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# PHYSICOCHEMICAL, MICROBIOLOGICAL QUALITY AND OXIDATIVE STABILITY IN SPICED LAMB MEAT BURGERS

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**ABSTRACT**

The effect of adding two powdered spices (rosemary and thyme) on the pH, colour coordinates, Cooking yield (CY) Cooking loss (CL), Diameter Reduction (DR), Shear Force (SF), microbiological levels and lipid oxidation (LO) in two types of lamb burgers (L= leg meat; LNB= leg+neck+breast meat) was assessed over a six day period. Both spices increased stability during the storage period, LO values being six times lower than those of the non-spiced control group at 6 days. L samples showed higher CY, lower CL and DR than LNB burgers, with significant differences at 6 d ($P < 0.001$). The length of storage only affected ($P < 0.01$) these parameters in L burgers. In general, SF was higher in LNB than in L burgers but did not vary with time. The colour coordinates showed lower values in L than in LNB samples. The formulation type affected TVC and Pseudomonas spp.

**Keywords:** Lamb burger, formulation, spice, physicochemical, shelf life

## INTRODUCTION

The sheep sector in the EU [127,306,839 heads of livestock; (**FAOSTAT, 2011**)] is concentrated in less favoured areas, where is plays a key environmental role. Among the main sheep breeds in Spain (the second EU country as regards the number of sheep), the Manchega breed is raised in Castilla-La Mancha region. Lamb is the most important product from this breed, which in 1996 attained its own official label "Manchego Lamb Denomination of Origin", thus enabling it to be advertised and guarantee its quality compared with other breeds. This lamb meat is highly appreciated (**Vergara and Gallego, 2001**) due to its organoleptic quality although consumption is remains low, 2.1 kg/per capita (**MAGRAMA, 2012**) probably as a result of its high price, caused by high production costs such as the cost of lamb feed. In addition lamb meat consumption is mainly associated with the pieces on the lamb carcass considered as first (leg or ribs) or second (shoulder) category. The other pieces, third category (neck and breast), are more difficult to sell because of their composition and the greater difficulty involved in their preparation. The European Parliament has expressed the need for product innovation in the small ruminants industry, especially in the case of lamb meat (**Aylward, 2008**). This study was carried out with this in mind and in order to optimize the use of the lamb carcass pieces to obtain new meat products for Manchega D.O. lamb. In an earlier paper (**Linares *et al.*, 2012**) we reported the nutritional composition, fatty acid (profile and indexes) and sensory properties in two types of lamb meat burger elaborated with different types of meat (leg vs. leg+neck+breast). Both types of burger raised nutritional indexes to a healthy threshold, and both possessed similar sensorial properties. Now we report on the physicochemical parameters (the dimensional changes in burgers after cooking are important because they affect the reactions of consumers) and microbiological-oxidative stability of these lamb meat products, which are largely unknown.

On the other hand many studies have examined the effect of the natural antioxidants found in plants on meat products. These may be in the form of an extract, whose effect has been analysed in beef patties (**Mohamed *et al.*, 2011**), beef meatballs (**Fernández-López *et al.*, 2005**), lamb patties (**Andrés *et al.*, 2010**; **Ibrahim *et al.*, 2010**) and pork burgers (**Lara *et al.*, 2011**) or in the form of essential oils the efficiency of which has been assessed in different types of frankfurters (**Estévez and Cava, 2006**) and in mortadella (**Viuda-Martos *et al.*, 2011**). However few papers, with the exception of those on minced pork (**Shahidi *et al.*, 1995**), minced chicken (**El-Alim *et al.*, 1999**), beef patties (**Sánchez-Escalante *et al.*, 2001**), and fresh pork sausages (**Martínez *et al.*, 2007**) have looked at the influence of the powdered spices (ground aromatic herbs) whose effect on lamb meat burgers has not been assessed. According to **El-Alim *et al.* (1999)** and **Estevez and Cava (2006)**, the matrix to which the antioxidants are added (type of meat, fat percentage) and format (essential oils, extract and powder) influence on the antioxidant capacity of the plants/herbs in question. Therefore, the aim of the present study was to evaluate the physicochemical quality (pH, colour, cooking characteristics, shear force) and the microbial-oxidative stability of two types of burger made with different parts of the carcass (leg vs. leg+neck+breast) spiced with rosemary or thyme or not spiced (control group).

## MATERIAL AND METHODS

### Preparation and Sampling of lamb burgers

Burgers were manufactured using meat obtained from light Manchega breed lambs which were slaughtered at 25 kg live weight (70 days old), having been fed with milk until weaning at 12 kg and then with a commercial concentrate and cereal straw *ad libitum* until slaughter. All carcasses were chilled at 4 ºC for 24 h in a conventional chiller after slaughter and dressed using standard commercial procedures.

Legs, neck and breast of the lamb carcasses were used in this experiment. These meat pieces were deboned and, the leg meat was minced separately from the remaining pieces (neck and breast), which were minced together. This provided two types of formulation according to the meat used to elaborate the burgers:

-Formulation type 1 (L): burgers completely made from minced hindleg meat.
-Formulation type 2 (LNB): burgers elaborated using minced hindleg meat (2/3) and the rest (1/3) minced neck and breast meat.

In both formulations (L and LNB) the ground meat was divided into three batches, adding (0.1 % w/w, for both spices) ground rosemary (Artemis, Alicante, Spain) or thyme (Artemis, Alicante, Spain) and leaving one non-spiced control (Control). In total, 6 different batches were obtained. From each batch, 48 burgers were prepared: 24 burgers each for the raw and cooked analyses (8 samples for each time and analysis).

After mixing by hand for 5 min with salt (1%, w/w) to ensure uniform distribution, lamb burgers weighting 100 g and 10 cm in diameter were formed using a commercial burger maker. The burgers were packed in white expanded

polystyrene trays (model 90M Coopbox Hispania S.L.U, Lorca, Murcia, Spain) and covered with a polyvinyl chloride (PVC) having an oxygen permeability 12500-13200 $cm^3/m^2$/24 h and water vapour permeability 200-230 $g/m^2$/24h. Samples were stored in darkness at 2 ºC.

**Analysis of lamb burgers**

The nutritional composition (published in **Linares *et al*., 2012**), physicochemical parameters, microbiological values and lipid oxidation were assessed in the burgers at 0, 3 and 6 days post manufacture. From each batch three replicates were made.

The following parameters were assessed in the raw lamb burgers:

-pH: measured using a Crison GLP 22 pH meter (Crison Instruments, S.A., Spain) in a suspension resulting from blending a 3 g sample with 27 ml distilled water for 10 seconds with a Ultra Turrax IKA model T25. Two measurements were taken for each sample.

-Colour coordinates (L*, lightness, a*, redness and b*, yellowness values): evaluated using a Minolta chromameter CR400 (Osaka, Japan) with a D65 illuminant and a 10º standard observer, calibrated against a standard white tile on the surface of the sample. Three readings were recorded for each burger and each value was the mean of three determinations.

-Microbiological analysis: samples (approximately 5 g) were transferred to a sterile bag with 45 ml of tryptone phosphate water (buffered peptone water; Scharlau Chemie, Barcelona, Spain) and blended for 60 seconds in a Stomacher (Masticator, IUL Instruments, Barcelona, Spain). Duplicate 100µl inoculums of 10-1 decimal solution (primal solution) were spread on Petri dishes using a spiral system (Eddy-Jet, IUL-Instruments, Barcelona, Spain), for enumerations of: total viable counts (TVC) on Plate Count Agar (PCA, Scharlau Chemie S.L., Barcelona, Spain), *Enterobacteriaceae* on Violet Red Bile Dextrose Agar (VRBD, Scharlau Chemie S.L, Barcelona, Spain) and *Pseudomonas* spp. on Pseudomonas Agar Base with a cetrimid, fucidin and cephaloridin supplement (Pseudomonas CFC, Oxoid LTD; Basingstoke, Hampshire, England). For all bacterial counts, plates were incubated at 32 ºC for 48 h (TVC) or 24 h (*Enterobacteriaceae*), except for *Pseudomonas* spp., which were incubated at 25 ºC for 48 h. An automatic colony counter (Countermat-Flash, IUL-Instrument, Barcelona, Spain) was used for counting. The results were expressed as log CFU/g.

-Lipid Oxidation: determined according to **Tarladgis *et al.* (1964)**, using a 5 g sample which was then homogenized with 25 ml of distilled water in an Ultraturrax T25 digital (IKA Works, Inc., USA) homogenizer for 2 minutes at 10,000 rpm. Absorbance was measured in duplicate and read with a Helios alfa spectrophotometer (THERMO, Electron Corporation, England) at 532 nm. The results are expressed as mg MDA/kg of meat.

To determine cooking characteristics, burgers were cooked in a conventional electric grill to an internal temperature of 72 ºC, determined at the centre of the burger using a digital food thermometer. After cooking, the burgers were equilibrated to room temperature until the samples reached an inner temperature of 25 ºC. The diameter and weight of each burger were measured before and after cooking. The following cooking characteristics were determined:

-Cooking yield (CY): estimated by the following equation: CY% = 100 × (cooked burger weight/raw burger weight).

-Cooking loss (CL): estimated by the following calculation: CL%= 100 - CY.

-Diameter reduction (DR): determined using a digital vernier caliper by the mean of four measurements in the same burger and calculated by the following equation: DR %= 100 × (raw diameter - cooked diameter)/raw diameter.

-Shear Force (SF): was analysed with Stable Micro system Mod TA.XT2 texturometer equipped with a Warner-Bratzler device. For this analysis we used the same cooked burgers cut into four replicate strips (1 $cm^2$ cross-section and 2 - 3 cm in length) were used after determining the above parameters. The results are expressed as $N/cm^2$.

**Statistical analysis**

The effect of different types of burger (L vs. LNB) and spice (rosemary, thyme and control) on the physicochemical parameters (pH, colour, CL, DL, CY, DR and SF), microbiological quality and lipid oxidation at 1, 4 and 7 days post-mortem (0, 3 and 6 d post manufacture of burgers) were analysed using a General Linear Model procedure with the Statistical Package SPSS 19.0 version statistical (SPSS Inc., Chicago, USA, 2010). The differences in burger parameters due to time of storage (0, 3 and 6 days) were analysed using an analysis of variance. When the differences between times were significant ($P < 0.05$), a Tukey's test at a significance level of $P < 0.05$ was carried out to check the differences between pairs of groups.

**RESULTS AND DISCUSSION**

**Physicochemical parameters**

Table 1 shows the values of pH, Cooking Yield (CY), Cooking loss (CL), Diameter Reduction (DR) and Shear Force (SF) in each group of burgers during the storage period studied.

-Value of pH

In general the type of formulation (L or LNB) did not affect pH value, which agrees with the findings of **Khalil (2000)** in beef burgers with different fat contents and **Pietrasik (1999)** in pork sausages.
Value of pH was also unaffected by the addition of spices, which reflects the data published by other authors with regard to the variation of this parameter using other natural antioxidants, such as **Naveena *et al*. (2008)** in chicken patties or **Andrés *et al*. (2010)** in lamb patties.
In general, the storage period increased this parameter, the pH being significantly higher in the burgers spiced with rosemary ($P < 0.01$ and $P < 0.05$ for L and LNB groups respectively) or thyme in the burgers manufactured with leg meat ($P < 0.05$). This increase in pH with time agrees with the results of the other authors who studied this parameter in meat products, such as **Ibrahim *et al.* (2010)** in lamb patties; **McCarthy *et al*. (2001)** in pork patties with rosemary; **Verma and Sahoo (2000)** in ground chevon. According to **Drosinos and Board (1995)** this increase in pH could be due to an increase in the microbial flora in aerobical conditions of storage because of the accumulation of bacterial metabolites and protein breakdown (**Karabagias *et al*., 2011**). **Babji *et al*. (2000)** associated high pH values (pH>6.0) in minced goat meat with high Enterobacteriaceae counts. However, in our study at the end of the storage period (6 d post-manufacture) and in all types of burgers, the pH values were lower than the level (pH= 6.4) considered as critical for consumption according to **Prost *et al*. (1985)**.

-Cooking characteristics [(Cooking Yield (CY), Cooking loss (CL) and Diameter Reduction (DR)]

The presence (rosemary, thyme) or absence of spices (control) did not have effect on these parameters. Three days after the elaboration of the samples, L burgers showed higher CY and lower CL and DR values than LNB burgers, while the differences were significant 6 d post storage ($P < 0.001$). A significant effect of storage time ($P < 0.01$) on CY, CL and DR was found in L burgers, CY increasing and the other two parameter decreasing. The differences between formulations (L or LNB) could be due to the differences ($P < 0.001$) in fat composition (5% vs. 10.7% for L and LNB, respectively; data from **Linares *et al*., 2012**) according to **Tornberg *et al.* (1989)**. These authors concluded that fat was more easily removed during cooking from higher-fat beef burgers thus lowering the cooking yield. In addition, some authors have reported that a decrease in fat improved the CY (**El-Magoli *et al*., 1996**; **Farouk *et al*., 1999**). Our findings showed that these parameters depend on the type of formulation of the meat product, suggesting that dimensional changes of lamb meat products, such as CY, CL or diameter reduction, improve when only first category carcass parts (such as leg) are used in the formulation.

-Shear Force (SF)

Only the type of formula (meat type used to elaborate the burgers) affected SF values ($P < 0.01$ and $P < 0.001$, at 0 and 3 days of storage, respectively). In general this parameter was higher in LNB than in L burgers. **Khalil (2000)**, showed that when the fat replacement level in beef patties increased (with modified corn starch and water), the SF decreased. According to **Cavestany *et al.* (1994)** there is a direct relationship between fat content and the values of SF. This could explain the lower SF found in L burgers. Curiously, in our previous paper (**Linares *et al*., 2012**), the formulation type did not affect the mouth texture attributes and these sensorial properties did not vary with time as occurred in the present study with the SF parameter.
SF values found in the present paper were higher than those shown by **Biswas *et al.* (2004)** in pork patties, **Kumar and Sharma (2004)** in low-fat pork patties but lower than those observed by **Jeong *et al.* (2007)** in pork patties with a different fat level.

**Table 1** Effect of formulation type (L *vs* LNB) and spice on the physicochemical parameters (Mean ± SE) during storage time storage in Manchega breed lamb burgers

| Parameters | Storage period (days) | Type L | | | Type LNB | | | GLM | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | Rosemary | Thyme | Control | Rosemary | Thyme | Type | Spice | Type x Spice |
| pH | 0 | 5.81±0.03 | 5.75±0.02$^{a}$ | 5.75±0.02$^{a}$ | 5.84±0.01 | 5.80±0.01$^{a}$ | 5.79±0.03 | NS | NS | NS |
| | 3 | 5.83±0.01$^{x}$ | 5.88±0.00$^{b,xy}$ | 5.90±0.02$^{ab,y}$ | 5.78±0.02 | 5.82±0.01$^{ab}$ | 5.83±0.05 | NS | * | NS |
| | 6 | 5.89±0.09 | 5.90±0.03$^{b}$ | 5.91±0.07$^{b}$ | 5.82±0.01 | 5.87±0.02$^{b}$ | 5.71±0.14 | NS | NS | NS |
| | Effect of storage period | NS | ** | * | NS | * | NS | | | |
| CY | 0 | 72.28±1.51$^{a}$ | 72.00±1.65$^{a}$ | 72.64±1.37$^{a}$ | 73.77±0.99 | 73.56±0.61 | 73.16±0.43 | * | NS | NS |
| | 3 | 77.85±1.49$^{b}$ | 77.62±1.43$^{b}$ | 77.72±1.31$^{b}$ | 74.63±1.58 | 74.41±0.92 | 74.51±0.61 | NS | NS | NS |
| | 6 | 79.62±1.12$^{b}$ | 78.67±0.90$^{b}$ | 79.87±1.21$^{b}$ | 75.48±2.13 | 74.52±1.67 | 75.00±1.17 | *** | NS | NS |
| | Effect of storage period | ** | ** | ** | NS | NS | NS | | | |
| CL | 0 | 27.72±1.51$^{b}$ | 28.00±1.65$^{b}$ | 27.36±1.37$^{b}$ | 26.23±0.99 | 26.44±0.61 | 26.84±0.43 | * | NS | NS |
| | 3 | 22.15±1.49$^{a}$ | 22.38±1.43$^{a}$ | 22.28±1.31$^{a}$ | 25.37±1.58 | 25.59±0.92 | 25.49±0.61 | NS | NS | NS |
| | 6 | 20.38±1.12$^{a}$ | 21.33±0.90$^{a}$ | 20.13±1.21$^{a}$ | 24.52±2.13 | 25.48±1.67 | 25.00±1.17 | *** | NS | NS |
| | Effect of storage period | ** | ** | ** | NS | NS | NS | | | |
| DR | 0 | 19.66±0.77$^{a}$ | 19.16±0.83$^{b}$ | 20.43±1.37 | 20.21±1.08 | 20.69±1.21 | 20.92±1.05 | NS | NS | NS |
| | 3 | 18.30±0.92$^{a}$ | 17.48±0.41$^{ab}$ | 17.94±0.57 | 19.40±0.99 | 19.87±0.64 | 20.22±0.41 | NS | NS | NS |
| | 6 | 14.56±1.03$^{b,x}$ | 15.71±0.37$^{a,xy}$ | 17.01±0.41$^{y}$ | 17.87±1.33 | 19.41±0.82 | 19.11±0.69 | *** | NS | NS |
| | Effect of storage period | ** | ** | NS | NS | NS | NS | | | |
| SF (N cm$^{-2}$) | 0 | 17.12±0.98 | 16.73±0.40 | 16.61±1.10 | 17.44±1.65 | 16.03±0.60 | 16.19±1.29 | ** | NS | NS |
| | 3 | 15.07±1.45 | 15.07±1.52 | 15.03±0.43 | 16.58±1.65 | 16.54±1.41 | 16.48±1.59 | *** | NS | NS |
| | 6 | 13.47±0.52 | 14.73±0.48 | 14.92±0.63 | 16.90±1.04 | 15.69±1.65 | 16.05±1.31 | NS | NS | NS |
| | Effect of storage period | NS | NS | NS | NS | NS | NS | | | |

**Legend:** NS: not significant. *, **, ***: indicates significance levels at 0.05, 0.01 and 0.001, respectively. $^{a, b}$: Values in the same column with different superscripts are significantly different ($P < 0.05$) for the different storage period (0,3 and 6 days). $^{x, y}$: Values in the same row with different superscripts are significantly different ($P < 0.05$) for the different spice at the same type burger (L or LNB).

-Colour coordinates (L*, a*, b)

The formulation type of the burgers affected the colour coordinates (Table 2). The differences in the L* coordinate ($P < 0.001$ at 0 and 3 d of storage) between L and LNB agree with the observations of **Pietrasik (1999)** that showed the highest brightness values in meat products (sausages) corresponded those with a high fat content. On the other hand our L* values were higher the values mentioned by **Hopkins (1996)** and **Khliji *et al.* (2010)**, who considered 34-35 as acceptable for the consumers of beef and lamb meat.

Neither the type of spice nor the storage time had any effect on this parameter. This stability of the L* coordinate was mentioned by other authors including **Cheng *et al.* (2007)** in pork burgers and **Andrés *et al.* (2010)** in lamb patties.

According to **Khliji *et al.* (2010)**, a* is the coordinate most strongly related with the consumer assessment of meat colour. For this reason the preservation of redness and the stability of this parameter are necessary to satisfy consumer demand. A decrease in the a* coordinate with storage time is related with the changes in colour from red to brown due to the oxidation of oxymyoglobin to metmyoglobin (**Cheng *et al.*, 2007**). In our study a* decreased with time ($P < 0.001$) in both formulations (L and LNB) and significant differences ($P < 0.001$) were found at the end of the experiment with the values in L burgers, being lower than in LNB. This decrease in a* during a period of 6 days agrees with the findings of **Lund *et al.* (2007)** in the half time of beef patties containing rosemary extract. In contrast the b* coordinate increased with time ($P < 0.001$) in all batches, although to a lesser extent in L burgers. The same trend has been observed in lamb meat (**Nieto *et al.*, 2011**) and in pork patties (**Lara *et al.*, 2011**). Although other studies mentioned that the red colour is preserved by the addition of natural extracts (**Georgantelis *et al.*, 2007**; **Hernández *et al.*, 2009**; **Doolaege *et al.*, 2012**), in the present study the addition of powered herbs had no effect on the variation in this parameter with time. According to our results, the effect of either spice on the colour stability depends on the format type and the matrix to which the spice is added.

**Table 2** Effect of formulation type (L *vs* LNB) and spice on colour (Mean ± SE) during time storage in Manchega breed lamb burgers.

| Parameters | Storage period (days) | Type L | | | Type LNB | | | GLM | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | Rosemary | Thyme | Control | Rosemary | Thyme | Type | Spice | Type x Spice |
| L* | 0 | 45.53±1.18 | 46.22±0.82 | 46.22±0.99 | 48.80±0.90 | 49.72±0.97 | 47.66±0.61 | *** | NS | NS |
| | 3 | 45.53±1.17 | 44.90±0.82 | 43.82±0.90 | 50.01±1.07 | 49.71±1.13 | 48.20±1.00 | *** | NS | NS |
| | 6 | 46.95±0.88 | 46.56±1.19 | 45.31±1.16 | 49.69±1.11 | 49.76±0.98 | 47.42±1.23 | NS | NS | NS |
| | Effect of storage period | NS | NS | NS | NS | NS | NS | | | |
| a* | 0 | 15.35±0.42$^{c}$ | 14.40±0.64$^{b}$ | 14.43±0.35$^{b}$ | 15.38±0.53$^{b}$ | 14.09±0.45$^{b}$ | 14.32±0.43$^{b}$ | NS | NS | NS |
| | 3 | 11.36±0.64$^{b}$ | 11.06±0.47$^{a}$ | 11.79±0.49$^{a}$ | 12.36±0.35$^{a}$ | 12.56±0.41$^{b}$ | 13.12±0.40$^{b}$ | NS | NS | NS |
| | 6 | 9.04±0.56$^{a}$ | 8.97±0.74$^{a}$ | 9.76±0.86$^{a}$ | 11.64±0.54$^{a}$ | 10.30±0.53$^{a}$ | 10.88±0.45$^{a}$ | *** | NS | NS |
| | Effect of storage period | *** | *** | *** | *** | *** | *** | | | |
| b* | 0 | 6.89±0.51$^{a}$ | 7.44±0.41$^{a}$ | 7.46±0.29$^{a}$ | 7.83±0.36$^{a}$ | 8.66±0.35$^{a}$ | 7.79±0.34$^{a}$ | *** | NS | ** |
| | 3 | 9.33±0.20$^{b}$ | 9.45±0.33$^{b}$ | 9.46±0.19$^{b}$ | 10.10±0.27$^{b}$ | 10.52±0.32$^{b}$ | 10.94±0.16$^{b}$ | * | NS | NS |
| | 6 | 9.49±0.24$^{b}$ | 9.05±0.27$^{b}$ | 9.18±0.29$^{b}$ | 10.82±0.25$^{b}$ | 10.25±0.32$^{b}$ | 10.79±0.36$^{b}$ | ** | NS | NS |
| | Effect of storage period | *** | *** | *** | *** | *** | *** | | | |

**Legend:** NS: not significant. *, **, ***: indicates significance levels at 0.05, 0.01 and 0.001, respectively. $^{a, b}$: Values in the same column with different superscripts are significantly different ($P < 0.05$) for the different storage period (0,3 and 6 days).

**Microbiological growth**

Table 3 shows the effect of the formulation, the added spice and storage time on the microorganisms analysed during the storage period studied.

Significant differences in TVC ($P < 0.05$ and $P < 0.001$, at 0 and 6 days of storage respectively) and *Pseudomonas* spp. values ($P < 0.001$ and $P < 0.05$ at 0 and 3 days respectively) were found between L and LNB samples.

Although the spices are known to prolong the storage life of foods through bacteriostatic or bactericidal activity (**Beuchat, 1994**) and the addition of natural extracts and essential oils has an even greater effect against microbial growth (**Tajkarimi *et al.*, 2010**), in our study the addition of powdered rosemary or thyme did not affect microbial stability. This way of adding the herb (ground leaves) or the dose used (0.1% w/w) was not able to inhibit the microorganism growth.

The length of storage caused a significant increase in TVC ($P < 0.001$ in L burgers and $P < 0.05$ in the LNB with rosemary or thyme) and in *Pseudomonas* spp. ($P < 0.001$ for control and thyme burgers and $P < 0.01$ in rosemary ones, in both L and LNB).

**Verma and Sahoo (2000)** observed that total bacteria increased with storage time in minced goat meat, where it was associated with a change in meat colour and increased lipid oxidation.

A value limit of 7 log CFU/g is considered the threshold of spoilage level for meat products (**Jackson *et al.*, 2001**). According to **EC Regulation Nº 1441/2007** the maximum values allowed in ground meat is 6.69 log CFU/g. In our study, only the L burgers came near to limit in TVC after 6 d of storage.

The level of *Enterobacteriaceae* has been used as an indicator of hygiene and contamination after processing (**Zeitoun *et al.*, 1994**). In the present study, none of the factors (formula, spice or time storage) had any effect on

*Enterobacteriaceae* count. Some authors found higher values than ones in minced lamb (**Drosinos and Board, 1995**), ground goat meat (**Babji *et al*., 2000**) and pork sausages with different extracts (**Georgantelis *et al*., 2007**). The low level in *Enterobacteriaceae* found in this suggests a high level of hygienic in these lamb burgers.

**Table 3** Effect of formulation type (L *vs* LNB) and spice on microbial growth (Mean ± SE) during storage time in Manchega breed lamb burgers.

| Microorganism | Storage period (days) | Type L | | | Type LNB | | | GLM | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | Rosemary | Thyme | Control | Rosemary | Thyme | Type | Spice | Type x Spice |
| TVC | 0 | 4.35±0.20$^{a}$ | 4.51±0.04$^{a}$ | 4.54±0.05$^{a}$ | 4.51±0.17 | 4.45±0.16$^{a}$ | 4.38±0.20$^{a}$ | * | NS | NS |
| | 3 | 4.94±0.07$^{a}$ | 5.01±0.09$^{a}$ | 4.94±0.08$^{a}$ | 4.89±0.30 | 4.92±0.46$^{ab}$ | 5.00±0.34$^{ab}$ | NS | NS | NS |
| | 6 | 6.40±0.25$^{b}$ | 6.37±0.22$^{b}$ | 6.49±0.17$^{b}$ | 5.18±0.03 | 5.80±0.41$^{b}$ | 5.58±0.16$^{b}$ | *** | NS | NS |
| | Effect of storage period | *** | *** | *** | NS | * | * | | | |
| *Enterobacteriaceae* | 0 | 2.05±0.06 | 2.16±0.04 | 2.21±0.08 | 2.53±0.32 | 2.40±0.39 | 2.13±0.37 | NS | NS | NS |
| | 3 | 2.30±0.05 | 2.33±0.09 | 2.18±0.06 | 2.56±0.33 | 2.80±0.44 | 2.49±0.38 | NS | NS | NS |
| | 6 | 2.63±0.25 | 2.63±0.24 | 2.53±0.18 | 2.69±0.29 | 3.17±0.77 | 2.96±0.50 | NS | NS | NS |
| | Effect of storage period | NS | NS | NS | NS | NS | NS | | | |
| *Pseudomonas* spp. | 0 | 4.07±0.16$^{a}$ | 4.04±0.23$^{a}$ | 4.07±0.18$^{a}$ | 3.68±0.18$^{a}$ | 3.73±0.22$^{a}$ | 3.61±0.23$^{a}$ | *** | * | * |
| | 3 | 5.40±0.27$^{b}$ | 5.60±0.21$^{b}$ | 5.42±0.24$^{b}$ | 4.87±0.19$^{b}$ | 5.02±0.30$^{b}$ | 4.95±0.13$^{b}$ | * | NS | NS |
| | 6 | 5.73±0.01$^{b}$ | 5.30±0.13$^{b}$ | 5.67±0.03$^{b}$ | 6.07±0.72$^{c}$ | 5.48±0.34$^{b}$ | 6.13±0.74$^{b}$ | NS | NS | NS |
| | Effect of storage period | *** | ** | *** | *** | ** | *** | | | |

**Legend**: NS: not significant. *, **, ***: indicates significance levels at 0.05, 0.01 and 0.001, respectively. $^{a, b}$: Values in the same column with different superscripts are significantly different ($P < 0.05$) for the different storage period (0,3 and 6 days).

**Lipid oxidation (LO)**

No significant differences between the burger formulations were found for this parameter (Table 4) despite the significant differences in fat and PUFA composition (data from **Linares *et al*., 2012**). Our results contrast with those of **Fernández-López *et al*. (2006)** in different types of ostrich burgers, where the highest TBA value corresponded to the fattest formula.

On the other hand, an increase in LO during the storage has been associated with mincing (**Verma and Sahoo, 2000**), because this process increases the surface area of the meat and its contact with oxygen (**Gray *et al*., 1996**). In addition the use of salt from 0.5 to 2.5% for the preparation of meat products is considered as a prooxidant promoting lipid oxidation (**Rhee and Ziprin, 2001**). These facts could explain the rapid and significant increase in MDA values (Figure 1) observed with storage time ($P < 0.001$) in the control burgers (both L and LNB), in which the initial level ranging from 1.12 to 0.99 rose to 2.63-3.03 mg MDA/kg at 6 d of storage, for L and LNB respectively.

The results of this study show (Figure 1) that both spices increased LO stability during the storage period, LO values being six times lower than those of the non-spiced control group at 6 days. The similar antioxidant activity showed by rosemary and thyme agrees with the mentioned by **Dorman *et al*. (2003)**. In addition, TBARS values did not vary during the storage time in spiced samples and their value (around 0.5 mg MDA/kg) reflects the quality of the products from a sensorial point of view (**López and Menoyo, 2000**). These TBARS values were similar to those found by **Andrés *et al*. (2010)** in lamb burgers with different plant extracts stored in modified atmosphere. Some authors on lipid oxidation such as **El-Alim *et al*. (1999)** have indicated that the dried spices are suitable for prevention of reactions of oxidative character on raw ground chicken meat. Our results agree with these studies. We consider that use powdered spices is an effective and cheap solution for lipid stabilization and it is not necessary to prepare an extract.

**Table 4** Effect of formulation type (L *vs* LNB) and spice on lipid oxidation (mg MDA kg$^{-1}$ of meat) on Manchega breed lamb burgers during storage time

| | Storage period (days) | GLM procedure | | |
|---|---|---|---|---|
| | | Type | Spice | Type x Spice |
| Lipid Oxidation | 0 | NS | *** | *** |
| | 3 | NS | *** | NS |
| | 6 | NS | *** | * |

**Legend:***, ***: indicates significance levels at 0.05 and 0.001, respectively. NS: not significant.

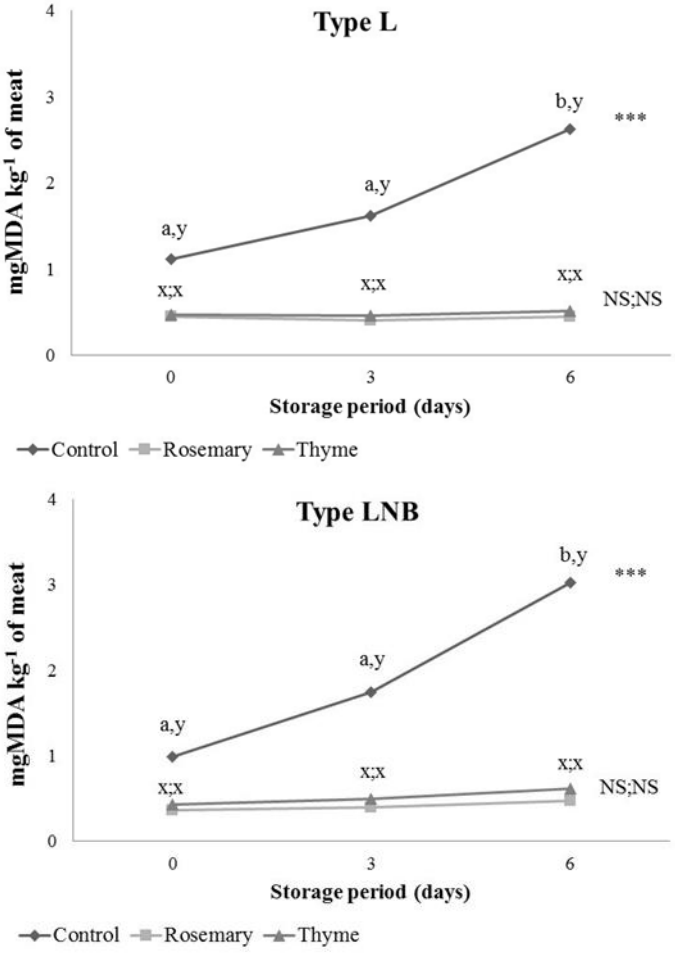


**Figure 1** Lipid oxidations (mg MDA kg$^{-1}$ of meat) in Manchega breed lamb burgers in each formulation type.

**Legend**: L: burgers completely made from minced hindleg meat; LNB: burgers elaborated using 2/3 hindleg minced meat and the rest (1/3) neck and breast minced meat. $^{x, y}$: Indicate significant differences ($P < 0.05$) among the different types of burgers due to the type of spice (Control, Rosemary, Thyme) used in the elaboration. $^{a, b}$: Indicate significant differences ($P < 0.05$) due to the storage period at the same burgers type. ***: Indicate significant differences ($P < 0.001$) due to the storage period in the not spiced burgers. NS: Indicate not significant differences due to the storage period in the spiced burgers with rosemary or with thyme

## CONCLUSION

According to the results we can conclude: (1) The use of lamb carcass parts of a minor category (neck or breast) to elaborate burgers lowered CY (up to 5%), but increased CL, DR and SF. (2) A similar trend with the storage time in the colour coordinates was shown in both formulations. (3) Both formulations and in the conditions of this study would maintain the product within the legally limit for up to 6 days. (4) The concentration and way of use (in ground herb) of the spices used in this work was effective against TBARS formation but did not vary other parameters analysed.

In addition and according to data obtained in this study and in our previous study (**Linares *et al*. 2012**) Manchego carcass pieces of the carcass of a minor category (neck and breast) could be considered suitable for the production of lamb burgers. Data from the current study indicate that the use of powdered rosemary or thyme in lamb meat products such as burgers that does not involve any synthetic antioxidant is an alternative solution.

Finally it might be interesting to study the effect of other natural additives on burger quality and to increase lamb meat product range and lamb meat consumption.

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