidant activity of RBR on TBARS after packaging. A possible explanation is found by Kahkonen et al. (1999), because red colour of the beet root extract interfered with the determination of the pink TBA chromogen. While the antimicrobial effects of lactates are well documented (Brewer et al., 1995), there are limited reports on the effect of lactates on lipid oxidation and meat pigments. Nnanna et al. (1994) indicated a suppression of lipid oxidation by lactates in raw ground pork during storage for up to 7 days at 0 and 5 ºC. At the last day of storage, all samples that remained with low TBARS increased their values to just above 1 mg/kg.

Microbial counts (Fig. 4). All samples started with very high microbial counts of near 5 $\log_{10}$ (CFU /g), which are common in fresh meat products and should be referred to intense manipulation during meat preparation, grinding and stuffing. Fresh sausages did not show significant differences among different treatments after 4 days of storage ($p>0.05$). Samples formulated without sodium lactate had rapidly growing counts, which reached 7 $\log_{10}$ (CFU /g) at 12th day ($p<0.05$). These samples had counts of about 8 $\log_{10}$ (CFU /g) at the end of the storage period. The rest of the samples with sodium lactate showed no significant differences among them ($p>0.05$) at 8th day, although they were significantly lower ($p<0.05$) than the rest of sausages during all the storage period. Those results clearly demonstrated the inhibitory effect on microbial growth of sodium lactate by extending the lag phase, in accordance with previous reports regarding other authors (Brewer et al., 1995).

Sensory evaluation (Table 1). Discoloration of fresh sausages markedly increased since the 8th day and throughout storage following all treatments. Control, RBR with or without NaL were given a score of 4, corresponding to 21-60 % discoloration, until the 12th day. Rosemary + AA + NaL with or without RBR, were given the lowest scores,

either 2.5 or 3 after 16th of storage. They would be considered acceptable according to their colour, while the rest of samples would be considered unacceptable. These results appeared to be in close agreement with those of metmyoglobin formation and a* index. After 20 days of storage all samples scored 5 points. Regarding off-odour, scores increased throughout storage for all samples. Sensory results of odour had a very close relationship with those of lipid oxidation. After 12th days only control, RBR and RBR + NaL overpassed the limit of acceptability (3 points). At 16^{th} day, all samples with rosemary + AA alone or with NaL added remained below 3 points, the lowest scores corresponded to RBR with antioxidants and NaL.

Conclusions

The use of a mixture containing red beet boot juice, rosemary powder, ascorbic acid and sodium lactate extended the shelf-life of fresh pork sausages stored in modified atmosphere. The samples improved their red colour, metmyoglobin, lipid oxidation and microbial indices after 16 days of storage, showing better sensory scores than the rest of samples. If red colour is used by consumers as freshness indicator and as willingness of purchasing, the use of this natural mixture enhanced the shelf life of this product.

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Table 1. Influence of different mixtures of rosemary, ascorbic acid, red beet root and sodium lactate on sensory panel scores (Predominant score) of fresh pork sausages packaged in modified atmosphere at 2 ± 1 ºC.

		Days of storage					
	Treatments	0	4	8	12	16	20
Discoloration (1)	Control	1	1	2	4	5	5
	Rosemary + AA	1	1	1	3	3,5	5
	NaL	1	1	1	3	3,5	5
	Rosemary + AA + NaL	1	1	1	3	3	5
	Red Beet Root	1	1	2	4	5	5
	Red Beet Root + Rosemary + AA	1	1	1	3	5	5
	Red Beet Root + NaL	1	1	1	4	5	5
	Red Beet Root + Rosemary + AA+ NaL	1	1	1	3	3,5	5
Off-odour (2)	Control	1	1	2	4	5	5
	Rosemary + AA	1	1	1	1	3	4
	NaL	1	1	1	3	4	5
	Rosemary + AA + NaL	1	1	1	1	1	4
	Red Beet Root	1	1	1	5	5	5
	Red Beet Root + Rosemary + AA	1	1	1	2	4	4
	Red Beet Root + NaL	1	1	2	4	5	5
	Red Beet Root + Rosemary + AA+ NaL	1	1	1	2	3	4

[1] Discoloration: 1 = none, 2 = 0 to 10%, 3 = 11 to 20%, 4 = 21 to 60%, and 5 = 61 to 100%.

[2] Off odor: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme.

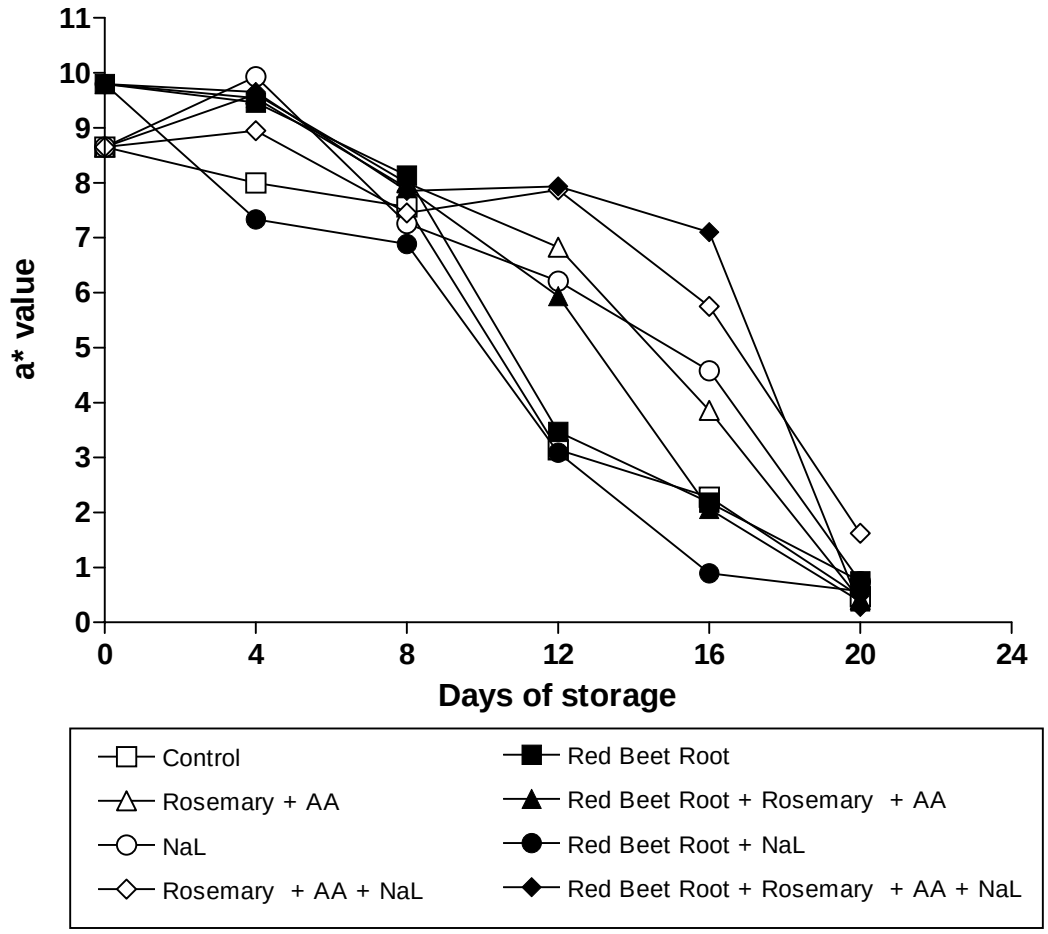

Figure 1. Evolution of a* values in fresh pork sausages treated with different mixtures of rosemary, ascorbic acid, red beet root and sodium lactate, packaged under modified atmosphere and stored at 2±1ºC.

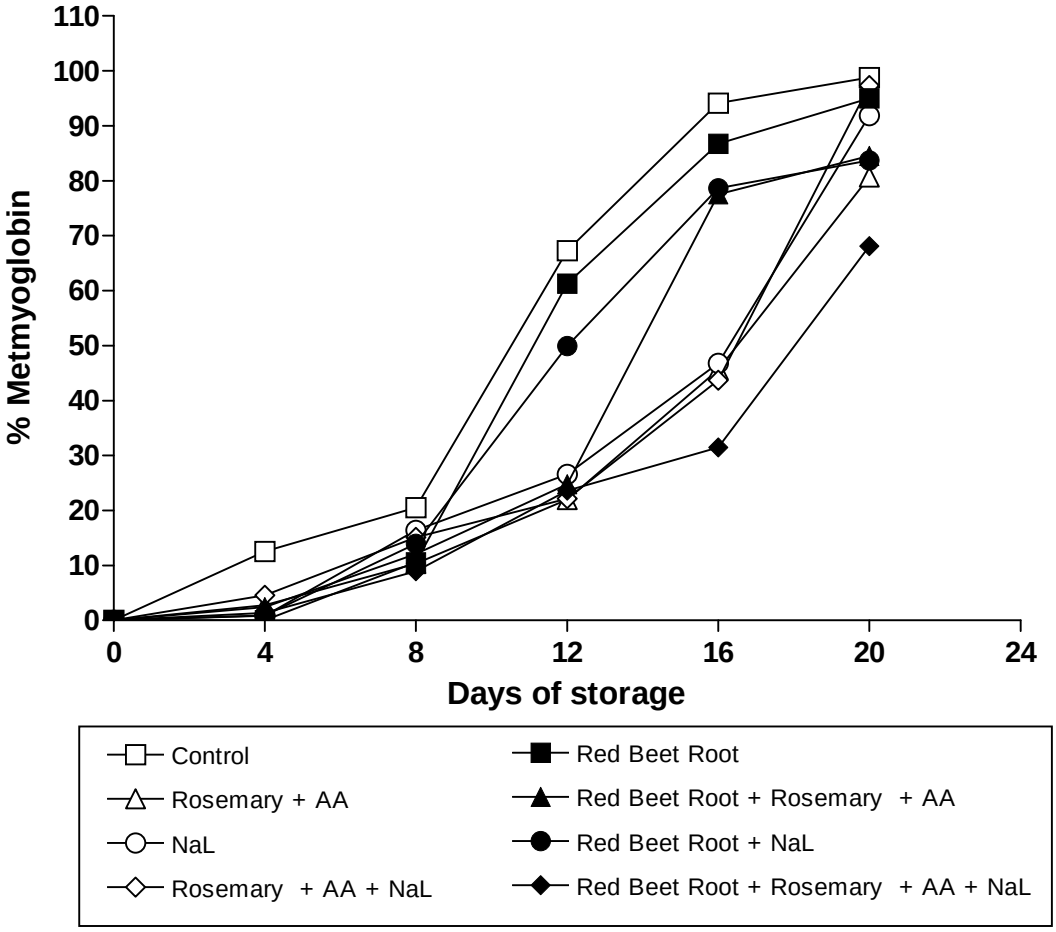

Figure 2. Evolution of metmyoglobin formation (%) in fresh pork sausages treated with different mixtures of rosemary, ascorbic acid, red beet root and sodium lactate, packaged under modified atmosphere and stored at 2±1ºC.

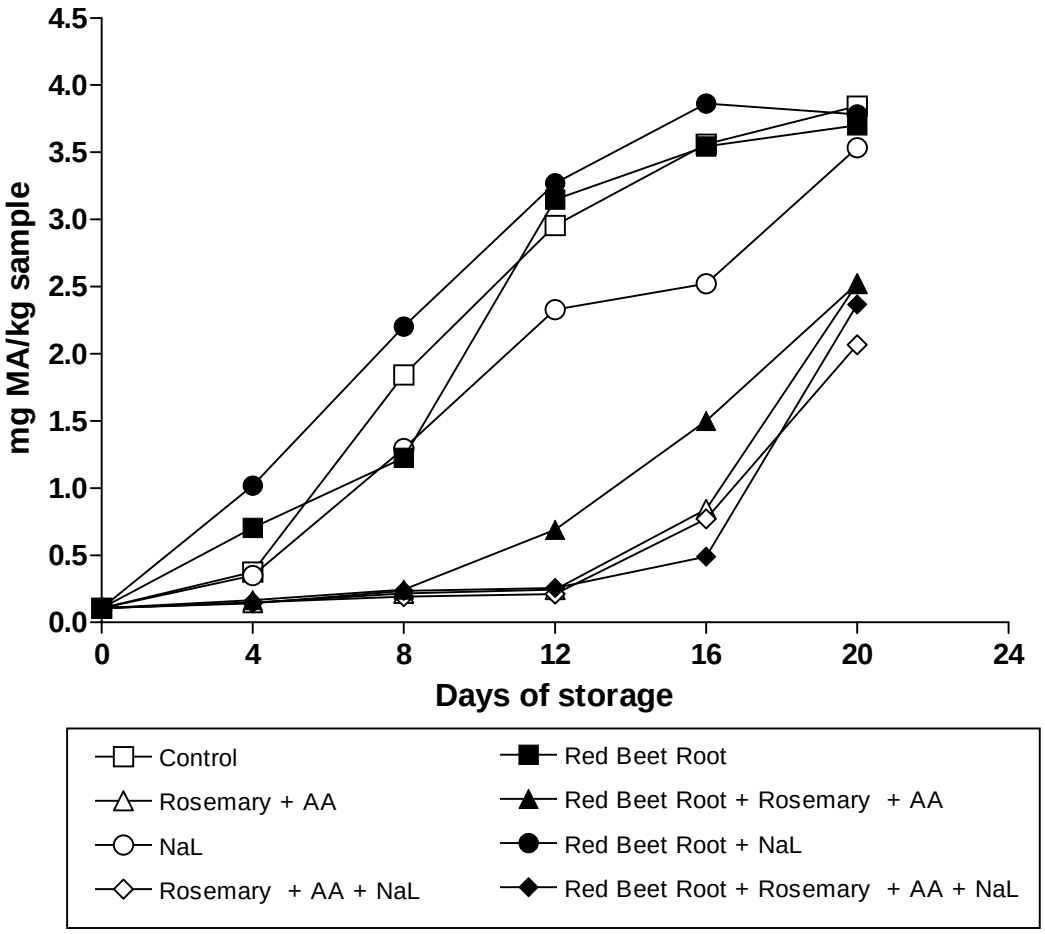

Figure 3. Evolution of TBARS values in fresh pork sausages treated with different mixtures of rosemary, ascorbic acid, red beet root and sodium lactate, packaged under modified atmosphere and stored at 2±1ºC.

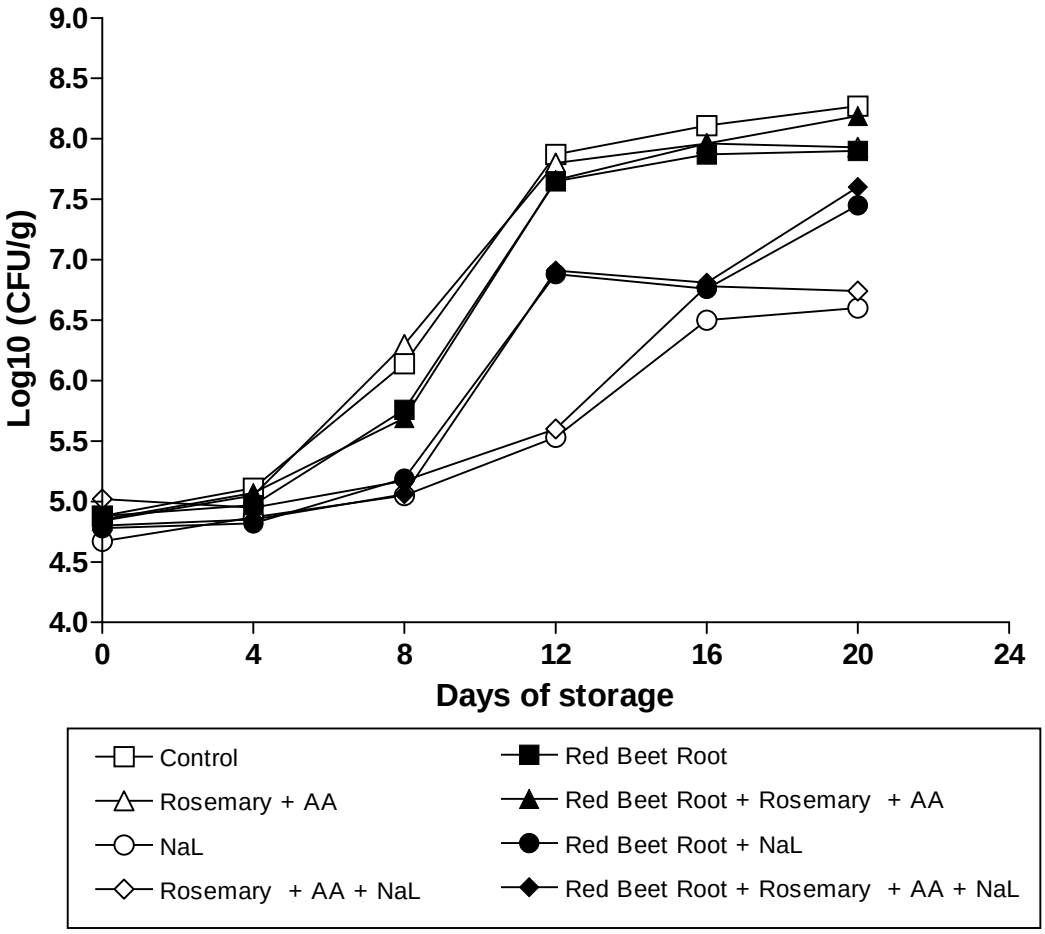

Figure 4. Evolution of psychrotrophic aerobes in fresh pork sausages treated with different mixtures of rosemary, ascorbic acid, red beet root and sodium lactate, packaged under modified atmosphere and stored at 2±1ºC.