Sulphite-free lamb burger meat: antimicrobial and antioxidant properties of green tea and carvacrol

Use of green tea and carvacrol to preserve lamb burger meat

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Keywords

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Abstract

Background

Sulphite is commonly used to preserve lamb burger meat in the European Union. Nevertheless, its consumption has been related to certain health problems which have increased consumer demand of free-sulphite products. Natural compounds with antioxidant and antimicrobial properties may be a feasible alternative to preserve lamb burger meat. This study evaluated the antimicrobial and antioxidant properties of carvacrol, green tea and their combination in preserving lamb burger meat. Their effect was also compared with that of 400 ppm sulphite.

Results

Lamb burger meat was mixed with different concentrations of the extracts, packaged aerobically and displayed through 8 days at 4 °C. Total polyphenols, TBARS, colour,

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microbial and sensory analyses were performed. Both green tea and carvacrol avoided lipid oxidation even at 300 ppm, while only carvacrol, which showed a concentration-dependent action, delayed discolouration and microbial growth. Carvacrol and green tea also limited the development of oxidation odour and flavour, but the former brought about herbal odours and flavours to the meat. On the other hand, sulphite provided a higher colour stability and lower microbial counts than both natural compounds, but presented a higher lipid oxidation.

Conclusion

Carvacrol seems to be a promising alternative to replace sulphite in lamb burger meat while green tea should be combined with an antimicrobial agent.

1. Introduction

The term sulphite encompasses several compounds containing the sulphite ion. Sulphite is largely used as a preservative in a wide variety of food products such as vegetables, wine, meat and seafood because of its powerful antioxidant and antimicrobial properties. In the European Union, the use of sulphite in meat and meat products is restricted to breakfast sausages and burger meat at a maximum concentration of 450 mg SO_2/kg.[1] Nevertheless, consumer concern about chemical additives continues to grow and sulphite is currently the subject of considerable debate. The consumption of sulphite has been related to certain health problems, including respiratory and allergic reactions, thiamine absorption deficit and disruption of carbohydrate metabolism.[2] Consequently, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) recently delivered a scientific opinion re-evaluating the use of sulphite for food preservation.[3] Moreover, several European projects are being carried out with the goal of reducing and/or replacing sulphite in food products.

Natural compounds emerge as a promising alternative to address consumer demand of clean label food without synthetic chemical preservatives. Herb extracts and essential oils, which are included in the Generally Recognized As Safe (GRAS) list of the American Food and Drug Administration, are perceived as natural and safe and possess interesting properties for meat preservation.[2] Antioxidant and antimicrobial compounds have been searched in different plant materials such as fruits, leaves, oilseeds, cereal crops, spices and herbs. Green tea composition includes catechin, flavones, anthocyan and phenolic acid, which are of great interest because of their potential antioxidant and antimicrobial properties.[4,5] Catechins, which constitute 30% of water-soluble solids of the dry weight, are the chief compounds of green tea while myricetin, quercetin and kaempferol are the main derivatives of the flavonol group.[6] Due to its chemical

composition, which is rich in polyphenols, green tea extract may be a feasible strategy to reduce oxidation in sulphite-free burger meat. On the other hand, carvacrol (2-metil-5-(1-metiletil) fenol) is a monoterpenic phenol present in many aromatic plants such as oregano and thyme. *In vitro* studies showed that carvacrol inhibits autoxidation in purified triacylglycerols, which suggests that it is likely to extend the shelf-life of lipid-containing food.[7] Moreover, carvacrol exerts a wide antimicrobial spectrum against both Gram-positive and Gram-negative bacteria isolated from food.[8]

Therefore, the aim of this work was to evaluate the preservative properties of green tea extract and carvacrol for increasing the shelf-life of sulphite-free lamb burger meat. The antioxidant and antimicrobial activities of both extracts were compared with those of sulphite. The sensory contribution of both extracts to cooked meat was also studied.

2. Materials and methods

2.1 Sampling

Nine carcasses were chosen at random among commercial lambs of Rasa Aragonesa breed. Lambs were raised intensively and fed with natural suckling up to 40 days of age and cereal straw *ad libitum* until they weighted between of 20 to 25 kg, when they were slaughtered in a commercial abattoir (Mercazaragoza) according to EU regulation. After dressing (15 min), carcasses were chilled during 24 h (4 ± 0.5 °C, 90% RH, 1-2 m/s) in the facilities of Casa de Ganaderos and Franco y Navarro S.A. Both legs of each carcass were excised and transported at 4 °C to the food technology pilot plant of the Faculty of Veterinary Science (University of Zaragoza), where they were deboned, minced through a 4-mm plate (M-94-32 Gesame, Vic, Spain), divided in five different batches (n = 5, batch is the sample) and mixed with potato starch (4% w/w) for 5 min using a AVT-50

mixer (Castellvall. Girona, Spain).Then, the mixture of each batch was distributed equally among the following treatments:

- Control (B): with no additives
- S400: 400 ppm of SO_2 added in the form of sodium metabisulphite
- T300: 300 ppm of green tea extract
- C300: 300 ppm of carvacrol
- C1000: 1000 ppm of carvacrol
- T+C300: 300 ppm of green tea extract and 300 ppm of carvacrol.
- T+C1000: 300 ppm of green tea extract and 1000 ppm of carvacrol.

Green tea extract (Sunphenon 90MB, TAIYO Europe, Filderstadt, Germany), sulphite (Merck, Darmstadt, Germany) and carvacrol (Sigma Aldrich, Misouri, USA) were dissolved in sterilized water and the resulting solutions were immediately added to the meat (10 ml of solution for 1 kg minced meat); each sample was thoroughly hand-mixed. The same amount of water was added to the control samples.

Burger meat was distributed in Polystyrene/EVOH/polyethylene trays (125 g per tray) (Linpac packaging S.A.U., Pravia, Spain) and covered with PVC film (O_2 transmission rate at 25 °C of 650-750 cm^3/m^2/24 h and 0% RH, Irma S.A., Zaragoza, Spain). One hundred and seventy five trays were maintained under commercial retail conditions (4 °C ± 0.5 °C with 14 h of artificial light per day) during 8 days. A standard supermarket fluorescent tube (Mazdafluor Aviva TF/36w; Philips, Eindhoven, Holland) with an UV-filter plate of polycarbonate was used. Light intensity (1000 lx) was measured with a luxometer (Chauvin Arnoux 810; Paris, France). Physicochemical, microbiological and sensory analyses were done at 0 (approximately 24 h post mortem), 1, 3, 6 and 8 days of display.

2.2 Total phenolic compounds

Total polyphenols were quantified following the methodology presented by Matthäus[9] with some modifications described in Bellés *et al.*[10] In brief, 2 ml of the extract was filled with 0.3% HCl (Panreac, Barcelona, Spain) to 5 ml. A 100 µl aliquot of the resulting solution was mixed with 2 ml of 2% Na_2CO_3 (Merck, Darmstadt, Germany) and then 2 ml 100 µl of Folin Ciocalteau reagent (diluted with methanol 1:1) (Sigma-Aldrich, Misouri, USA) was added. After 30 min of incubation, the absorbance was measured at 750 nm using a spectrophotometer. Gallic acid (Sigma-Aldrich, Misouri, USA) was used as a standard, expressing the results as milligrams of gallic acid equivalents (GAE) per g of extract. The content of polyphenols was determined by triplicate in the extracts before their application.

2.3 Lipid oxidation

The Thiobarbituric Acid Reactive Substances (TBARS) assay was carried out to determine lipid oxidation of meat samples according to the methodology described by Alonso *et al.*[11] Briefly, 20 ml of tricloracetic acid (TCA 10%) (VWR) were added to 10 g of meat and homogenized using an ultraturrax for 90 seconds at 2000 r.p.m. (T-25 basic, IKA-WERKE, Staufen, Alemania).Then, it was centrifuged at 4000 r.p.m for 30 minutes at 10˚C (Jouan CR 411, USA). Supernatant was filtered (Machere-Nagel, Alemania) and 2 ml was mixed with 2 ml of TBA 20 mM (Sigma-Aldrich). Tubes were vortex (Heidolph REAX 2000, Schwabach, Germany) and incubated in a thermostatic bath at 97 ˚C for 20 minutes (Grant W14, Cambridge, UK). After that, samples were cooled in tap water at ambient temperature and absorbance was measured at 532 nm with a spectrophotometer (Unicam 5625 UV/VIS, Cambridge, UK). The TBA-reactive substances (TBARS), mainly malondialdehyde (MDA), values were calculated from a standard curve of 1, 1, 3, 3-Tetramethoxypropane (TMP) (Sigma-Aldrich) because the

MDA can be obtained by acid hydrolysis from TMP in an equimolecular reaction, so the lipid oxidation was expressed as mg MDA/kg of meat.

2.4 Instrumental colour

Instrumental colour was measured on the surface of meat using a Minolta CM- 2002 reflectance spectrophotometer (Osaka, Japan) with an aperture of 30 mm and a D65 illuminant. The instrument was calibrated at the beginning of each session. Each value was the mean of 10 consecutive determinations. Meat pigment proportions were calculated as described by Krzywicki[12], while the values of the oxymyoglobin/metmyoglobin ratio were determined as the quotient of light reflectance at 630 and 580 nm.

2.5 Microbial analyses

Ground meat (25 g) was aseptically collected from each package, placed in a stomacher bag, diluted with 0.1% peptone water (225 ml) (Biolife) and homogenized for 3 min in a stomacher. Decimal dilutions were carried out using the same diluent. One ml of the proper dilution was plated in the following manner: aerobic total viable counts (ATVC) on plate count agar (PCA) (Merck, Darmstadt, Germany) at 37 °C for 24 h, *Enterobacteriaceae* on violet red bile dextrose agar (VRBD) (Merck, Darmstadt, Germany) at 37 °C for 48 h and lactic acid bacteria (LAB) on Man Rogosa Sharpe agar (MRS, Merck, Darmstadt, Germany) at 37 °C for 96 h in anaerobiosis. Anaerobic conditions were generated in an incubator jar using a commercial kit (Anaerocult A) (Merck, Darmstadt, Germany) and checked with an indicator strip (GazPack^{TM}). For the investigation of *Pseudomonas spp*. and *Brochotrix thermospacta*, 0.1 ml of the proper dilution was inoculated on the surface of Cephalothin-Sodium Fusidate-Cetrimide Agar (CFC, Merck, Darmstadt, Germany) and streptomycin thallous acetate actidione agar

(STAA) (STAA, Oxoid, Unipath Ltd., Basingstoke, UK) and incubated at 20 °C for 24 h and 30 °C for 48 h, respectively. Results were presented as base-10 logarithm of colony-forming units per g of meat (log CFU/g).

2.6 Sensory analyses

Meat for the sensory analyses was thawed at 4 °C during 12 h before cooking and the samples were prepared as described by Bellés *et al*.[10] The panel involved 9 members who were trained following the methodology proposed in the study cited above. Panellists rated the samples on a 10-point structured scale in which intensity was from low (0) to high (10). The descriptors, definitions and references of the parameters evaluated in the sensory analyses are shown in Table 1. Sessions took place in individual booths with red light. Nine sessions of approximately 40 minutes were required to evaluate the samples.

2.7 Statistical analyses

Differences in the content of polyphenols between the extracts were assessed using the independent-samples T-test of SPSS, version 19.0 (IBMSPSS, 2010) with the level of significance established at $P \leq 0.05$.

Data from colour, lipid oxidation and microbial analysis were analysed using the General Linear Model (GLM) procedure of SPSS, version 19.0 (IBMSPSS, 2010) and mean separation was carried out using Tukey's post hoc test with the level for statistical significance set at $P \leq 0.05$. The model was as follows:

$$Yij = \mu + Ti + Dj + (Ti \times Dj) + eij$$

where Yij is the dependent variable; μ is the population average; Ti is the fixed effect of treatment; Dj is the fixed effect of display duration; (Ti ×Dj) is the interaction effect of treatment and display duration; and eij is the aleatory error.

The same model was employed for analyzing the results from the sensory evaluation, including "panellist" and "session" as fixed effects and their interactions. "Panellist" resulted in a significant effect ($P \leq 0.001$) on all of the sensory parameters evaluated but the effect of "session" was not significant. No significant interactions were found.

3. Results

3.1 Polyphenol content

Total content of phenolic compounds was significantly higher in green tea extract (108.25 ± 3.2 mg GAE/g extract) than in carvacrol (77.78 ± 2.8 mg GAE/g of carvacrol) ($P < 0.001$).

3.2 Lipid oxidation

Mean TBARS values of lamb burger meat during display are presented in Figure 1. As it could be expected, oxidation spread significantly in all the batches ($P < 0.001$). However, the propagation of lipid oxidation differed among treatments: the addition of both carvacrol and green tea resulted in lower TBARS values than those measured in control and sulphite treatments from 3 days onwards ($P < 0.001$). Differences between tea and carvacrol batches were only found at eight days post-packaging, when the former showed higher values than those observed in the carvacrol and the carvacrol plus green tea groups ($P < 0.001$). Increasing the concentration of carvacrol from 300 to 1000 ppm did not modify significantly the rate of lipid oxidation of lamb burger meat. Otherwise, the addition of sulphite also inhibited the spread of oxidative reactions compared to control; nonetheless, its antioxidant action was weaker than that exerted natural antioxidants ($P < 0.05$).

3.3 Instrumental colour and meat pigment proportions

The evolution of the ratio 630/580 and meat pigment proportions are presented in Figure 2. Sulphite protected the meat from discolouration, thereby exhibiting higher values of the ratio 630/580 than the control batch from three days post-packaging onwards ($P < 0.001$). On the other hand, natural antioxidants did not modify the colour immediately after its application, but also retarded colour fading in comparison to control: Treatments including 1000 ppm carvacrol (C1000, T+C1000) showed higher values of the ratio 630/580 than control at 3 and 6 days of display, while the addition of carvacrol at lower dosage (300 ppm) led only to significant differences after 6 days of refrigeration. In contrast, green tea at 300 ppm did not exert any significant effect on meat colour at any sampling day.

The relative content of the different forms of myoglobin did not differ among treatments up to three days of display ($P > 0.05$) (Figure 2). Both sulphite (S400) and carvacrol (C300, C1000, T+C300, T+C100) reduced the oxidation of the pigment, therefore leading to a lower percentage of metmyoglobin than control at 6 days of display ($P < 0.001$). Moreover, metmyoglobin content in S400 was also lower at 3 and 8 days of refrigeration ($P = 0.006$). Differences in the proportion of deoxymyoglobin among treatments were only observed after 6 days ($P = 0.046$), when sulphite showed a higher percentage of deoxymyoglobin than control. Similarly, burger meat containing 400 ppm sulphite or 1000 ppm carvacrol had a higher percentage of oxymyoglobin than the control batch at 6 days post-packaging ($P = 0.001$) (Figure 2). By contrast, differences in the proportion of oxymyoglobin and deoxymyoglobin between the treatments with green tea or carvacrol were not registered at any sampling point.

Significant changes in the percentage of metmyoglobin, oxymyoglobin and deoxymyoglobin of lamb burger meat throughout display were detected in all the

batches, except for the percentage of deoxymyoglobin in the T+C100 treatment ($P < 0.05$). Indeed, as metmyoglobin accumulated, the proportion of deoxymyoglobin and oxymyoglobin decreased.

3.4 Microbial growth

The mean of total aerobic (ATVC), *Enterobacteriaceae*, lactic acid bacteria (LAB), *Pseudomonas spp*. and *Brochotrix thermospacta* counts for each day in each treatment are shown in Figure 3. Microbial growth remained stable during the first 24 h post-packaging but, as it could be expected, it further increased throughout display in all the batches ($P < 0.001$).

Nevertheless, the growth rate of the genera investigated differed among treatments. Differences in ATVC were not detected at the 1st day ($P > 0.05$) but the counts in S400, C1000, T+C1000, and C300 were lower than those determined in the control treatment after 3 days ($P < 0.001$). The antimicrobial effect of sulphite and carvacrol (C1000 and T+C100) was also detected at 6 days post-packaging ($P < 0.001$). Tukey's post hoc test identified four groups which differed significantly the 8th day: burger meat with 400 ppm sulphite presented the lowest ATVC, followed by those included 1000 ppm of carvacrol (C1000 and T+C1000) and 300 ppm of carvacrol (C300). The control batch reached the highest ATVC, while the treatments T300 and T+C300 neither differed from control nor from those with 1000 ppm carvacrol (C1000 and T+C1000) and C300.

Enterobacteriaceae and *Pseudomonas* spp. growth followed a similar pattern. Differences among batches were not observed up to three days post-packaging, when sulphite and 1000 ppm carvacrol-added treatments registered lower counts of both genera than control ($P < 0.001$). The antimicrobial effect of carvacrol (1000 ppm) was also detected at 6 and 8 days of storage ($P < 0.001$). Furthermore, the T+C300 treatment

showed a significant inhibition of both *Enterobacteriaceae* and *Pseudomonas spp.* growth after 6 days, when the C300 treatment also provided lower counts of *Pseudomonas spp.* than control.

Lactic acid bacteria and *Brochotrix termosphacta* showed a lower growth rate. In fact, growth in the latter was not detected up to the 3rd day of refrigeration. The addition of sulphite and carvacrol both at 300 and 1000 ppm resulted in lower LAB counts than the control treatment after 3 days of display ($P < 0.001$). The effect of sulphite and carvacrol at 1000 ppm was also significant the 6th day, while only the former led to lower LAB counts after 8 days ($P < 0.001$). Similarly, sulphite and 1000 ppm of carvacrol delayed the growth of *Brochotrix thermosphacta*, thereby providing lower counts than those found in the control treatment both at 6 and 8 days of display ($P < 0.001$).

3.5 Sensory evaluation

Results of sensory evaluation of grill-cooked lamb are presented in Figure 4. Carvacrol was perceived by panellists, who detected a higher herbal odour and flavour in the treatments which contained both 300 and 1000 ppm of this compound at all sampling days ($P < 0.001$). Moreover, they were capable of discriminating between these concentrations ($P \leq 0.05$). In contrast, herbal odour and flavour were not detected in the other treatments. As it could be expected, microbial odour and flavour increased throughout display in all the batches ($P < 0.001$). Differences among treatments were neither detected at 0 nor at 1 days of storage, but arose from 3 days post-packaging onwards. The addition of sulphite and 1000 ppm carvacrol decreased the development of microbial odour and flavour, without significant differences between them. On the other hand, both carvacrol and green tea reduced the development of oxidation odour and flavour, thus registering lower scores than the control treatment at 3, 6 and 8 days

of display ($P \leq 0.05$). Similarly, sulphite diminished oxidation related odour and flavour, although scores attributed to this batch were higher than those recorded in the treatments with carvacrol (from 6 days onwards) or in those with green tea at 8 days post packaging.

4. Discussion

4.1 Lipid oxidation

Lipid oxidation is one of the main causes that limit the shelf life of aerobically packaged lamb. Indeed, oxidation spread quickly in the control treatment, in which the limit of acceptability for oxidised lamb (1 mg MDA/kg) proposed by Ripoll et al.[13] was already exceeded after 3 days of display. By contrast, the addition of both carvacrol and green tea extract maintained lipid oxidation below this threshold during the eight days of storage. In fact, the antioxidant properties of these compounds were already noted 24 hours post-packaging, when the treatments with green tea and carvacrol showed a lower content of MDA than both control and sulphite ($P < 0.001$) (Figure 1).

The effect of both compounds on inhibiting oxidative reactions is related to their content of polyphenols. The polyphenol content of green tea extract was in accordance with the data previously reported by Stapornkul *et al.*[14] Tea polyphenols have been described to possess antioxidant properties by acting as hydrogen donors, reducing agents, nascent oxygen quenchers, and chelating metal ions.[15] These mechanisms would explain the great inhibition of lipid oxidation observed in the batch with 300 ppm of green tea. In this regard, Mitsumoto *et al.*[16] and Liu *et al.*[17] also concluded that tea catechins at concentrations close to 300 ppm were capable of effectively retarding lipid oxidation. The amount of phenolic compounds quantified in carvacrol was lower than that registered in green tea. Nevertheless, carvacrol reduced lipid oxidation to a similar

degree, showing even lower TBARS values than those registered in T300 after eight days of display.

The antioxidant properties of an extract are strongly related to its content of polyphenols; nonetheless, the type of phenolic compound is also a key factor, as it determines its mechanism of action. Carvacrol is an oxygenated monoterpene that possesses a phenol group in its chemical structure. It may inhibit peroxidation of phospholipid liposomes in the presence of iron (III) and ascorbate, and has been also described to be a good scavenger of peroxyl radicals.[8] As far as we know, the antioxidant properties of carvacrol had not been tested in meat matrix but an active film containing 4 % of an oregano extract showed a three-fold reduction in the degree of lipid oxidation in lamb.[18] Neither increasing the concentration of carvacrol to 1000 ppm nor the combination with 300 ppm of green tea extract improved the effects reached with 300 ppm of carvacrol, thus the latter concentration would be enough to inhibit lipid oxidation of lamb burger meat under the conditions tested.

On the other hand, the addition of 400 ppm sulphite also delayed lipid oxidation compared to control, but its effect was lower than that exerted both carvacrol and green tea. Differences between sulphite and carvacrol or green tea-added treatments were registered from 1 days post-packaging onwards ($P < 0.001$). Sulphite acts as a secondary antioxidant by decomposing hydroperoxides in a non-radical way.[19] However, data obtained revealed that 400 ppm of sulphite were not able to control lipid peroxidation during eight days of display, since the threshold of acceptability (1 mg MDA/kg) was already overpassed in this batch after 6 days. Likewise, Bañón *et al*.[20] pointed out noticeable lipid oxidation in low sulphite (100 ppm) beef patties. Therefore, lipid oxidation is likely to be a limiting factor to the shelf-life of sulphite added burger meat.

4.2 Colour

Colour is the chief sensory property of lamb during retail display because of consumer purchasing decision relies on meat appearance. Indeed, consumers associate a bright red colour with freshness and superior meat quality.[21] Colour is highly related to the quantity and the chemical state of meat myoglobin; but it transforms to metmyoglobin when the central iron atom is oxidised, changing meat colour from a red fresh appearance to a less desirable brown colour and therefore decreasing consumer acceptance. The 630/580 ratio has been widely used to describe meat discolouration: larger ratios and differences indicate more redness while a ratio of 1.0 would indicate essentially 100% MMb.[22] Sulphite provided higher values of the index 630/580 than control from 3 days onwards, as well as than the treatments with green tea and carvarol at both 6 and 8 days of display. Indeed, sulphite kept meat colour above the limit of acceptability proposed by Ripoll *et al.*[13] throughout the eight days of display. These results may be explained by the effect of sulphite on inhibiting myoglobin oxidation. In this sense, sulphite showed a lower proportion of metmyoglobin than the control batch from 3 days onwards. According to Djenane *et al.*[23], consumers reject beef when the percentage of metmyoglobin exceeds 40%. As it could been seen in Figure 2, sulphite maintained the relative content of metmyoglobin below this threshold even after 8 days. Colour stability of sulphite-added burger meat may be related to the ability of this compound to reduce the myoglobin haem group, thereby lessening the formation of metmyoglobin and providing a fresh appearance to red meat.[24] Similarly, Bañón *et al.*[20] and Ortuño *et al.*[25] also identified a protective effect of SO_2 against the discolouration of beef and lamb minced meat throughout display.

Regarding to the effect of carvacrol on meat colour, it was able to diminish myoglobin oxidation at a concentration of 1000 ppm, thereby showing higher values of the 630/580

index than control at 3 and 6 days of display. Nevertheless, both the percentage of metmyoglobin and the values of the 630/580 index exceeded their limits of acceptability at the end of display. As it could be seen in Figure 2, the effect of carvacrol at 300 ppm on meat colour was limited, and both C300 and T+C300 treatments showed values of the index 630/580 below the threshold proposed by Ripoll et al.[13] at 3 days post-packaging. Similarly, Matromatteo *et al.*[26] and Al-Hijazeen *et al.*[27] did not register any effect of carvacrol on keeping the colour of poultry patties and ground chicken enriched with up to 300 ppm of this compound. Therefore, the effect of carvacrol on meat colour seems to be concentration-dependent. Otherwise, no effect of green tea on preventing pigment oxidation was detected.

Lipid oxidation may enhance meat discolouration, since secondary-by-products of lipid oxidation have been described to promote myoglobin oxidation and thereafter colour fading.[28] Nevertheless, both the percentage of metmyoglobin and the values of the ratio 630/580 did not differ between control and T300 treatments despite having different degrees of lipid oxidation. Likewise, C1000 accumulated lower metmyoglobin than T300, regardless of their similar TBARS values. Hence, myoglobin oxidation does not seem to have been mediated by lipid oxidation-by-products. It has been demonstrated that in low pO_2 atmospheres oxymyoglobin is rapidly converted to metmyoglobin while these conditions provide a high stability to lipids.[28] In fact, myoglobin can interact directly with oxygen reactive species and become oxidised. In this regards, it could have been expected that green tea extract, being water soluble, might had been more likely to permeate the cytoplasm and thus would have protected water-soluble myoglobin from oxidation. In contrast, carvacrol, which has a hydrophobic nature, may have had more difficulties to protect myoglobin from direct oxidation. Nevertheless, our results did not confirm this hypothesis. In agreement with our data, Liu *et al.*[17] did not observe any

effect of tea catechins on preventing from myoglobin oxidation. Moreover, Lorenzo *et al.*[29] registered that green tea catechins accelerated the accumulation of metmyoglobin in pork patties during display. Mitsumoto *et al.*[16] threw light on this matter, pointing out that tea catechins may cause discolouration by binding with the iron component of myoglobin. Therefore, tea catechins should not be recommended to prevent meat discolouration.

4.3 Microbial growth

Initial aerobic total viable counts in lamb retail cuts tend to be comprised between 2.50 and 4.00 log UFC/g (Berruga *et al.*[21]; Karabagias *et al.*[30]; Nieto *et al.*[31]). In our study, counts were slightly higher, which may have been as a result of the deboning, grounding and mixing procedures. As it could be expected, microbial counts increased throughout display. *Pseudomonas spp*. growth exceeded the limit for acceptable quality meat (7 log CFU/cm^2) proposed by ICMFS[32] in all batches, except for that containing 400 ppm sulphite. The effectiveness of sulphite to inhibit microbial growth was higher than that showed both green tea and carvacrol for all the microorganisms investigated. Previously, Bañón *et al.*[20] reported a remarkable inhibition of total viable and total coliform counts in low sulphite beef patties (100 ppm). The antimicrobial properties of sulphite are widely known, indeed. The mechanisms by which sulphite inhibits microbial multiplication are very complex since it can interact with many critical components of the microbial cell. Sulphite may modify several bacterium metabolic systems such as energy synthesis, energy production, DNA replication and membrane functions.[33]

Carvacrol also demonstrated relevant antimicrobial properties against the microorganisms determined. The addition of 1000 ppm carvacrol reduced at least 1.00 log UFC/g final total viable counts compared to the control. Moreover,

Enterobacteriaceae and *Pseudomonas* spp. growth followed a similar trend, while the inhibition of LAB and *Brochothrix thermosphacta* was slightly lower. However, the antimicrobial activity of carvacrol seemed to be concentration-dependent, since the addition of a lower concentration (300 ppm) did not provide significant reductions in most sampling points. According to Lambert *et al.*[34] the minimum inhibitory concentration (MIC) of carvacrol against *Staphylococcus aureus* and *Pseudomonas aeruginosa* are 140 and 385 ppm, respectively. It should be pointed out that these MIC were calculated *in vitro* and hence may be expected to be lower than those required in *vivo*. Therefore, these data support the low inhibition reached with 300 ppm carvacrol. In contrast, Mastromatteo *et al.*[26] registered a reduction of the cell load of about 1–1.5 log CFU/g in poultry patties with 300 ppm carvacrol. A feasible explanation may be the lower temperature or the combined effect of carvacrol and modified atmosphere packaging used in that study. The antimicrobial effect of carvacrol is based on its hydrophobicity: carvacrol accumulates in the cell membrane and thereafter induces its conformational modification which results in cell death.[35]

Otherwise, tea catechins have been described to manifest certain antimicrobial activity by interacting with bacterial enzymes or proteins.[36] Wu *et al.*[37] reported a noticeable reduction of microbial counts by using 1000 ppm of tea catechins while Lorenzo et al.[29] observed also an antimicrobial effect on pork patties using the same concentration. Nevertheless, we did not observe any antimicrobial effect of green tea extract at 300 ppm, which suggests that higher concentration of tea catechins may be required to make profit of green tea antimicrobial properties.

4.4 Sensory properties

Panellists were not able to identify oxidation odour and flavour in any treatment during the first 48 hours post-packaging. Furthermore, Mean values of the treatments C300,

C1000, T+C300 and T+C1000 were generally lower than 1 point during display, which demonstrated the great effect of carvacrol on inhibiting lipid oxidation (Figure 4). These results agreed with Dunshea *et al*.[38], who proposed 0.5 mg MDA/kg as borderline level for detection of rancidity by trained sensory panellists. The panel also detected low levels of rancidity in the batch including 300 ppm of green tea extract, while the scores assigned to the sulphite batch increased from the 3rd day onwards and were close to the central value at the end of display. These results agreed with TBARS values and pointed out the limited effect of sulphite on inhibiting lipid oxidation. Panellists reported the highest oxidation odour and flavour in the control samples after eight days of display (7.33 and 5.80 respectively), but they assigned to this treatment values above the central point at 6 days of display, when the limit of rejection for oxidised lamb (1 mg MDA/kg) proposed by Ripoll *et al*.[13] was already exceeded.

As it could be expected, microbial odour and flavour increased with bacteria counts. Hence, the control and T300 treatments were assigned the highest scores. In fact, both treatments showed values above the central point at 6 days post-packaging, when their counts of *Pseudomonas spp.* counts exceeded the limit for acceptable quality meat (7 log CFU/cm^2) proposed by ICMFS[32]. Panellist assigned values lower than 5 to the other treatments up to the 8th day, when the ICMFS[32] threshold was overpassed in all the batches except in that with 400 ppm sulphite. It has been demonstrated that microbial metabolism produces volatile compounds which are perceived as off odours and flavours and ultimately result in meat rejection.[39] Treatments including 1000 ppm carvacrol were given scores close to 5 despite having *Pseudomonas spp.* counts higher than 7 Log CFU/g. A feasible explanation to these results could be the characteristic odour and flavour of carvacrol, which was perceived by panellists. This compound could have overpowered microbial odour and flavour and hence lower scores were

assigned to the latter. Moreover, the perception of this compound is likely to be concentration dependent since higher values were attributed to the samples with 1000 ppm of carvacrol compared to those with 300 ppm. Several researchers have previously described a significant impact of essential oils and herb extracts on meat sensory properties. Latoch and Stasiak[40] noticed that mint extract bring about strange odours and flavours to pork sausages which compromise their acceptability. In contrast, herbal odours and flavours were not related to green tea extract, showing that it did not provide strange odours or flavours to burger meat. Similarly, neither Mitsumoto *et al.*[16] nor Bellés *et al.*[10] detected herbal flavours and odours in the samples with green tea.

5. Conclusion

The addition of a preservative to lamb minced meat seems to be required due to its limited shelf-life. Sulphite demonstrated to have strong antimicrobial properties and prevented lamb from discoloration. It also reduced lipid oxidation compared to control but to a lower degree than both green tea and carvacrol. Indeed, these natural additives almost avoided lipid oxidation while only carvacrol showed a significant effect against discolouration and microbial growth. The antimicrobial properties of carvacrol are likely to be concentration-dependent, hence the inhibition obtained using 1000 ppm was significantly higher than that observed at 300 ppm. Moreover, both green tea extract and carvacrol limited the development of oxidation odour and flavour while the latter also decreased the perception of microbial odour and flavour. Nevertheless, carvacrol provided herbal odour and flavour, which were detected by panelists. In contrast, neither green tea extract nor sulphite were sensory detected. Therefore, carvacrol seems to be a promising alternative to replace sulphite in lamb burger meat while green tea should be combined with an antimicrobial agent. Nevertheless, carvacrol may bring about herbal odours and flavours to meat, which must be taken into account.

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8. Conflict of interests

None.

9. References

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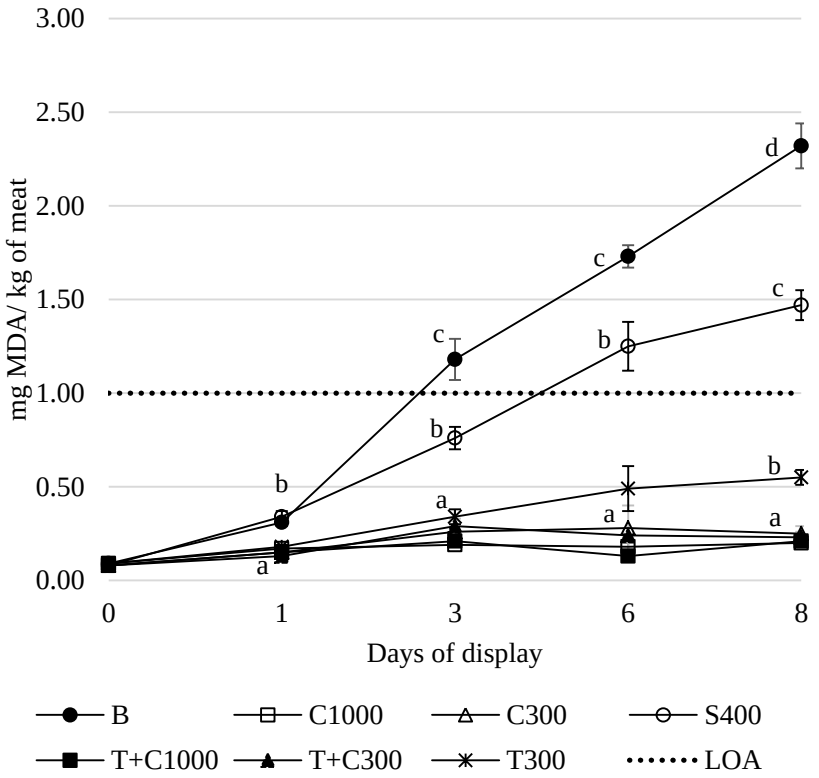

Figure 1. TBARS values of lamb burger meat during display at 4 °C. Treatments: B - control -; S400 - sulphites 400 ppm -; T300 - green tea extract 300 ppm -; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -; C1000 – carvacrol 1000 ppm -; T + C1000 - green tea extract 300 ppm + carvacrol 1000 ppm -. Markers represent the mean of malondialdehyde (MDA) values and the error bars represent standard error of the mean. Means with different letters (a, b, c) in the same day of storage indicate significant differences among mean values ($P \leq 0.05$).

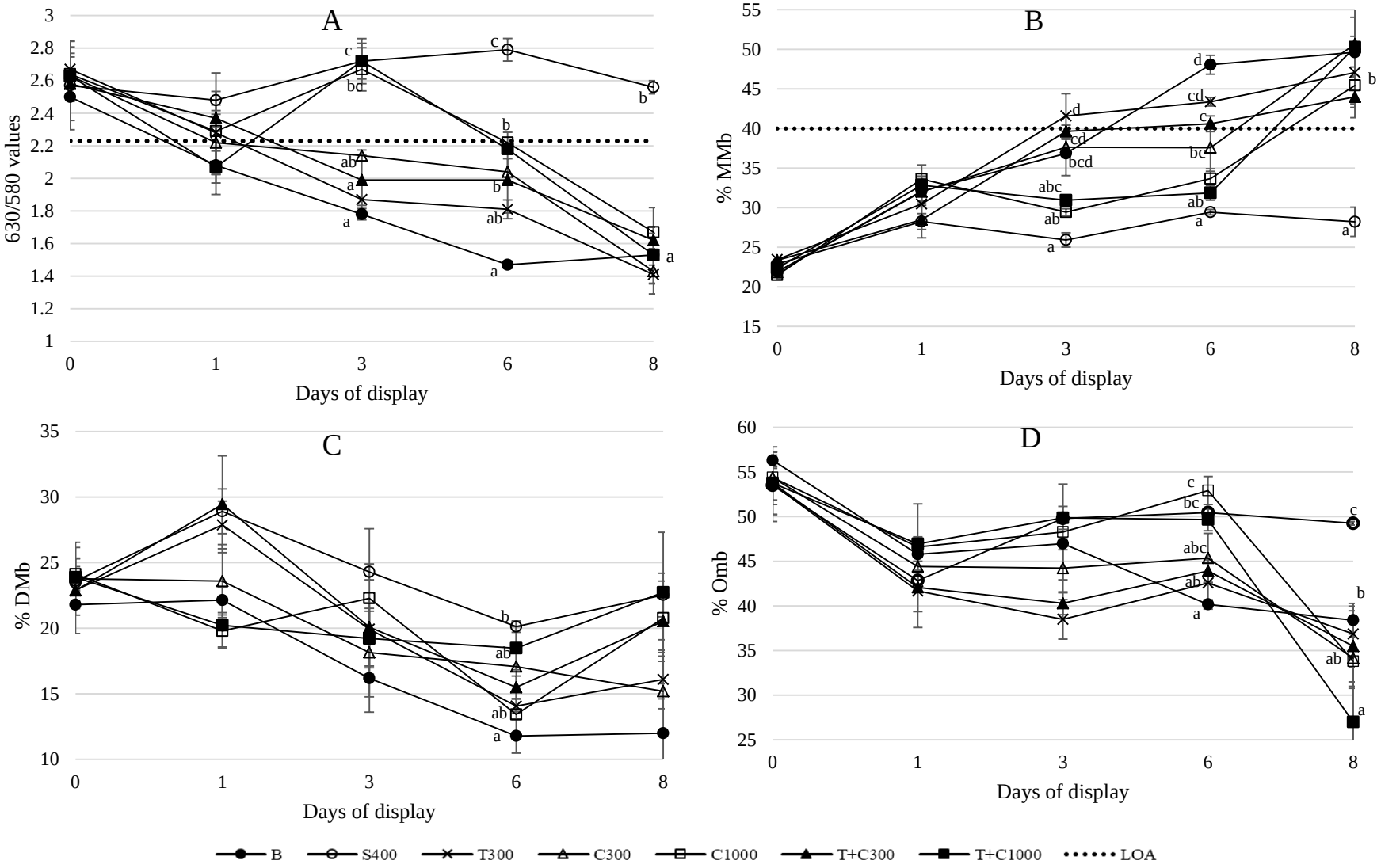

Figure 2. Values of the 630/580 ratio (A) and the percentage of metmyoglobin (B), deoxymyoglobin (C) and oxymyoglobin (D) of lamb burger meat during display. Treatments: B -control -; S400 - sulphites 400 ppm -; T300 - green tea extract 300 ppm -; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -; C1000 – carvacrol 1000 ppm -; T + C1000 - green tea extract 300 ppm + carvacrol 1000 ppm -. Markers represent the mean and error bars represent standard error of the mean. Means with different letters (a, b, c) in the same day of storage indicate significant differences among mean values ($P \leq 0.05$).

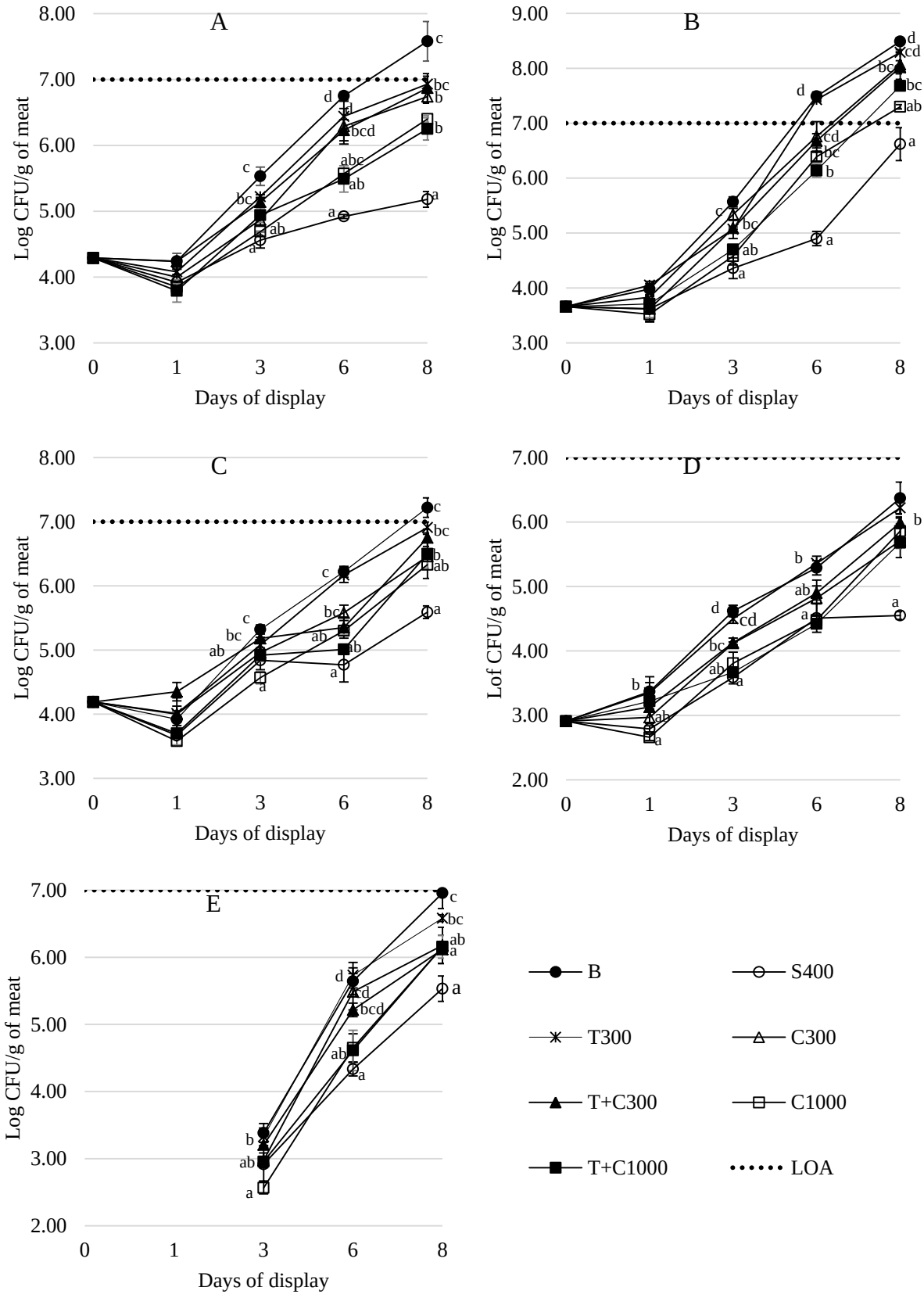

Figure 3. Aerobic total viable (A), *Pseudomonas* spp. (B), *Enterobacteriaceae* (C), Lactic acid bacteria (D) and *Brochotrix thermosphacta* (E) counts of lamb burger meat during display at 4 °C. Treatments: B - control -; S400 - sulphites 400 ppm -; T300 - green tea extract 300 ppm -; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -; C1000 – carvacrol 1000 ppm-; T + C1000 - green tea extract 300 ppm + carvacrol 1000 ppm -. LOA: Limit of acceptability. Markers represent the mean and the error bars represent standard error of the mean. Means with different letters (a, b, c) in the same day of storage indicate significant differences among treatments ($P \leq 0.05$).

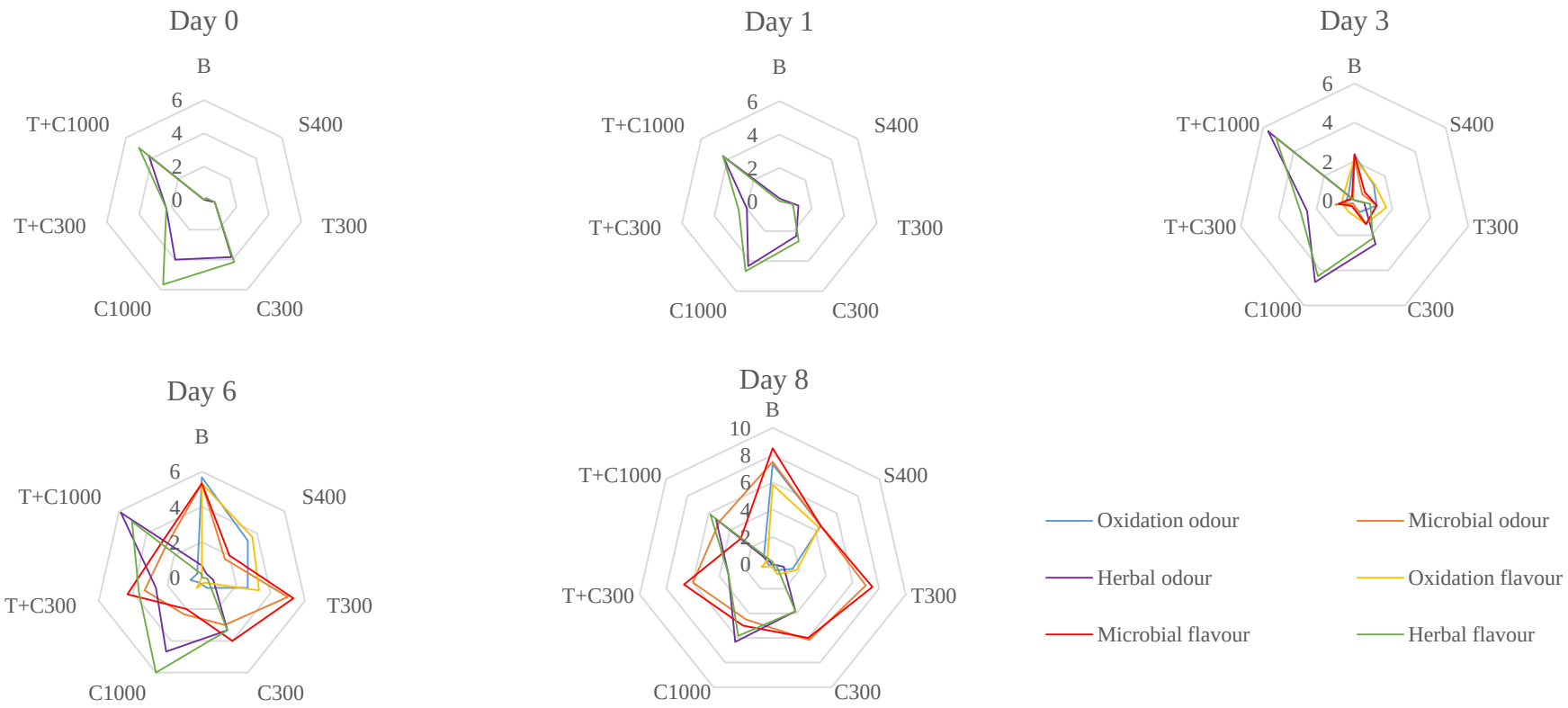

Figure 4. Values assigned by panellists for the sensory attributes of grill-cooked lamb during display. Treatments: B - control -; S400 - sulphites 400 ppm -; T300 - green tea extract 300 ppm -; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -; C1000 – carvacrol 1000 ppm-; T + C1000 - green tea extract 300 ppm + carvacrol 1000 ppm -. A 10 point scale (0 = low, 10 = high).

Table 1. Description and references of the attributes used for the sensory analyses.

Attribute	Definition	References
Odour		
Oxidation	Odour associated with oxidation compounds derived from fat	Rancid seed oil
Herbal	Odour associated with herbs	Tea leaves
Microbial	Putrid odours derived from meat spoilage	Spoiled meat
Flavour		
Oxidation	Flavour associated with oxidation compounds derived from fat	Rancid seed oil
Herbal	Flavour associated with herbs	Tea leaves
Microbial	Putrid flavours derived from meat spoilage	Spoiled meat

Table 2. Evolution of TBARS values of lamb burger meat throughout display.

	Days of display						
Treatments	0	1	3	6	8	$P_{display}$	SEM
B	0.09x	0.31b,x	1.18c,y	1.73c,y	2.32d,z	< 0.001	0.062
C1000	0.09x	0.17a,y	0.19a,y	0.18a,y	0.2a,y	< 0.001	0.008
C300	0.09	0.15a	0.26a	0.28a	0.25a	0.085	0.042
S400	0.09x	0.34b,x	0.76b,y	1.25b,z	1.47c,z	< 0.001	0.060
T+C1000	0.09x	0.15a,x	0.21a,y	0.13a,x	0.21a,y	< 0.001	0.012
T+C300	0.09x	0.13a,x	0.29a,y	0.24a,y	0.23a,y	< 0.001	0.018
T300	0.09x	0.18a,x	0.34a,y	0.49a,yz	0.55b,z	< 0.001	0.024
$P_{treatment}$	0.09	< 0.001	< 0.001	< 0.001	< 0.001	-	-

Mean of TBARS values and standard error of the mean (SEM). Treatments: B -control -; S400 - sulphites 400 ppm -; T300 - green tea extract 300 ppm -; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -; C1000 – carvacrol 1000ppm-;T + 1000C - green tea extract 300 ppm + carvacrol 1000 ppm -. Means with different letters (a, b, c) in the same column indicate significant differences among treatments ($P \leq 0.05$). Means with different letters (x, y, z) in the same row indicate significant differences among days of storage ($P \leq 0.05$).

Table 3. Colour and relative content of the different forms of myoglobin of lamb burger meat during display at 4 °C.

	Treatments	Days of display					$P_{display}$	SEM
		0	1	3	6	8		
630/580 ratio	B	2.50z	2.08y	1.78a,x	1.47a,w	1.53a,wx	< 0.001	0.084
	S400	2.57xy	2.48x	2.72c,y	2.79c,y	2.56b,xy	< 0.001	0.067
	T300	2.67z	2.28z	1.87a,y	1.81ab,y	1.41a,x	< 0.001	0.092
	C300	2.63z	2.22y	2.14ab,y	2.04b,y	1.43a,x	< 0.001	0.017
	C1000	2.64yz	2.29y	2.67bc,z	2.22b,y	1.67a,x	< 0.001	0.076
	T+C300	2.58z	2.37z	1.99a,y	1.99b,y	1.62a,x	< 0.001	0.073
	T+C1000	2.63z	2.07xy	2.72c,z	2.18b,yz	1.53a,x	< 0.001	0.124
	$P_{treatment}$	0.623	0.798	< 0.001	< 0.001	0.003	-	-
% MMb	B	21.90x	32.08y	36.85bcd,y	48.05d,z	49.62b,z	< 0.001	2.371
	S400	22.90x	28.25yz	25.92a,y	29.43a,z	28.22a,yz	< 0.001	0.607
	T300	23.45w	30.46x	41.58d,y	43.37cd,yz	47.06b,z	< 0.001	1.897
	C300	21.85w	32.01x	37.64cd,y	37.58bc,y	50.66b,z	< 0.001	0.374
	C1000	21.49w	33.61xy	29.44ab,x	33.66ab,y	45.44b,z	< 0.001	1.542
	T+C300	23.36w	28.48x	39.63d,y	40.60c,yz	43.99b,z	< 0.001	1.666
	T+C1000	22.36x	32.84y	30.94abc,xy	31.86ab,xy	50.26b,z	< 0.001	2.642
	$P_{treatment}$	0.491	0.237	< 0.001	< 0.001	0.006	-	-
% DMb	B	21.79y	22.14y	16.18xy	11.78a,x	11.99x	< 0.001	1.208
	S400	23.59y	28.91z	24.29y	20.12b,x	22.53xy	< 0.001	0.809
	T300	22.91y	27.87y	19.92xy	14.06ab,x	16.09x	< 0.001	1.539
	C300	23.77y	23.59y	18.14xy	17.07ab,xy	15.19x	< 0.001	0.193
	C1000	24.12y	19.79y	22.28y	13.42ab,x	20.76y	< 0.001	0.905
	T+C300	22.87y	29.45z	20.07xy	15.49ab,x	20.53xy	< 0.001	1.887
	T+C1000	23.88	20.22	19.21	18.47ab	22.73	0.785	1.080
	$P_{treatment}$	0.502	0.120	0.626	0.046	0.155	-	-
% OMb	B	56.31z	45.78y	46.97y	40.17a,x	38.39b,x	< 0.001	1.364
	S400	53.51y	42.84x	49.80y	50.45bc,y	49.25c,y	< 0.001	0.626
	T300	53.64y	41.67x	38.49x	42.57ab,x	36.85ab,x	< 0.001	1.069
	C300	54.38z	44.41y	44.22y	45.35abc,y	34.15ab,x	< 0.001	0.274
	C1000	54.39z	46.6y	48.28y	52.92c,yz	33.8ab,x	< 0.001	1.788
	T+C300	53.77z	42.07y	40.29y	43.91ab,y	35.48ab,x	< 0.001	1.524
	T+C1000	53.76z	46.94y	49.86y	49.67bc,y	27.00a,x	< 0.001	2.937
	$P_{treatment}$	0.611	0.104	0.063	0.001	< 0.001	-	-

Treatments: B - control -; S400 - sulphite 400 ppm -; T300 - green tea extract 300 ppm - ; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -;C1000 – carvacrol 1000 pp, -; T + 1000C - green tea extract 300 ppm + carvacrol 1000 ppm -. Means and standard error of the mean (SEM). Different letters (a, b, c) within a column indicate significant differences among packaging conditions ($P \leq 0.05$). Different letters (x, y, z) within a row indicate significant differences among days of

display ($P \leq 0.05$). – = not determined. % MMb: percentage of metmyoglobin; % DMb: percentage of deoxymyoglobin; % OMb: percentage of oxymyoglobin.

Table 4. Microbial counts of lamb burger meat during display.

Treatments		Days of display						
		0	1	3	6	8	$P_{display}$	SEM
Aerobic total viable counts	B	4.29w	4.24w	5.53c,x	6.75d,y	7.58c,z	$P < 0.001$	0.056
	S400	4.29xy	3.92x	4.56a,yz	4.92a,z	5.18a,z	$P < 0.001$	0.066
	T300	4.29x	4.08x	5.22bc,y	6.44d,z	6.93bc,z	$P < 0.001$	0.066
	C300	4.29x	4.00x	4.87ab,y	6.29cd,z	6.74b,z	$P < 0.001$	0.052
	T+C300	4.29w	4.24w	5.14bc,x	6.23bcd,y	6.87bc,z	$P < 0.001$	0.028
	C1000	4.29w	3.85v	4.69a,x	5.57abc,y	6.40b,z	$P < 0.001$	0.034
	T+C1000	4.29w	3.79w	4.94ab,x	5.49ab,y	6.25b,z	$P < 0.001$	0.057
	$P_{treatment}$	-	0.0530	$P < 0.001$	$P < 0.001$	$P < 0.001$	-	
Enterobacteriaceae	B	4.19w	3.92w	5.32c,x	6.22c,y	7.22c,z	$P < 0.001$	0.071
	S400	4.19xy	3.67x	4.84ab,yz	4.77a,yz	5.59a,z	$P < 0.001$	0.09
	T300	4.19w	4.01w	5.08bc,x	6.17c,y	6.91bc,z	$P < 0.001$	0.068
	C300	4.19w	4.00w	4.96bc,x	5.58bc,y	6.46bc,z	$P < 0.001$	0.031
	T+C300	4.19x	4.35x	5.18bc,y	5.35ab,y	6.75bc,z	$P < 0.001$	0.029
	C1000	4.19wx	3.58w	4.57a,x	5.30ab,y	6.33b,z	$P < 0.001$	0.071
	T+C1000	4.19x	3.70w	4.92ab,y	5.01ab,y	6.50bc,z	$P < 0.001$	0.052
	$P_{treatment}$	-	0.0650	0.0050	$P < 0.001$	$P < 0.001$	-	-
Pseudomonas spp.	B	3.66w	3.98w	5.57c,x	7.49d,y	8.49d,z	$P < 0.001$	0.04
	S400	3.66x	3.62x	4.36a,xy	4.90a,y	6.62a,z	$P < 0.001$	0.08
	T300	3.66w	4.05w	5.07bc,x	7.43d,y	8.29cd,z	$P < 0.001$	0.056
	C300	3.66w	3.83w	5.34c,x	6.76c,y	8.07cd,z	$P < 0.001$	0.036
	T+C300	3.66w	3.62w	5.09bc,x	6.68bc,y	8.02bcd,z	$P < 0.001$	0.043
	C1000	3.66w	3.52w	4.58ab,x	6.39bc,y	7.30ab,z	$P < 0.001$	0.077
	T+C1000	3.66w	3.71w	4.70ab,x	6.14b,y	7.68bc,z	$P < 0.001$	0.059
	$P_{treatment}$	-	0.1550	$P < 0.001$	$P < 0.001$	$P < 0.001$	-	-
Lactic acid bacteria	B	2.91w	3.37b,w	4.62d,x	5.29b,y	6.37b,z	$P < 0.001$	0.064
	S400	2.91wx	2.79ab,w	3.59a,xy	4.51a,yz	4.05a,z	$P < 0.001$	0.079
	T300	2.91v	3.35b,w	4.50cd,x	5.36b,y	6.22b,z	$P < 0.001$	0.044
	C300	2.91w	2.97ab,w	4.12bc,x	4.82ab,y	5.71b,z	$P < 0.001$	0.033
	T+C300	2.91w	3.13ab,w	4.13bc,x	4.90ab,y	5.99b,z	$P < 0.001$	0.053
	C1000	2.91w	2.66a,w	3.81ab,x	4.48a,y	5.86b,z	$P < 0.001$	0.051
	T+C1000	2.91w	3.22ab,wx	3.67ab,x	4.42a,y	5.68b,z	$P < 0.001$	0.058
	$P_{treatment}$	-	0.0040	$P < 0.001$	$P < 0.001$	$P < 0.001$	-	-

Treatments		Days of display						
		0	1	3	6	8	$P_{display}$	SEM
Brochotrix termosphacta	B	nd	nd	3.38b,x	5.64d,y	6.96c,z	$P < 0.001$	0.069
	S400	nd	nd	2.92ab,x	4.33a,y	5.53a,z	$P < 0.001$	0.037
	T300	nd	nd	3.33b,x	5.73d,y	6.58bc,z	$P < 0.001$	0.07
	C300	nd	nd	2.97ab,x	5.49cd,y	6.18ab,z	$P < 0.001$	0.048
	T+C300	nd	nd	3.20b,x	5.22bcd,y	6.12a,z	$P < 0.001$	0.047
	C1000	nd	nd	2.56a,x	4.65abc,y	6.15ab,z	$P < 0.001$	0.066
	T+C1000	nd	nd	2.96ab,x	4.61ab,y	6.16ab,z	$P < 0.001$	0.047
	$P_{treatment}$	-	-	$P = 0.005$	$P < 0.001$	$P < 0.001$	-	-

Mean of microbial counts and standard error of the mean (SEM). Treatments: B -control -; S400 - sulphites 400 ppm -; T300 - green tea extract 300 ppm -; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -; C1000 –carvacrol 1000 ppm-; T + C1000 - green tea extract 300 ppm + carvacrol 1000 ppm -. Means with different letters (a, b, c) in the same column indicate significant differences among treatments ($P \leq 0.05$). Means with different letters (x, y, z) in the same row indicate significant differences among days of storage ($P \leq 0.05$). nd: not detected.

Table 5. Means and standard error of the mean (SEM) for sensory attributes of grill-cooked lamb.

Attributes	Treatments	Days of display					$P_{display}$	SEM
		0	1	3	6	8		
Oxidation odour	B	0.00w	0.00w	2.33b,x	5.67c,y	7.33d,z	<0.001	0.559
	S400	0.00w	0.00w	1.27ab,x	3.33b,y	4.50c,z	<0.001	0.350
	T300	0.00 x	0.00x	1.17ab,xy	2.67b,y	1.50b,xy	0.001	0.262
	C300	0.00	0.00	0.67a	0.67a	0.50ab	0.010	0.089
	C1000	0.00	0.00	0.33a	0.33a	0.00a	0.146	0.063
	T+C300	0.00	0.00	0.50a	0.67a	0.30ab	0.025	0.088
	T+C1000	0.00x	0.00x	0.40a,xy	0.33a,xy	0.83ab,y	0.004	0.088
	$P_{treatment}$	-	-	<0.001	<0.001	<0.001	-	-
Microbial odour	B	0.00w	0.00w	2.17b,x	5.33c,y	7.50c,z	<0.001	0.563
	S400	0.00x	0.00x	0.50a,xy	1.67a,y	4.50a,z	<0.001	0.344
	T300	0.00w	0.00w	1.17ab,x	5.00bc,y	7.00c,z	<0.001	0.541
	C300	0.00w	0.00w	1.37ab,x	3.00ab,y	6.17bc,z	<0.001	0.439
	C1000	0.00w	0.00w	0.17a,w	2.33a,x	4.50a,y	<0.001	0.348
	T+C300	0.00x	0.00x	1.00a,x	3.33abc,y	6.00abc,z	<0.001	0.462
	T+C1000	0.00x	0.00x	0.10a,x	2.67a,y	5.00ab,z	<0.001	0.383
	$P_{treatment}$	-	-	<0.001	<0.001	<0.001	-	-
Herbal odour	B	0.00a,x	0.17a,x	0.00a,x	0.67a,y	0.00a,x	<0.001	0.069
	S400	0.00a	0.17a	0.00a	0.33a	0.00a	0.222	0.056
	T300	0.67ab	1.17ab	0.50a	0.67a	0.83a	0.604	0.133
	C300	3.83cd,y	2.33b,x	2.50b,x	3.33b,y	3.83b,y	<0.001	0.145
	C1000	4.00cd,x	4.33c,xy	4.67c,xy	4.67c,xy	6.33c,y	0.028	0.256
	T+C300	2.33bc	2.00b	2.50b	2.67b	3.33b	0.123	0.140
	T+C1000	4.25d,x	4.33c,x	5.67c,y	5.87c,y	5.33c,xy	0.005	0.172
	$P_{treatment}$	<0.001	<0.001	<0.001	<0.001	<0.001	-	-
Oxidation flavour	B	0.00x	0.00x	2.17b,y	5.33c,z	5.83d,z	<0.001	0.475
	S400	0.00x	0.00x	1.33ab,y	3.67b,z	4.33c,z	<0.001	0.358
	T300	0.00x	0.00x	1.67ab,xy	3.33b,y	1.83b,xy	<0.001	0.297
	C300	0.00x	0.00x	1.33ab,z	0.33a,xy	0.83ab,yz	<0.001	0.125
	C1000	0.00x	0.00x	0.67a,y	0.67a,y	0.17a,xy	0.004	0.085
	T+C300	0.00x	0.00x	0.67a,y	0.00a,x	0.83ab,y	<0.001	0.085
	T+C1000	0.00	0.00	0.67a	0.00a	0.50a	0.027	0.092
	$P_{treatment}$	-	-	0.015	<0.001	<0.001	-	-
Microbial flavour	B	0.00w	0.00w	2.37b,x	5.33c,y	8.50e,z	<0.001	0.615
	S400	0.00x	0.00x	0.67a,x	2.00a,y	4.50b,z	<0.001	0.328
	T300	0.00w	0.00w	1.17ab,x	5.33c,y	7.50d,z	<0.001	0.578
	C300	0.00w	0.00w	1.37ab,x	4.00bc,y	6.00c,z	<0.001	0.454
	C1000	0.00w	0.00w	0.33a,w	2.00a,x	5.00b,y	<0.001	0.364
	T+C300	0.00x	0.00x	0.83a,x	4.33bc,y	6.67cd,z	<0.001	0.513
	T+C1000	0.00x	0.00x	0.17a,x	3.00ab,y	3.00a,y	<0.001	0.270
	$P_{treatment}$	-	-	<0.001	<0.001	<0.001	-	-
Herbal flavour	B	0.00a	0.00a	0.00a	0.17a	0.17a	0.86	0.069
	S400	0.17 a	0.00 a	0.00 a	0.00a	0.00a	0.861	0.069
	T300	0.67ab	0.83a	0.83 a	0.33 a	0.27a	0.404	0.133
	C300	4.17c,z	2.67b,x	2.17b,xy	3.33b,yz	3.83b,z	<0.001	0.177
	C1000	5.67c	4.67c	4.33c	6.00c	5.83c	0.088	0.240
	T+C300	2.33b,x	2.50b,xy	2.83b,xyz	3.67b,z	3.33b,yz	<0.001	0.126
	T+C1000	5.00c,xy	4.33c,x	5.17c,xy	5.07c,xy	5.83c,y	0.008	0.139
	$P_{treatment}$	<0.001	<0.001	<0.001	<0.001	<0.001	-	-

Treatments: B - control -; S400 - sulphites 400 ppm -; T300 - green tea extract 300 ppm -; C300 - carvacrol 300 ppm -; T+C300 - green tea extract 300 ppm + carvacrol 300 ppm -; C1000 – carvacrol 1000 ppm-; T + 1000C - green tea extract 300 ppm + carvacrol 1000 ppm -. Means and standard error of the mean (SEM). Different letters (a, b, c, d) within a column indicate significant differences among packaging conditions ($P \leq 0.05$). Different letters (w, x, y, z) within a row indicate significant differences among days of display ($P \leq 0.05$). A 10 point scale (0 = low, 10 = high); – = not determined.