Antioxidant and antibacterial activities of natural extracts: application in beef meatballs

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Abstract

The antioxidant and antibacterial effect of rosemary, orange and lemon extracts was investigated in cooked Swedish-style meatballs. Activity in a lard system was established for all the extracts and further determination of the development of rancidity as thio-barbituric acid reactive substances consistently showed that about 50% of the rancidity can be controlled by the citrus preparations. Two of the rosemary extracts (water soluble and oil soluble) were more effective with practically complete elimination of rancidity (TBA values) after a period of 12 days. Rosemary extract activity against lactic acid bacteria and Listeria but not Brochothrix thermosphacta was demonstrated in an agar diffusion test, but in the product only lactic acid bacteria counts were slightly reduced. Sensory analysis results, particularly aroma and acceptability scores, indicated the significant advantages in using rosemary and citrus extracts in rancidity-susceptible meat products.

Keywords: Rosemary; Garlic; Antioxidant; Antimicrobial; Citrus; Spoilage; Lactic acid bacteria

1. Introduction

The appearance of foods is one of the major determinants of its appeal to consumers and consequently, sales of the product. Lipid oxidation and bacterial contamination are the main factors that determine food quality loss and shelf-life reduction. Therefore, delaying lipid oxidation and preventing bacterial cross-contamination are highly relevant to food processors. The growth of microorganisms in meat products may cause spoilage or foodborne diseases. Oxidative processes in meat lead to the degradation of lipids and proteins which, in turn, contribute to the deterioration in flavour, texture and colour of displayed meat products (Decker, Chan, Livi-say, Butterfield, & Faustman, 1995). Several authors have postulated that in meat and meat products pigment and lipid oxidation are interrelated (Anton, Salgues, & Renerre, 1993; Yin & Faustman, 1993). Genot, Borel, Metro, Gandemer, and Renerre (1991) concluded that O₂ can initiate lipid peroxidation, leading to the formation of prooxidant substances capable of reacting with oxymyoglobin (OMb) and resulting in metmyoglobin (MMb) formation. Anton et al. (1993) postulated that OMb could be oxidized not only by lipid-oxy radicals but by other pro-oxidant radicals generated by O₂. Several investigators reported that the susceptibility of myoglobin to autoxidation is the main factor in explaining colour stability in meat and meat products (Renerre, Anton, & Gatellier, 1992).

Although synthetic additives have been widely used in the meat industry to inhibit both, the process of lipid oxidation and microbial growth, the trend is to decrease their use because of the growing concern among
consumers about such chemical additives (Chastain, Huffman, Hsieh, & Cordray, 1982; Chen, Pearson, Gray, Fooladi, & Ku, 1984). Consequently, search for natural additives, especially of plant origin, has notably increased in recent years (Lölliger, 1991). Compounds obtained from natural sources such as grains, oilseeds, spices, fruit and vegetables have been investigated (Chen, Muramoto, Yamauchi, & Huang, 1996). Therefore, the development and application of natural products with both antioxidants and antibacterial activities in meat products may be necessary and useful to prolong their storage shelf life and potential for preventing food diseases.

Rosemary (Rosmarinus officinalis L.) has been reported to contain certain compounds including, rosmarinol, rosmarinquinone, rosmaridiphenol and carnosol, which may be up to four times as effective as butylated hydroxy anisole (BHA) and equal to butylated hydroxy toluene (BHT) as antioxidants (Houlihan, Ho, & Chang, 1984, 1985; Nakatani & Inatani, 1984). Moreover, several authors reported that some compounds present in rosemary extracts could have antibacterial activity (Cuvelier, Richard, & Berset, 1996). Del Campo, Amiot, and Nguyen-The (2000) reported that the compounds responsible for the antibacterial action seemed presumably to be the phenolic di-terpenoids, which are the main compounds of the apolar fraction of the rosemary extracts.

Garlic extracts have also been shown to have antioxidant activity in different in vitro models. The antioxidant activity of Allium plants has mainly been attributed to a variety of sulphur-containing compounds and their precursors (Kim, Kubota, & Kobayashi, 1997; Lampe, 1999; Nuutila, Puupponen-Pimiä, Aarni, & Oksman-Caldentey, 2003). These compounds have been also reported as responsible for their in vitro antibacterial activity (Harris, Cottrell, & Plummer, 2001; Tsao & Yin, 2001).

Citrus fruits are an important source of bioactive compounds (flavonoids and vitamin C). The main flavonoids found in citrus species are hesperidin, narirutin, naringin and eriocitrin (Mouly, Arzouyan, Gaydou, & Estienne, 1994; Schieber, Stintzing, & Carle, 2001). Ascorbic acid, a well known natural antioxidant, together with natural flavonoids are also attracting more and more attention not only due to their antioxidant properties, but as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects (Cuvelier, Richard, & Berset, 1996). Del Campo, Amiot, and Nguyen-The (2000) reported that the compounds responsible for the antibacterial action seemed presumably to be the phenolic di-terpenoids, which are the main compounds of the apolar fraction of the rosemary extracts.

2. Materials and methods

2.1. Natural extracts

Rosemary and garlic extracts: Three different commercial rosemary extracts and one of garlic were provided by Kalsec® (Kalsec Mildenhall, UK): Herbalox® seasoning Type HT-O which is an rosemary oil miscible extract, Duralox® oxidation management Blend CN-2 which is a rosemary water miscible extract, Herbalox® seasoning Type W which is a rosemary oil and water miscible extract, and Aquaresin® garlic which is a garlic water miscible extract.

Citrus extracts (orange and lemon): these extracts were obtained from by-products of lemon and orange juice industries. As these by-products have a high water content (80%) which would make their application in the food industry difficult, it was necessary to process them to obtain a dry powder of aprox. 7% moisture. The process described by Fernández-Ginés, Fernández-López, Sayas-Barberá, Sendra, and Pérez-Alvarez (2003) included some built-in precautions to minimize the losses of the associated bioactive compounds; flavonoids and ascorbic acid.

2.2. Bacterial cultures

The eleven foodborne bacteria used as test organisms were selected as follows: Listeria innocua 4202, Listeria monocytogenes 5105, Lactobacillus sake 550, Leuconostoc mesenteroides subsp. mesenteroides 824; Leuconostoc mesenteroides subsp. dextranicum 882; Leuconostoc carnosum 558, Lactobacillus curvatus 860 Brochothrix thermosphaeta CRA 7883, Brochothrix thermosphaeta CRA 7884, Brochothrix thermosphaeta CRA 3235, and Lactoccus lactis FMRD 492. All isolates from vacuum packed spoiled meat products (Borch & Molin, 1988; Davies et al., 1999) except the last one (FMRD 492). FMRD strain was obtained from the Agricultural and Food Research Centre (DARDNI, Belfast, UK) and
CRA strains are from Campden and Chorleywood (Food Research Association, Chipping Campden, UK). All of them were selected as markers for meat products deterioration.

2.3. Determination of antioxidant effect in natural extracts (Rancimat method)

The American Oil Chemists' Society (AOCS) air oxidation method (AOA-OCS Cd 12b-92) was used to determine the antioxidant effect of each extract (Läubli & Bruttel, 1986). All experiments were performed with a 679 Rancimat (Metrohm, Herisau, Switzerland). Each extract (at the percentages showed in Table 1) was added to 2.5 g of melted commercial pork lard (Co-op, UK) into the reaction vessel. Then, they were inserted into the heating block for 10 min to preheat the sample (120 °C). The air supply and the absorption vessels were connected and recording of the conductivity curves started. Lard (without extract added) was used as control sample. The results are expressed as stability index (SI), calculated as a ratio of the induction time of the treatment sample and the induction time of the control. Three replications of this experiment were made.

2.4. Determination of antibacterial effect in natural extracts

Antimicrobial activity of natural extracts against 11 bacteria was tested using the agar diffusion method (Daeschel, 1992; Kuri, 1998). Stock cultures of all tested bacteria were grown in nutrient broth (Oxoid Unipath Ltd., Basingtoke, UK) into the reaction vessel. Then, they were inserted into the heating block for 10 min to preheat the sample (120 °C). The air supply and the absorption vessels were connected and recording of the conductivity curves started. Lard (without extract added) was used as control sample. The results are expressed as stability index (SI), calculated as a ratio of the induction time of the treatment sample and the induction time of the control. Three replications of this experiment were made.

2.5. Meatball manufacture

2.5.1. Product formulation

Swedish-style meatballs were manufactured according to a conventional formula: 78% minced beef (20% fat content), 14.5% flake potatoes, 5% water and 2.5% salt. Sunflower oil (1.5%) was used as a carrier for the extracts. A set of 6 treatment samples differing only by the extract added were prepared (Table 1).

Garlic extract was not used in meatballs manufacture due to their pro-oxidant activity showed with rancimat method and to their strong aroma showed in previous assays.

Rosemary extracts were used at the concentrations recommended by the supplier for its use in meat product. Citrus extracts were used at the concentration suggested by previous studies (Fernández-Ginés et al., 2003; Fernández-López et al., 2004).

2.5.2. Product processing

The products were prepared in a pilot plant resembling to commercial processing conditions. All ingredients were homogenized in a bowl mixer with a spiral dough hook (Silverson, Birmingham, UK) during 5 min. For each treatment, the corresponding extract was added at the concentrations shown in Table 1, and then mixed again for 5 min. Meatballs were formed by hand (15 g, 20–25 mm) and then subjected to a two-stage cooking process. First the meatballs were flash fried into sunflower oil at 190 °C for 30 s. The objective of this stage was to seal the surface of the ball and produce the characteristic browned look. They were then thoroughly cooked in a forced draught oven (Zanussi, Italy) at 250 °C during 4 min to reach an internal temperature of 72 °C in the center of the meatball. The temperature was monitored using an Omega digital thermometer (Omega Engineering, Inc., Stamford, CT) with a chromel–alumel (Omega K) thermocouple probe positioned in the geometric center of the product samples. When the endpoint temperature was achieved, the samples were immediately placed in a chiller (2–5 °C) to reach a product temperature below 12 °C. Three replications of this experiment were made.

2.5.3. Storage conditions

After reaching the packing temperature the samples were placed into plastic containers, sealed with one layer of a semi-permeable film (Polyvinylidene chloride, Freshcling, Plymouth, UK), and stored in darkness at

Table 1

<table>
<thead>
<tr>
<th>Type</th>
<th>Amount of each natural extract used in this study</th>
<th>Percentage (%, w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosemary oil extract</td>
<td>Herbalox® Type HTO</td>
<td>0.10</td>
</tr>
<tr>
<td>Rosemary water extract</td>
<td>Duralox®</td>
<td>0.15</td>
</tr>
<tr>
<td>Rosemary oil and</td>
<td>Herbalox® Type W</td>
<td>0.25</td>
</tr>
<tr>
<td>water extract (OWR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garlic extract (GR)</td>
<td>Aquarexin® garlic</td>
<td>0.05</td>
</tr>
<tr>
<td>Lemon fiber (LF)</td>
<td>Lemon extract</td>
<td>5.00</td>
</tr>
<tr>
<td>Orange fiber (OF)</td>
<td>Orange extract</td>
<td>5.00</td>
</tr>
</tbody>
</table>
8 ± 1 °C for 12 days to follow an accelerated shelf life determination protocol (IFST, 1993). Sampling and storage conditions records from each treatment took place at 1, 3, 6, 9 and 12 days (storage time) and every sample was analysed promptly as follows.

2.6. Meatballs analysis

2.6.1. Colour measurements

Meat colour was measured using a Minolta colorimeter CR-200 (Minolta Camera Co., Osaka, Japan) with illuminant D65, 2° observer, Diffuse/O mode, 8 mm aperture of the instrument for illumination and 8 mm for measurement. The colorimeter was standardized with a white tile. Colour was described as coordinates: lightness (L*), redness (a*), ±yellow-blue (b*). Colour differences (ΔE* = 1.89). Nine replicate measurements were taken for each sample, following the guidelines for colour measurements from American Meat Science Association (Hunt et al., 1991).

2.6.2. TBA values

Lipid oxidation was assessed in triplicate by the 2-thiobarbituric acid (TBA) method of Tarladgis, Watts, and Younathan (1960) with minor modifications. A 10 g sample was blended with 50 ml distilled water for 2 min and then transferred to a distillation tube. The cup used for blending was washed with an additional 47.5 ml distilled water, which was added to the same distillation tube with 2.5 ml 4N HCl and a few drops of antifoam agent silicone o/w (Fisher Scientific, Loughborough, UK). The mixture was distilled and 50 ml distillate was collected. Five ml of 0.02 M 2-thiobarbituric acid in 90% acetic acid (TBA reagent) was added to a vial containing 5 ml of the distillate and mixed well. The vials were capped and heated in a boiling water bath for 30 min to develop the chromogen and cooled to room temperature. The absorbance was measured at 538 nm against a blank prepared with 5 ml distilled water, which was added to the same distillation tube. Reagents were obtained from Sigma (UK). The TBA numbers were calculated as mg MA/kg sample.

2.6.3. Microbiological analysis

A composite sample (10 g) was formed with portions of at least 3 meatballs and homogenized with sterile 1.5% peptone water, in a Stomacher 400 (Colworth, London, UK) for 1 min. Aliquotes were serial diluted in peptone water and plated out following standard methodologies (Gerhardt et al., 1994). Lactic acid bacteria (LAB) counts were determined on MRS Agar (pH 5.6), with the plates incubated under anaerobic conditions (Gas generating kit anaerobic system, Oxoid Unipath Ltd., Basingtoke, Hampshire, UK) at 30 °C for 2 days. Psychrotrophic microbial counts were determined on Plate Count Agar (PCA) with plates incubated at 7 °C for 10 days. Coliform organisms were enumerated on MacConkey Agar (MCA) plates incubated at 30 °C for 48 h. Moulds and yeasts were determined on RBC (Rose bengal cloramphenicol) agar plates incubated at 22 °C for 5 days. Culture media were from Oxoid (Oxoid Unipath Ltd., Basingtoke, Hampshire, UK). Results were expressed as log10 cfu/ml.

2.6.4. Sensory evaluation

Sensory evaluation followed standard guidelines ISO 6658-1995 and ISO 6564-1985 (British Standards, 1986a, 1986b). A preliminary descriptive evaluation was performed on commercial Swedish meatballs (Crown Brands International, Ltd, Swindon, UK) and samples made as described above to identify and define principal sensory attributes and find consensus. Each meatball sample was evaluated by a trained 4-member panel. The same panel evaluated samples at each storage time (1, 6, 9 and 13 days storage). The sensory questionnaires measured intensity on a 7-point balanced semantic scale (weak to strong) for the following attributes colour (cruma), brightness, surface-slime, aroma (rancidity), aroma (putrefaction), aroma (off odour), aroma (acid), aroma (sour) and overall acceptance.

2.7. Statistical analysis

Conventional statistical methods were used to calculate means and standard deviations. Statistical analysis (ANOVA) was applied to determine significant differences (P < 0.05). To discover where there were significant differences between the levels of the main factor, contrasts (Tukey test) between means were made (Afifi & Azen, 1979). For the antioxidant and antimicrobial properties of natural extracts ANOVA with two factors (extract with seven levels: control, OR, WR, OWR, GR, OF and LF, and replication with three levels) were applied. For the meatball analysis, ANOVA with three factors (treatment with six levels: control, OR, WR, OWR, OF and LF, storage time with four levels: 1, 6, 9 and 12 days, and replication with three levels) were applied for each parameter. Statgraphics Plus 5.1 for Windows (Statgraphics Co., Tulsa, OK, USA) was used for statistical analysis.

3. Results and discussions

3.1. Antioxidant activity of natural extracts

Antioxidant activities of each natural extract expressed as stability index (SI) are shown in Table 2.
The only extract that did not show any antioxidant properties was garlic extract (GR), which showed a SI lower than that of the control, which could indicate pro-oxidant activity linked to the garlic product. The antioxidant activity of Allium plants has been attributed to a group of sulphur-containing compounds, from which allicin (diallyl thiosulphinate) appeared to be the main component (Kim et al., 1997). Animal tests have shown that in low concentrations, allicin can be responsible for the antioxidative properties of garlic, although it can also act as a pro-oxidant in high concentrations (Lawson, 1998). Also, controversial results about antioxidant properties of garlic extracts have been found to be dependent on the sample extraction method. These differences have been attributed to the spectrum of compounds extracted with the different solvents used (Nuuutila et al., 2003; Yin & Cheng, 1998).

The extracts with the highest (P < 0.05) antioxidant activity were those obtained from rosemary (OR, WR, OWR). There were no significant differences (P > 0.05) between the oil extract of rosemary (OR) and the water miscible rosemary extract (WR), which showed the highest SI. However, the oil and water miscible rosemary extract (OWR) had a lower SI.

Citrus extracts (LF, OF) showed antioxidant activity but lower (P < 0.05) than rosemary extracts (OR, WR, OWR). The SI of orange extract (OF) was higher (P < 0.05) than lemon extract (LF).

The antioxidant activity of phenolic compounds from plants is well known (Pokorny, 1991; Shahidi, 2000). This activity has been mainly attributed to flavonoids and ascorbic acid in citrus fruits (hesperidin, neohesperidin and eriocitrin) and to carnosol and rosmarinic acid in rosemary (Schwarz et al., 2001). All of these polyphenolic compounds have the ability to act as antioxidants by a free radical scavenging mechanism and also through their known ability to chelate transition metals (inactivation of iron ions) (Martin et al., 2002).

3.2. Antibacterial activity of natural extracts

The antibacterial activity of natural extracts against the spoilage bacterial strains tested is shown in Table 3. The water (control) was ineffective. Lemon extracts (LF), orange extracts (OF) and garlic extracts (GR) were only active against one, two and three bacterial strain/species, respectively. On the other hand, rosemary extracts (OR, WR, OWR) were active against all bacteria tested. From rosemary extracts studied, the oil miscible extract (OR) showed the highest inhibitive effect against each bacterium. Water miscible extract (WR) showed higher antibacterial effect than water and oil miscible extract (OWR) for all bacteria tested except for Brochothrix spp. For all rosemary extracts (OR, WR, OWR), Brochothrix spp. were the most sensitive bacteria. Some reports found that the most apolar phenolic compounds from rosemary extracts are presumably responsible of their antibacterial activity (Del Campo et al., 2000; Karamanoli, Vokou, Menkissoglu, & Constantinidou, 2000). Davidson (1993) reported that gram-positive bacteria are generally more susceptible to nonpolar phenolic compounds than gram-negative microorganisms. This could explain some of the

<table>
<thead>
<tr>
<th>Spoilage bacterial strains</th>
<th>Diameter of the zones of inhibition in mm (6 mm well)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
</tr>
<tr>
<td>Br. thermosphacta CRA 7883</td>
<td>28.1</td>
</tr>
<tr>
<td>Br. thermosphacta CRA 7884</td>
<td>25.5</td>
</tr>
<tr>
<td>Br. thermosphacta CRA 3235</td>
<td>26.4</td>
</tr>
<tr>
<td>L. innocua 4202</td>
<td>20.5</td>
</tr>
<tr>
<td>L. monocytogenes 5105</td>
<td>25</td>
</tr>
<tr>
<td>Lb. sake 550</td>
<td>21.4</td>
</tr>
<tr>
<td>Lc. mesenteroides subsp mesenteroides 824</td>
<td>21</td>
</tr>
<tr>
<td>Lc. mesenteroides subsp dextranicum 882</td>
<td>21</td>
</tr>
<tr>
<td>Lb. carnosum 558</td>
<td>26.2</td>
</tr>
<tr>
<td>Lb. curvatus 860</td>
<td>23.1</td>
</tr>
<tr>
<td>Lb. lactis FMRD 492</td>
<td>21.8</td>
</tr>
</tbody>
</table>

*–, no inhibition.
differences in the antibacterial activity between the rosemary extracts studied (OR, WR, OWR), if we assume that the oil extract (OR) is richer in nonpolar phenolic compounds than the others.

Frazier (1980) reported that citrus bioflavonoids also had antimicrobial properties. These compounds have reportedly wide-ranging antimicrobial properties effective against a broad range of human pathogens, fungi and food spoilage organisms (Cho, Seo, Choi, & Joo, 1990; Harich, 1997; Morgan, Andersen, & Hankinson, 1991). These compounds have had antimicrobial properties. These compounds have reportedly wide-ranging antimicrobial properties effective against a broad range of human pathogens, fungi and food spoilage organisms (Cho, Seo, Choi, & Joo, 1990; Harich, 1997; Morgan, Andersen, & Hankinson, 1991).

3.3. Antibacterial and antioxidant properties of natural extracts in meatballs

The effect of the natural extracts on lipid oxidation of cooked meatballs during storage is shown in Fig. 1. The analysis of variance for the TBARS data indicates that the TBA values were significantly affected \((P < 0.05)\) by both the storage period and the extract treatments. Initial (day 1) TBA values for all extract samples were significantly lower than those for the control \((P < 0.05)\). These results suggest that these antioxidants retarded lipid oxidation during and immediately after cooking. These results agree with that reported by Ahn, Grün, and Fernando (2002) and Fernández-López et al. (2003) for other natural antioxidants applied to cooked beef. Sato and Hegarty (1971) reported that non-heme iron was the active catalyst in cooked meats. Chen et al. (1984) also reported that iron was released from heme pigments during cooking and proposed that the resultant increase in non-heme iron was responsible for lipid oxidation.

At the end of storage time (day 12) all treatments resulted in significantly lower \((P < 0.05)\) TBA values when compared to the control, which indicates that all the tested natural extracts added to meatballs showed antioxidant properties. The product samples with rosemary extracts (OR, WR, OWR) showed the lowest \((P < 0.05)\) TBA values at each time of storage. Only treatments with orange extracts (OF) and rosemary water miscible extracts (WR) maintained \((P > 0.05)\) the initial TBA values during the 12 day storage period, and no differences were found between them \((P < 0.05)\). Samples treated with rosemary oil and water miscible extracts (OWR) had slightly increased TBA values only during the first 6 days of storage and became stable after that period. The products with added lemon extracts (LF) reached higher \((P < 0.05)\) TBA values than those with orange extracts (OF) by the end of storage. Consistently, this difference in antioxidant properties between orange and lemon extracts (OF and LF) has also been detected by using the Rancimat method, as explained before.

All colour coordinates showed differences \((P < 0.05)\) between treatments and storage days, except yellowness which only showed differences between treatments (Table 4). In all samples, lightness increased with storage time \((P < 0.05)\), and the highest values of \(L^*\) were obtained in control samples. Some authors reported that this increase could be related to the increase in MMb formation (Anton et al., 1993; Genot et al., 1991). These results suggest that the presence of antioxidant compounds in the natural extracts could retard MMb formation in meatballs and so \(L^*\) values decreased. For this reason, it could be expected that treatments with orange extracts (OF) and rosemary water miscible extracts (WR) would have lowest lightness values because they had the highest antioxidant capacity (Table 2), but in this study, treatments with lemon extracts (LF) and orange extracts (OF) showed the lowest \(L^*\) values. This could be explained by the increased water retention associated with hygroscopic materials, and because these extracts were prepared as a dry powder, they have absorbed free water within the product, subsequently decreasing lightness values. This relation between free water and lightness in meat and meat products has been reported by several authors (Fernández-López, Pérez-Alvarez, & Aranda-Catalá, 2000; Kauffman, Warner, & Russell, 1991).

![Fig. 1. Rancidity (TBA) evolution in meatballs with different natural extracts added, during storage time. For sample denomination see Table 1.](image-url)

Table 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(L^*_{\text{day} 1})</th>
<th>(L^*_{\text{day} 12})</th>
<th>(a^*_{\text{day} 1})</th>
<th>(a^*_{\text{day} 12})</th>
<th>(b^*_{\text{day} 1})</th>
<th>(b^*_{\text{day} 12})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.26(^{ab})</td>
<td>46.97(^{a})</td>
<td>9.52(^{ab})</td>
<td>7.13(^{a})</td>
<td>8.88(^{ab})</td>
<td>9.14(^{a})</td>
</tr>
<tr>
<td>OR</td>
<td>42.32(^{ab})</td>
<td>44.73(^{b})</td>
<td>9.53(^{ab})</td>
<td>8.33(^{b})</td>
<td>8.78(^{ab})</td>
<td>9.07(^{ab})</td>
</tr>
<tr>
<td>WR</td>
<td>42.48(^{ab})</td>
<td>44.61(^{b})</td>
<td>9.39(^{ab})</td>
<td>8.42(^{b})</td>
<td>8.50(^{ab})</td>
<td>8.78(^{ab})</td>
</tr>
<tr>
<td>OWR</td>
<td>42.25(^{ab})</td>
<td>44.89(^{b})</td>
<td>9.33(^{ab})</td>
<td>8.45(^{b})</td>
<td>9.11(^{ab})</td>
<td>9.22(^{ab})</td>
</tr>
<tr>
<td>LF</td>
<td>39.46(^{ab})</td>
<td>42.08(^{b})</td>
<td>10.22(^{ab})</td>
<td>8.83(^{b})</td>
<td>10.30(^{ab})</td>
<td>10.54(^{ab})</td>
</tr>
<tr>
<td>OF</td>
<td>39.28(^{ab})</td>
<td>41.57(^{b})</td>
<td>10.37(^{ab})</td>
<td>8.92(^{b})</td>
<td>10.29(^{ab})</td>
<td>10.33(^{ab})</td>
</tr>
</tbody>
</table>

For sample denomination see Table 1.  
\(^{a,b}\) Means within a column with different letters are significantly different \((P < 0.05)\).  
\(^{a,b}\) Means within a row with different letters are significantly different \((P < 0.05)\).
In all samples redness decreased as the storage time progressed ($P < 0.05$) but red colour ($a^*$ values) of the control sample faded very rapidly. Phillips, Mancini, Sun, Lynch, and Faustman (2001) reported a decrease in $a^*$ values of cooked ground beef linked to storage time. This is not surprising as meat which has been stored longer would be expected to have predominantly either OMb or MMb, as opposed to deoxymyoglobin (DMb), which in turn would predispose the meat to a faster browning rate (Hunt, Sorheim, & Slinde, 1995, DMb), which in turn would predispose the meat to a stored longer would be expected to have predominantly time. This is not surprising as meat which has been studied the effect of different antioxidants on the colour of meat and meat products (Higgins, Kerry, Buckley, & Morrissey, 1998; Lee, Hendricks, & Cornforth, 1998) and have reported that meat oxidation decreases $a^*$ values. Therefore, it was expected that samples with citrus extracts (LF and OF), which were the treatments with lower antioxidant activity (Table 2), would have the lowest $a^*$ values. Probably, this decrease in redness associated with the oxidation process is being compensated by the presence of red component pigments such as carotene in these citrus extracts. To confirm this, a significant effect of carotenes increasing $a^*$ values by about one unit was observed after preparation of the samples (day 1) (Table 4). If adjustments were made by subtracting the contribution of added pigments, the results would be consistent with previous observations.

In all samples yellowness values were not modified ($P < 0.05$) by storage time. Therefore, the differences in $b^*$ values observed between treatments incorporating citrus extracts (LF, OF) and the others, can be attributed to the presence of pigments in the citrus extracts and not to the oxidation processes.

In this work, the presence of coliforms, moulds and yeasts, and psychrotrophic microorganisms was not detected in any cooked meatball samples, regardless of storage time. This product was prepared following safe practices resembling ‘commercial aseptic conditions’, with a heat treatment that guaranteed pasteurization effectively inactivating vegetative cells and then stored under conditions that would limit the growth bacterial groups. Cross contamination from the environment (i.e. airborne or food handlers) or the survival of spores or resistant cells was possible, as it is in commercial operations. Lactic acid bacteria were detected at low levels in the control and samples with rosemary water and oil miscible extracts (OWR) from day 1 (data not shown). Some spore formers and heat resistant strains which have been linked with spoilage of meats (including VP and MAP) are likely to contribute to LAB counts (Borch, Kant-Muermans, & Blixt, 1996; Kuri, 1998). After 12 days storage, the growth of LAB to levels of $10^3$ log cfu/g was similar ($P > 0.05$) for control samples and treatments with rosemary extracts (OR, WR and OWR) (Table 5). These bacterial groups were not detected in samples from any treatment with citrus extracts (LF and OF) during storage time. Despite the presence of lactic acid bacteria, there was no evidence of strong lactic fermentation in any product, as confirmed by very low (<15 mM) lactic acid as determined by HPLC (data not shown), which also indicated the absence of significant amounts of sugars. Therefore, some bacteria may be present but their growth on the product is controlled under storage conditions. While there was in vitro antilisterial activity when the rosemary extracts (OR, WR, OWR) were tested using the agar diffusion test, this was not clear when used in the meatballs at the concentrations tested. Pandit and Shelef (1994) found rosemary extracts to have an effect against L. monocytogenes growth in a pork liver sausage. As can be seen, the antibacterial activity obtained for rosemary extracts (OR, WR, OWR) (Table 3) have not been manifested during the storage of cooked meatballs with added extracts. This could be explained by the dilution of the rosemary extracts necessary for its use in meat products, but there is also a possibility of reduction of the effectiveness of the antimicrobial extract due to physical interactions with the food matrix, which has been observed before for nisin in meat (Davies et al., 1999; Kuri, Collins, & Madden, 1998). Doyle (1999) notes a reduced effect of plant extracts related to fat in meat. Also, it is important to observe that citrus extracts (LF and OF) which showed the lowest antibacterial activity (Table 3) in the previous assay, appear to be more effective than the others to control LAB growth during the storage of meatballs. This is likely to be related with the lowered water activity within the product that would be the result of adding the citrus fruit preparations as a dry powder which contain fibre with high water absorption, as reported by Lario et al. (2003). Reduced water activity could reduce microbial growth but the presence of fermentable hydrocolloids in meat products may result in the development of off flavours (Kuri, Ponce, & Guerrero, 1994).

**Table 5**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Control</th>
<th>OR</th>
<th>WR</th>
<th>OWR</th>
<th>LF</th>
<th>OF</th>
</tr>
</thead>
<tbody>
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<td>n.d.*</td>
<td>n.d.*</td>
<td>2.30*</td>
<td>n.d.*</td>
<td>n.d.*</td>
</tr>
<tr>
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<td>2.69*</td>
<td>2.84*</td>
<td>n.d.*</td>
<td>2.69*</td>
<td>n.d.*</td>
<td>n.d.*</td>
</tr>
<tr>
<td>9</td>
<td>2.77*</td>
<td>3.00*</td>
<td>2.84*</td>
<td>2.69*</td>
<td>n.d.*</td>
<td>n.d.*</td>
</tr>
<tr>
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<td>3.00*</td>
<td>3.30*</td>
<td>3.30*</td>
<td>n.d.*</td>
<td>n.d.*</td>
</tr>
</tbody>
</table>

For sample denomination see Table 1. n.d.: not detected (<1.69; detection limit).

*Means within a column with different letters are significantly different ($P < 0.05$).

**Means within a row with different letters are significantly different ($P < 0.05$).**
From the eight sensory attributes evaluated, only three (aroma rancidity, surface lime and overall acceptance) provided useful information on the changes due to treatment and storage day, while for brightness, citrus aroma, putrefaction and sour aroma no differences ($P > 0.05$) were detected for any of the factors. The products were fairly stable for a week, with some differences becoming apparent between day 6 and day 9. In Fig. 2 it can be observed that at the end of the storage period (day 12), the differences in rancidity perception between treatments ($P < 0.05$) are consistent with differences measured as TBA values (Fig. 1). One exception is the lower score for orange extracts (OF) in comparison to lemon extracts (LF). This correlation between the sensory and the chemical methods is less evident at day 6, because at this point, changes in TBA values seem to show differences before panellist could perceive the increased rancidity. While these results suggest that the panellist may not be sensitive enough to detect differences of TBA values below a threshold, sensory scores provided better discrimination at day 9 in comparison to TBA values. Interestingly, samples with citrus extracts (LF, OF) were scored at equivalent terminal levels from day 9. Another factor to consider is the presence of off flavours in lemon extracts (LF), which could contribute to perceived rancidity, but are not identifiable by the TBA reaction. Overall acceptability scores for each treatment (Fig. 2) showed an inverse relation with TBA values and rancidity perception (Figs. 1 and 2, respectively). Product surface slime was not detected in any treatment during the first days of storage, but in control samples it was detected at day 9, increasing by day 12, when it was also detected in samples from treatments with rosemary extracts (OR, WR and OWR). No perception of surface slime was detected in citrus treatments (OF, LF) during storage time (results not shown). These observations agree with LAB enumeration results (Table 5).

The only attribute that showed differences ($P < 0.05$) between treatments but not between storage days was aroma off odour, which suggests that it was mainly due to the type of extract added and not to the deterioration of meatballs during storage. So, this off odour perception can be attributed to the peculiar composition of each extract, which is detected at its incorporation and was not modified during storage. The highest ($P < 0.05$) scores for off odours were obtained for citrus treatments (LF, OF), the lowest for control samples and there were not differences ($P > 0.05$) in off odours perception between rosemary treatments (OR, WR, OWR). Therefore, it can be observed that this off odour perception may not to have any influence upon the overall acceptability of the products.

4. Conclusions

The use of rosemary extracts has proved to be effective as an antioxidant in cooked swedish-style meatballs. The application of rosemary and citrus extracts improved the acceptability of the product. The application of rosemary preparations could be useful to control the development of rancidity and off flavours, while orange and lemon serve the same purpose, but may have some additional effects such as water binding that need to be managed. Some antibacterial activity was detected, but additional measures would be needed to control spoilage. The effect of antibacterial interaction with fat and the physical availability and compatibility of the fatty and lean phases and the active compounds need to be established in order to control better the deterioration of meat products.

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References


