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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ROSEMARY EXTRACT, MINT EXTRACT AND A MIXTURE OF TOCOPHEROLS IN BEEF SAUSAGE DURING STORAGE AT 4C

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ABSTRACT

The effects of extracts of *Rosmarinus officinalis* (RE), *Mentha longifolia* (L.) Hudson (ME) and a mixture of tocopherols (Toc) on microbiological parameters and lipid oxidation were investigated in beef sausages stored for 25 days at 4C. The addition of RE resulted in significant ($P \leq 0.05$) inhibition of microbial growth and the lowest microbial counts were obtained in samples containing both RE and ME, indicating a possible synergistic effect. RE and its combination with Toc or ME, but especially the latter, showed the greatest antioxidative effect ($P \leq 0.05$); the use of RE alone was comparable ($P > 0.05$) with ME and its combinations. Shelf lives of samples containing RE were almost doubled compared with the remaining samples and the best antimicrobial and antioxidative effects were obtained from the combination of RE with ME.

PRACTICAL APPLICATION

Food spoilage is considered to be one of the most important economic and health-related matters in the food industry; thus, preservatives are used in food products to inhibit chemical deterioration and the growth of microorganisms. Concerns over the safety of some chemical preservatives and the negative consumer reaction to those perceived as being of chemical and/or artificial origin have prompted increased interest in more natural or "green" alternatives for the maintenance or extension of product shelf life. Many spices and herbs exert antioxidant and antimicrobial activities conferred by compounds present in extracts of these plants or in their essential oil fractions, some of which are classified as generally recognized as safe substances. These compounds could therefore be of considerable interest for use in the food industry as natural preservatives.

INTRODUCTION

Sausage is a meat product that is present in the diets of a range of cultures and is gaining popularity because of its convenience, variety and economy. It takes little time to prepare, with some sausages made "ready to serve" and others that need only to be heated (O'Grady *et al.* 2006). However, there is increasing awareness of the risks posed by microbiological contamination of meat and meat products as this constitutes a major source of pathogens that cause foodborne illness in humans (Haugaard *et al.* 2014).

Due to the high fat content and the comminuted nature of the raw materials, such products are prone to spoilage by both lipid oxidation and microbial contamination. Lipid oxidation contributes to the development of unacceptable organoleptic characteristics, whereas microbial growth may cause both spoilage and foodborne diseases. Delaying lipid oxidation and preventing microbial growth are therefore factors that can make a significant contribution toward the extension of shelf life. In order to achieve these goals, meat product manufacturers have used a number of chemical/synthetic food additives over the past few decades that have antioxidative and antimicrobial properties, such as nitrites.

Nowadays, however, increasing consumer awareness and health consciousness is resulting in pressure to avoid the use of synthetic additives. This trend is also reflected in a recently issued European Union Directive (2006) referring to the necessity to reduce the use of nitrites (European Union Directive 2006/52/EC). Thus, there is a need for research into the use of natural additives or alternative methods in order to extend shelf life and/or improve food safety. Natural antioxidants could provide such a solution as many of them exhibit antimicrobial activity in addition to their capacity to prevent lipid oxidation.

Rosemary (*Rosmarinus officinalis*) extracts (RE) have a potent antioxidant activity and are widely used in the food industry. This activity has been associated with the presence of several phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which terminate free radical chain reactions by hydrogen donation (Almela *et al.* 2006; Azizkhani and Zandi 2010; Zhang *et al.* 2010). A number of researchers have reported the effectiveness of rosemary extracts for retarding lipid oxidation in various foods: Jordan *et al.* (2014) used 500–1000 mg/kg in beef steaks; Ortuno *et al.* (2014) recommended concentrations of 200 or 400 mg/kg to improve meat preservation, while Sebranek *et al.* (2005) reported that the addition of 1000 mg/kg was just as effective as butylated hydroxyanisole/butylated hydroxytoluene in maintaining low thiobarbituric acid reactive substances (TBARS) values in precooked frozen sausage. In addition to the inhibition of lipid oxidation, several authors have reported that some of the compounds present in rosemary extracts also possess antibacterial properties (Djenane *et al.* 2002; Fernandez-Lopez *et al.* 2005; Del Campo *et al.* 2010).

Free radical scavenging activity and lipid oxidation inhibition by *Mentha longifolia* extracts (ME) have also been investigated by several researchers. Scavenging activities were measured in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays and the reaction was seen to follow a concentration-dependent pattern, free radical scavenging capacity increasing as the extract concentration rises. Similarly to RE, ME antioxidant activity might be related to the extract's phenolic content in the form of phenolic acids and polyphenols as reported in previous studies (Jaimand and Rezaee 2002; Yadegarinia *et al.* 2006; Gulluce *et al.* 2007; Kamkar *et al.* 2010). The antimicrobial activities of an extract of *M. longifolia* (L.) Hudson as well as the essential oil of this plant have also been qualitatively and quantitatively assessed as determined by the presence or absence of bacterial inhibition zones, zone diameter and minimum inhibitory concentration (MIC) values. The results obtained indicate that the essential oil of *M. longifolia* (L.) Hudson may be used as a natural preservative in food against well-known causal agents of foodborne diseases and food spoilage such as *Escherichia coli*, *Bacillus* spp., *Salmonella* spp., *Staphylococcus aureus* and *Enterococcus faecalis* (Jaimand and Rezaee 2002; Gulluce *et al.* 2007; Azizkhani and Ataee 2011).

Tocopherols (Toc) are effective natural antioxidants for lipid-containing foods. A mixture of Toc behaves like a chain-breaking electron donor by competing with the substrate for chain-carrying peroxyl radicals and Toc mixtures have been associated with retarding the decomposition of hydroperoxides (Frankel 1998; Azizkhani and Zandi 2010; Azizkhani *et al.* 2011). Although much research has focused on supplementing animal feeds with Toc mixtures in order to improve myoglobin and lipid stability in beef (Sohn *et al.* 2009; Liu *et al.* 2010), turkey (Nam *et al.* 2003) and broiler bird meats (Yasin *et al.* 2012), limited research has been conducted on the *in vitro* treatment of fresh beef sausages. Several studies have demonstrated the beneficial effects of these natural antioxidants in counteracting lipid oxidation and/or the inhibition of microbial growth when applied individually. Due to the interaction of these compounds with the substrate, investigation of their activities across a range of food systems is still needed for successful application to meat products. To the best of our knowledge, the use of these natural antioxidants, either individually or in combination, has not been studied in fresh beef sausages. The objective of the present research was therefore to determine the effect of rosemary extract, mint extract and a mixture of Toc, applied either individually or in combination, on both microbiological parameters and lipid oxidation during refrigerated storage (4C) of fresh beef sausages.

MATERIALS AND METHODS

Natural Antioxidants and Chemicals

The Toc mix oil preparation (670 mg/g; 1000 IU/g; storage at −20C) was obtained from Sigma (Sigma-Aldrich Inc., St. Louis, MO). Rosemary extract containing 30 ± 3% phenolic diterpenes (carnosic acid, carnosol, rosmanol and rosmarinic acid) was purchased from Danisco, Copenhagen, Denmark. All other chemicals used were of analytical grade or the highest grade available and were obtained either from Sigma-Aldrich or Merck (Darmstadt, Germany).

M. longifolia (L.) Hudson plants at the flowering stage were collected from Band-e-pey, a village in the region of Babol, Mazandaran, northern Iran. The taxonomic identification of plant materials was confirmed by the pharmacology department of Mazandaran University of Medical Sciences.

A portion of plant material (500 g) was successively extracted with 1 l of methanol (Merck, Darmstadt, Germany) using a Soxhlet extractor (Isolab, Wetheim, Germany) for 72 h at a temperature not exceeding the boiling point of the solvent (Jaimand and Rezaee 2002; Gulluce *et al.* 2007). The methanol extracts were filtered

using Whatman filter paper (No. 1), concentrated under vacuum at 40C using a Rotary Evaporator (Buchi, Flawil, Switzerland) and subsequently stored at −80C until tested.

Sausage Preparation

Boneless beef meat and fat were obtained fresh from a local market. Beef meat was cut into cubes and stored at 4C for 24 h in order to drain off excess liquid. The raw materials were comminuted separately at −2C and then thoroughly mixed at an appropriate ratio to achieve a fat content of 10 ± 1%. The mixture was comminuted again through a 4-mm steel plate and divided into six equal parts by weight in order to prepare the experimental treatments (Table 1). Five of these were transferred to a commercial grinder/cutter (Seas-nve_Strommen, Hovedgaden, Denmark) where they were mixed with salt (23 g/kg), sunflower oil (6 g/kg) and the appropriate antioxidants; the sixth was retained as the control (Table 1). Sunflower oil was used as the carrier for the Toc mix in order to achieve better distribution in the meat blend and it was also added to the remaining treatments for experimental uniformity. Throughout the procedure, the temperature of the meat blend was kept constant at 4–6C. RE was added to a final concentration of 250 mg/kg and ME was used at a concentration (62 mg/kg) similar to that used (61.30 mg/kg) successfully *in vitro* by Gulluce *et al.* (2007) and Azizkhani *et al.* (2011). The final concentration chosen for Toc mix was 150 mg/kg, slightly greater than the 100 mg/kg successfully applied to beef steaks by Djenane *et al.* (2002) because of the higher fat content of our products. Although the use of other ingredients, including nitrites, phosphates or spices, is permitted in these products, none were added in the present research because some of them have antioxidant and/or antimicrobial properties (Baber and Dilger 2014) that could interfere with the effects of the natural antioxidants added. The meat blends for each treatment were stored at 4C for 8 h. After determining pH values, the blends were stuffed into synthetic casings (cellophane) to achieve a diameter of approximately 2.5–3 cm. The sausages were heated so that their central part reached 70C after which they were cooled and stored at 4C in the dark for 25 days. All experiments were performed in triplicate. The mean percentage contents of protein, fat, moisture and ash for the raw material (meat mixture) used for the preparation of experimental sausages were 17.3%, 11%, 68.2% and 2.1%, respectively. These data are in accordance with Iranian National Standard for Beef Sausages (2011).

Sampling

For all parameters tested, determinations were performed after 0, 5, 10, 15, 20 and 25 days of refrigerated storage on two separate samples and in duplicate. Analyses on day 0 were conducted only for the control samples, except for the determination of the pH value which was conducted for all treatments.

Microbiological Analyses

A 20 g portion from each experimental treatment was homogenized in a Stomacher (Stomacher 400, Lab. Blender, London, U.K.) with 180 mL of sterile chilled 1 g/L peptone (BBL, Sigma-Aldrich Inc., St. Louis, MO) water for 2 min. Serial 10-fold dilutions were prepared in 9 mL volumes of peptone water. Lactic acid bacteria were counted using duplicate 1 mL volumes of suitable dilutions in overlaid pour-plates of MRS (de Man, Rogosa and Sharpe) agar (Merck) incubated inverted at 30C for 3 days (De Man *et al.* 1960; ISO 1996). Enterobacteriaceae counts were determined in overlaid pour-plates of violet red bile glucose agar (Merck) incubated at 37C for 24 h (ISO 1997). For yeast and mold counts, duplicate 0.2 mL volumes of suitable dilutions were spread onto the dry surface of prepoured plates of potato dextrose agar (Merck; pH 3.5) and were incubated at 25C for 5 days. Finally, total viable counts were determined using 1 mL of suitable dilutions on pour-plates of plate count agar (Merck) incubated at 32C for 3 days. All results were reported as $\log_{10}$ colony forming units per gram (cfu/g).

pH Determination

The pH values of the sausage samples were measured after homogenization with distilled water at a 2:8 ratio using a

TABLE 1. ANTIOXIDANT TREATMENTS APPLIED TO THE MEAT MIXTURE FOR THE PREPARATION OF EXPERIMENTAL SAUSAGES

	Treatments					
Antioxidant	RE	ME	Toc	RE + ME	RE + Toc	Con
Rosemary extract (250 mg/kg)	+	−	−	+	+	−
Mentha longifolia extract (62 mg/kg)	−	+	−	+	−	−
Tocopherols mix (150 mg/kg)	−	−	+	−	+	−

Con, control; ME, mint; RE, rosemary; RE + ME, rosemary + mint; RE + Toc, rosemary + tocopherols mix; Toc, tocopherols mix.

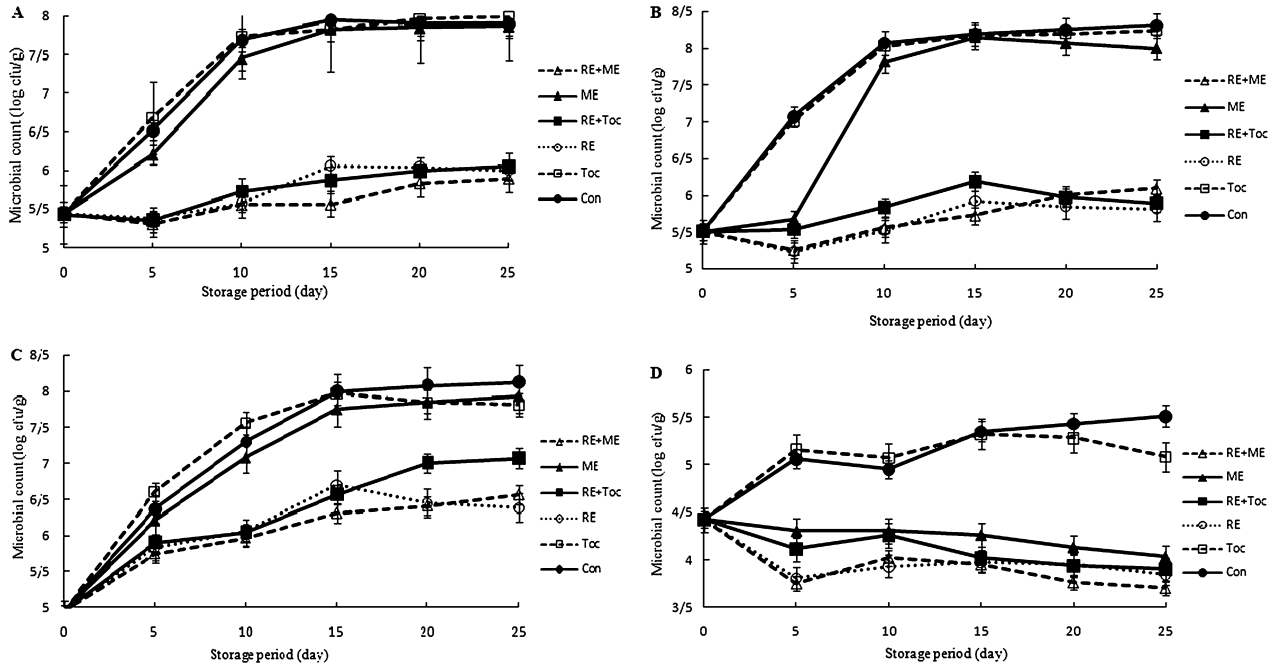

FIG. 1. POPULATIONS OF MICROBIAL GROUPS (CFU/G) DURING REFRIGERATED STORAGE OF THE EXPERIMENTAL SAUSAGES AT 4C FOR 25 DAYS; (A) TOTAL VIABLE COUNT, (B) LACTIC ACID BACTERIA, (C) YEASTS AND MOLDS, (D) ENTEROBACTERIACEAE

digital pH meter (Omega Instruments, Stamford, Connecticut, United States). The means of two measurements were recorded for each sample.

Chemical Analyses

Lipid oxidation was assessed by determination of the primary (hydroperoxides) and secondary (malondialdehyde, MDA) oxidation products formed during refrigerated storage. Lipids were extracted from the sausages on each sampling day according to the procedure of Folch *et al.* (1957) and the determination of lipid hydroperoxides was conducted following the International Dairy Federation standard method 74A:1991 (IDF 1991) for measurement of peroxide value (PV), as modified by Shantha and Decker (1994). Results were expressed as milli-equivalents of hydroperoxides per kg of lipids (mequiv/kg lipids). The concentration of MDA in sausage samples was determined by a selective third-order derivative spectrophotometric method and results were expressed as g MDA/kg wet weight (Bostoglou *et al.* 1994).

Statistical Analysis

All data were analyzed using the general linear model of analysis of variance with treatment and time as factors, after normality and homogeneity of variances were confirmed. The statistical significance of the differences was checked using the Student–Newman–Keuls' test at the 0.05 level. All statistical analyses were conducted using the SPSS statistical package (SPSS 22: IBM, New York, United States).

RESULTS

The results of the microbiological analyses of the sausages over the course of the 25-day storage period are represented graphically in Fig. 1. It can be seen that the counts recorded for both control sausages (Con) and those containing Toc mix alone increased similarly in each of the four microbial groups during the course of storage. These indicators were, however, markedly ($P \leq 0.05$) affected by the presence of RE (either alone or in combination with Toc or ME). Total viable counts (Fig. 1A) and the counts of lactic acid bacteria (LAB; Fig. 1B) and also those of yeasts and molds (Fig. 1C) all showed notably reduced rates of increase in samples treated with RE compared with controls ($P \leq 0.05$).

In the case of the Enterobacteriaceae, all treatments except that of Toc alone resulted in a clear tendency of decreasing counts over time (Fig. 1D). Indeed, while ME did not affect the other microorganisms tested it did have a pronounced effect on the Enterobacteriaceae ($P \leq 0.05$), and counts were even more reduced ($P \leq 0.05$) when RE and ME were used in combination thus indicating a synergistic effect between the two.

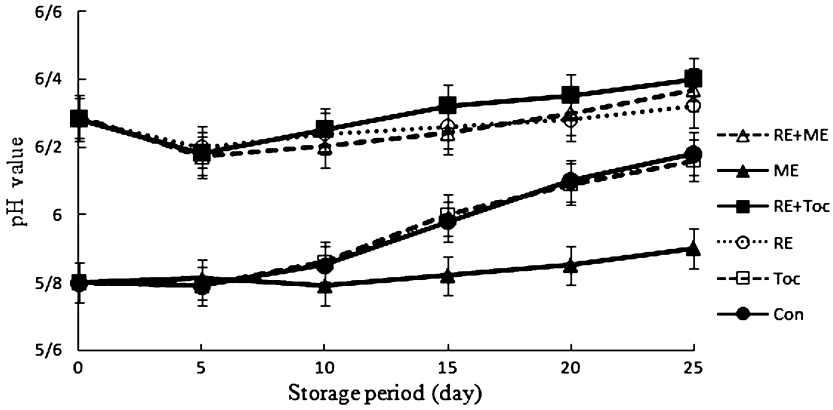

FIG. 2. EFFECT OF NATURAL ANTIOXIDANTS ON pH VALUES OF THE EXPERIMENTAL SAUSAGES DURING THE 25-DAY STORAGE PERIOD

Changes in pH values during the 25-day storage period are presented in Fig. 2. Initially, pH values decreased slightly in all samples until day 5, after which there was a gradual increase that was more accentuated in the controls and those samples containing Toc alone. All samples containing rosemary extract (RE, RE + ME and RE + Toc) showed similar ($P > 0.05$) pH values throughout this period and were significantly ($P > 0.05$) higher compared with those for mint and Toc alone or controls. This may be attributed to both the pH value (7.1) of the rosemary extract itself and the lower LAB populations in these experimental sausages (Fig. 1B).

To assess the impact of the treatments on lipid oxidation during the 25 days of storage, the concentrations of primary oxidation products in the lipid fraction of the sausages were measured as PV together with those of MDA, and these are presented graphically in Fig. 3. Samples containing rosemary extract and its combinations (RE, RE + ME, RE + Toc) along with those containing mint extract alone exhibited the lowest ($P \leq 0.05$) values for both oxidation parameters compared with Toc samples or the controls. The greatest antioxidant effect ($P \leq 0.05$) was obtained with the combination of rosemary and mint extracts (RE + ME), for which the values of both PV and MDA were lower at the end of the storage period (day 25) than those obtained as early as day 5 in controls ($P \leq 0.05$). Furthermore, samples containing the combinations of antioxidants RE + ME and RE + Toc had lower PV concentrations ($P \leq 0.05$) than those containing the individual antioxidants (RE, ME, Toc).

DISCUSSION

Herbal extracts and essential oils have been employed traditionally as flavoring agents in foods and beverages but their use as natural food preservatives has increased considerably during the past few decades and this has been followed by a rapid increase in demand for them throughout the world (Bart 2004; Oussalah *et al.* 2007). The antimicrobial and antioxidative activities of rosemary and mint in a number of food products has been documented (Shahidi *et al.* 1999; Kamkar *et al.* 2010; Azizkhani and Ataee 2011). The deterioration of thermally sensitive products such as beef sausages is a frequent problem, sometimes occurring in a large proportion of the same production batch. Sausages deteriorate because of both microbial spoilage and oxidative rancidity. To examine the possible benefits of herbal extracts as

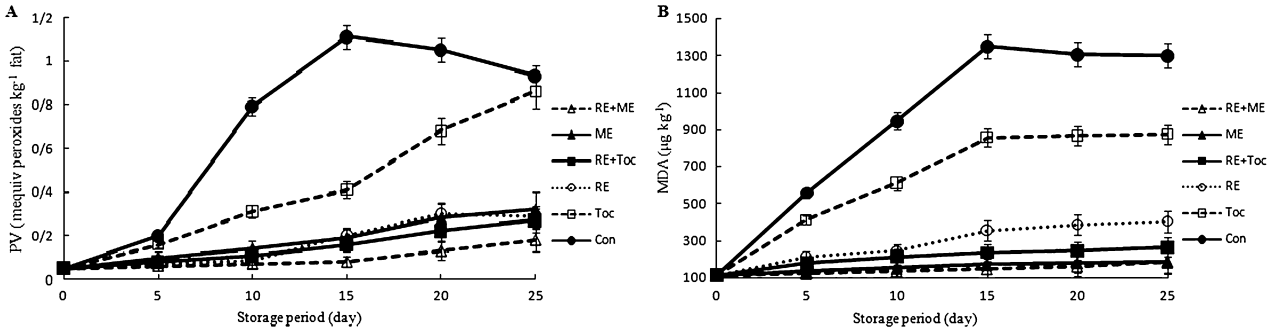

FIG 3. (A) PEROXIDE VALUES (PV) AND (B) MALONDIALDEHYDE (MDA) CONTENT DURING REFRIGERATED STORAGE OF THE EXPERIMENTAL SAUSAGES AT 4C FOR 25 DAYS

preservatives for extending this food's shelf life, the antimicrobial and antioxidative activities of rosemary, mint and a mixture of Toc were assessed in this study.

Yeasts and molds have been pointed out to be major determinants of spoilage in beef sausages when they reach a level of about 7 $\log_{10}$ cfu/g (Samelis and Metaxopoulos 1998). In the present study, the counts of these organisms reached this level in control sausages as well as those treated with Toc and ME alone on the 10th day of cold storage ($P > 0.05$). Counts of samples containing RE, however, were well below this level until the end of the storage period. In addition, samples containing RE had lower microbial counts compared with the other samples regardless of their Gram-positive or Gram-negative classification ($P \leq 0.05$). The antimicrobial activity of RE has been documented both *in vitro* and *in situ* against a number of food spoilage and pathogenic microorganisms, including (among others) *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Bacillus cereus*, *Proteus vulgaris*, *Escherichia coli*, *Vibrio spp.* and *Salmonella typhimurium* as well as several yeasts and molds, with MIC or minimum bactericidal concentrations ranging between 50 and 10000 mg/kg (Shahidi *et al.* 1999).

During the first 5 days of storage, mild acidification of all samples took place followed by a steady rise in pH which was most evident in the controls and those samples containing Toc. This phenomenon is associated mostly with an increase ($P \leq 0.05$) in Gram-negative bacterial populations (Verma and Sahoo 2000) such as Enterobacteriaceae, but also with the growth of yeasts and molds which cause protein and amino acid degradation resulting in the formation of ammonia and a consequent increase in pH (Nychas *et al.* 1998). The development of the larger microbial populations seen in Toc samples and controls over the course of the storage period is consistent with their greater increase in pH compared with the other treatments ($P \leq 0.05$). Similar pH values to those for RE and Toc samples in the current study have been reported in fresh beef patties containing rosemary extract (Mc Carthy *et al.* 2001) and in ground chevon containing Toc mix (Verma and Sahoo 2000). However, pH values in our control samples were higher than the average pH values (5.76 and 5.48) reported in the literature (Ambriosiadis *et al.* 2004; Baber and Dilger 2014). The lower values measured in the latter studies are indicative of fermentation occurring during storage of these products. Sugars are usually added to the sausage mixture and carbohydrates contained in spices which are common ingredients of sausages could also be used as substrates for LAB metabolism resulting in the production of organic acids and a consequent lowering of the pH (Baber and Dilger 2014). The absence of sugar in our experimental sausage mixture could thus explain the higher pH.

With regard to lipid oxidation, Mc Carthy *et al.* (2001) demonstrated that rosemary extract added to a level of 1000 mg/kg in fresh beef patties significantly ($P \leq 0.05$) inhibited oxidation during 9 days of storage at 4C as assessed by TBARS, the corresponding TBARS values for control samples being almost eightfold higher. Similar effects were observed for the addition of various concentrations of rosemary essential oil to beef frankfurters and refrigerated storage for 24 h (Estevez *et al.* 2005; Estevez and Cava 2006). St. Angelo *et al.* (1990) indicated that the antioxidative effect of Toc was weaker than that of rosemary in ground beef patties and that the greatest effects were obtained using antioxidants capable of acting as chelating agents. The superiority of the latter type of antioxidant was also pointed out by Murphy *et al.* (1998) in the case of the refrigerated storage of precooked roast beef slices and could explain the better overall performance of rosemary combinations compared with the individual use of rosemary or Toc in the present study. In this regard, Azizkhani and Zandi (2010) previously reported that the PV of margarine samples containing 200 mg/kg rosemary and 200 mg/kg Toc were at approximately the same level after 14 days of storage at 4C as on day 0 compared with control samples in which PV values increased.

Azizkhani and Ataee (2011) reported that mint extract was able to reduce the stable free radical DPPH with an IC_{50} (concentration providing 50% inhibition) of 55.3 mg/kg, a very similar result to that obtained by Gulluce *et al.* (2007). Lipid oxidation in the beef sausage controls in the current study was found to be greater ($P \leq 0.05$) than that of treated samples, except for those samples containing just Toc on day 25. Maximum values for PV were reached on day 15 of storage and subsequently a decline was observed. After day 15, it is thus possible that the rate of hydroperoxide decomposition exceeded the rate of its formation. Similarly, Antequera *et al.* (1992) reported maximum PV levels in Iberian pig hams on the 15th day of storage at 4C, followed by a decline until the end of the storage period. As regards the MDA content in controls, a peak value was obtained on the 15th day of storage, after which a decline was observed. This could be attributed to either decomposition of MDA by bacteria, such as pseudomonads or Enterobacteriaceae that possess the ability to selectively attack and utilize carbonyl compounds including MDA (Casaburi *et al.* 2014; Fregonesi *et al.* 2014) or to further oxidation of MDA to yield other organic products of lipid oxidation (alcohols and acids) which cannot be determined by reaction with thiobarbituric acid (Almandos *et al.* 1986; Fernandez *et al.* 1997; Georgantelis *et al.* 2007). Sheard *et al.* (2000) indicated an MDA concentration of 500 μg/kg as a threshold for rancidity perception by consumers. Control samples in the present study would therefore be perceived as rancid after the 10th day of storage. MDA contents in

samples containing Toc mix were near the threshold value on the 25th day whereas in the remaining four treatments, MDA content did not exceed 500 μg/kg until the end of the storage period.

The shelf life of meat and meat products corresponds to the storage time until spoilage. The point of spoilage may be defined by a certain maximum acceptable level of a microbial group and/or chemical indicators as well as by an unacceptable off-odor/off-flavor or appearance (Casaburi *et al.* 2014). An estimation of sample shelf life in the present study can be made based on the following criteria: (1) attainment of a level of 7 $\log_{10}$ cfu/g for yeast and mold counts (Samelis and Metaxopoulos 1998; Georgantelis *et al.* 2007) and (2) a threshold value of 1000 μg/kg MDA for the perception of rancidity (Georgantelis *et al.* 2007). By combining these criteria, the shelf life of our samples was approximately 8–10 days for the controls, 7–10 days for Toc samples, 10–18 days for RE samples and more than 22 days for RE + ME and RE + Toc treatments. Furthermore, samples containing the combinations of antioxidants had lower PV and MDA concentrations than those containing the individual antioxidants ($P \leq 0.05$), indicating the occurrence of a synergistic effect. While the positive effects of the individual use of rosemary, mint and Toc in the prevention of lipid oxidation are well documented, to the best of our knowledge, ours is the first report on the combined use of rosemary extract with mint.

Finally, it has been observed that the shelf life of our control samples is shorter than that of commercial beef sausages. This is probably attributable to the absence of nitrites or other additives such as phosphates and spices from our samples which contribute antimicrobial and/or antioxidative activities (Ambriosiadis *et al.* 2004; Baber and Dilger 2014). In that regard, however, the positive effect of individual antioxidants combined with the use of rosemary extract was remarkable as the shelf life of samples containing rosemary extract was considerably increased and comparable with the shelf life of commercial beef sausages which contain added nitrites, phosphates and spices.

CONCLUSION

The results of the present study demonstrate the effectiveness of rosemary extract, added either individually or in combination with mint extract or Toc, on microbial growth inhibition, the retardation of lipid oxidation and the extension of the shelf life of fresh beef sausages during refrigerated storage (4C). The combination of rosemary extract with mint showed the greatest effect on limiting both microbial growth and lipid oxidation and could thus have potential for commercial use in order to improve the preservation of these products without the need for nitrites or other additives. Further research could examine the utility of the combined application of rosemary extract and mint in different meat products as well as the use of different quantities/ratios for the optimization of their antimicrobial and antioxidative effects.

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