Lamb meat burgers shelf life: effect of the addition of different forms of rosemary (Rosmarinus Officinalis L.)

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ABSTRACT
The effect of different rosemary formats (powdered: RP; extract: REX; essential oil: ROE; oleoresin: ROL) on vacuum-packed lamb meat raw burgers shelf-life was studied. pH, color coordinates (L*, a*, b*), microbial count (Total viable count, Enterobacteriaceae, Pseudomonas spp., Lactic acid bacteria), and lipid oxidation were analysed during 14 d. A control group, without spice was used. pH decreased (p < .001) in all samples, reaching the lowest values in ROL. L* remained stable, except in ROL (p < .05), a* showed variations in RP (p < .05), and b* in RP (p < .01) and in ROL (p < .001). Differences (p < .001) were found among treatments on lipid oxidation, with the lowest values in RP. The microbial counts increased with the storage (p < .001) in all microorganism groups in all batches. Shelf life was 10 days for RP and control, 14 days for REX, REO and ROL.

RESUMEN
El efecto de diferentes formatos de romero (molido: RP; extracto: REX; aceite esencial: ROE; o oleoresina: ROL) en la vida útil de hamburguesas de cordero envasadas al vacío fue estudiado. pH, color (L* a* b*), crecimiento microbiano (recuento total de aerobios, Enterobacteriaceae, Pseudomonas spp y bacterias ácido-lácticas) y oxidación de lipídica fueron analizados durante 14 días. Un lote control sin especia fue usado. pH descendió (p < .001) en todas las muestras, alcanzando los valores más bajos en ROL. L* permaneció estable, excepto en ROL (p < .05), a* mostró variación en RP (p < .05), y b* en RP (p < .01) y en ROL (p < .001). Hubo diferencias (p < .001) entre grupos en la oxidación lipídica, con los valores más bajos en RP. La carga microbiana aumentó con el almacenamiento (p < .001) en todos los microorganismos y en todos los lotes. La vida útil fue 10 días en RP y control y 14 días en REX, ROE y ROL.

1. Introduction
Meat and meat products contribute to our diet with natural and essential elements, such as vitamins, amino acids, high-quality proteins and minerals (Estévez et al., 2005). However, meat is a highly perishable food, mainly due to its high water activity, nutritional composition, unsaturated lipids presence and incorporation of oxygen in the manufacturing (Shah et al., 2014; Soumia et al., 2012), which contribute to increase lipid oxidation and microbial load. Both factors have been considered the main causes of meat spoilage (Fernández-López et al., 2005; Smeti et al., 2013) and shelf-life reduction, along with the formation of toxic compounds, appearance of off-flavors, off-odors, color changes and foodborne illnesses. This deterioration is even faster in manufactured meat products (Honikel, 2014), such as hamburgers, whose consumption has increased considerably (Vergara et al., 2020). Therefore, it is necessary to apply preservation methods, chemical (additives or natural substances) or physical, such as vacuum packaging, or combined, to maintain the quality of food and extend its shelf life (Vergara & Cózar, 2015). In the last years food consumption trends have changing (Vasilatos & Savvaidis, 2013), requiring healthier and high-quality products, free of synthetic additives (Rožman & Jeršek, 2009) with appropriate taste and attractive colour (Soumia et al., 2012).

Spices and herbs, whose antioxidants and antimicrobials properties have been extensively studied in different meat matrices, could be an alternative to the synthetic additives to extend meat shelf life, reducing losses attributed to spoilage and by allowing the products to reach distant and new markets (Ntzimani et al., 2010). Among them, the Labiatae family is well known, with rosemary (Rosmarinus Officinalis L.) one of the most important (Jordán et al., 2013; Ržnar et al., 2006) and studied of the Mediterranean herbs. In addition, spices are available in many forms (Raghavan, 2006) such as whole, ground, extracts, essential oils, oleoresins, whose properties, active compounds concentration and form of interaction with the meat matrix depends on the extraction method, as well as environmental and agronomic factors (Ojeda-Sana et al., 2013). Many works have dealt with the antimicrobial or antioxidant activity of the rosemary in several meat matrix shelf life. Sirochi et al. (2017) studied the effect of essential oil on beef meat; Vasilatos and Savvaidis (2013) on turkey; Sebranek et al.
(2004) on pork sausage; Bowser et al. (2014) on dry fermented lamb-meat sausages manufactured with rosemary extract. However, no studies have compared the influence of all different forms of rosemary on lamb meat preparations quality, such as burgers. It is the first work in which the effect of the four different forms of this rosemary (powdered, extracted, essential oil, and oleoresin) on lamb burgers is compared.

Thus, the objective of this study was to analyze and compare the effect of different forms (essential oil, oleoresin, powdered or extract) of rosemary on lamb meat raw burgers quality (assessed by color stability, pH, lipid oxidation and microbial growth), preserved under vacuum, at 2°C, during 14 d of storage.

2. Material and methods

2.1. Materials and experimental design

Ground legs meat of Spanish Manchega breed male lambs, with official label ‘Manchego Lamb PGI’, was used in this study. Animals (n = 18) were slaughtered at 70 days (25 Kg of live weight), having been fed with milk until 12 Kg of weight and after that, with a commercial concentrate and cereal straw ad libitum until slaughter, which was carried out through standard commercial procedures. All carcasses were chilled at 4°C for 24 h. Dried rosemary (Artemisia, Alicante, Spain) was purchased in a local market and ground in the laboratory. The rosemary extract, rosemary essential oil and rosemary oleoresin were obtained from Hausmann S.A. (Sant Andreu de la Barca, Barcelona, Spain), Hermes Aromatics S. A. and Tecnufar Iberia S.A. (Montornes del Valles, Barcelona, Spain), respectively, and keep at 2°C following the recommendations of the manufacturer.

Ground meat was distributed in five batches. Four batches of meat had salt (1% w/w) and one of the four rosemary-form [0.1% of commercially dried powdered rosemary (RP); 0.08% of commercial rosemary extract (REX); 0.08% of commercial rosemary essential oil (REO) or 0.08% of commercial rosemary oleoresin (ROL)]. A Control group (C) with 1% w/w of salt and without spice was used. Then, each batch was manually blended for 5 minutes and raw burgers were formed (100 g/10 cm diameter) with a conventional burger machine (MODEL 2 R, BECAM, J. Bernard S.L., Albacete, Spain). After manufacture, the raw burgers were vacuum packed with a packaging machine (Selecta Vacuum saler, Sealcom-V, Barcelona, Spain) in vacuum bags (150 μm of thickness, oxygen permeability of 30 cm²/m² and CO₂ permeability of <130 cm³/m² at 1 atm and 23°C; Ind. Pargon, José Bernard S.L., Albacete, Spain). Samples remained at 2°C until their analysis, carried out at 0, 7, 10 and 14 days after processing. Three replicates were made in each batch and eight samples were analyzed per batch type and per sampling time.

2.2. Samples analysis

2.2.1. Physicochemical parameters

pH. 3 g of sample was homogenized with 27 mL of distilled water for 10 seconds with an Ultra Turrax T25 digital (IKA Works, Inc., USA), then the pH was measured with a pH-meter connected to a penetrating electrode (Crison GLP 22 + pH & Ion-meter-Crison Instruments, S.A., Barcelona, Spain) in duplicate. 

Color coordinates. After five minutes of opening packages, lightness (L*), redness (a*) and yellowness (b*) were registered on the surface of each burger, in triplicate, using a Minolta CR-400 chromameter (Osaka, Japan) with a D65 illuminant and a 100 standard observer angle and using a standard white tile for calibrating.

2.2.2. Microbiological analysis

Five grams of burger samples were transferred to a sterilized bag containing 45 mL of tryptone phosphate water (buffered peptone water; Biokar diagnostics, Beauvair, France) and homogenized for 60 s in a Stomacher (Masticator, IUL-Instruments, Barcelona, Spain) and serial 10-fold dilutions of homogenates were prepared in peptone water. One mL of decimal solution inoculums were spread by duplicated, manually, on Petrifilm TM (3MTM S.A., Spain) for enumeration of total viable counts (TVC) and Enterobacteriaceae, and then were incubated at 32°C, for 48 h or 24 h, respectively. Furthermore, 100 μL of inoculum aliquots were spread by duplicates on Petri dishes by using a spiral system (Eddy-Jet, IUL-Instruments, Barcelona), for enumerations of lactic acid bacteria (LAB) in Man, Rogosa and Sharpe agar (MRS, Scharlau Chemie S.L.) and Pseudomonas spp. on Pseudomonas Agar Base with a cetrimide, Fucidin, cephaloridine supplement (Pseudomonas CFC, Oxoid LTD; Basing-stoke, Hampshire, England). Plates were incubated, at 32°C – 72 h for LAB and at 25°C – 72 h for Pseudomonas spp. The counting of TVC and Enterobacteriaceae was manual while an automatic colony counter (Countermat-Flash, IUL-Instrument, Barcelona, Spain) was used for LAB and Pseudomonas spp. Results were expressed as log CFU/g of meat.

2.2.3. Lipid oxidation

Lipid oxidation of raw burgers samples was measured according to Tarladgis et al. (1964), in which the reactive substances to thiobarbituric acid (TBARS) was analyzed. Five grams of sample was homogenized with 25 mL of distilled water with an Ultra Turrax T25 digital (IKA Works, Inc., USA) homogenizer for 2 min at 10000 rpm. Then, 25 mL of 10% trichloroacetic acid (TCA) was added and the homogeneous mixture was filtered using Whatman No. 1 filter paper. One mL of 0.8% Thiobarbituric acid was added to 4 mL of clear filtrate and incubated at 80°C for 90 min by duplicate. The pink colored resulting substance was measured at 532 nm with a Helios Alfa spectrophotometer (THERMO, Electro Corporation, England) and the results were expressed as mg malondialdehyde (MDA)/Kg of meat.

2.2.4. Statistical analysis

Data were analyzed with the Statistical Package SPSS 22.0 version (IBM Corp, 2013). First, a Shapiro–Wilk test was performed to check the normality and homogeneity of variance of all variables. Then, an ANOVA test was performed to determine the effect of the forms of rosemary (RP, REX, REO, and ROL) added to the raw burgers on the assessed quality parameters, in each time of analysis. In addition, within each batch, an ANOVA test was used to analyze the effects of time of storage (0, 7, 10 and 14 days) on the parameters studied. When the differences were significant (p < .05), a Tukey test was carried out to check the differences between pairs of groups.
3. Results

3.1. Physicochemical parameters

Table 1 shows the differences due to the added form of rosemary (Rosmarinus Officinalis L.) on lamb raw burgers pH and color coordinates values and its evolution with the storage time. Significant differences (p < 0.01 at 0, 10 and 14 d; p < 0.05 at 7 days) were observed among the batches on the pH. At 14 d post-packing, the ROL samples presented the lowest pH (5.47), while in the C and REX groups were found the highest values (5.67–5.69). RP and REO showed intermediate values (= 5.63). In general, this parameter registered a significant decrease (p < .001) until the end of the experiment (from 10 days after manufacture in C, REO and ROL batches and from 7 days in RP and REX burgers).

In general, in all samples, L* coordinate showed a great stability with the storage period. Redness increased in RP (p < .05) and yellowness decreased in the RP and ROL samples (p < .01 and p < .001, respectively). Significant differences (p < .05) were found among the groups for L* parameter at the end of experiment with the highest value in ROL burgers and the lowest in Control group. In general, there were significant differences among batches on a* (p < .001 at 0 d; p < .05 at 10 and 14 d post-packing) and b* coordinates (p < .01 at 0 and 14 d; p < .001 a 10 d) with the highest value, in both a* and b*, on RP samples.

3.2. Microbiology

Table 2 shows the microbial count (TVC, Enterobacteriaceae, Pseudomonas spp and, LAB) on the different batches of burgers. The count of all microorganisms increased significantly (p < .001) with the storage period, reaching the highest values for TVC and Enterobacteriaceae values in ROL samples (p < .05 and p < .001, respectively, at 14 days). The lowest values for Pseudomonas spp. were found in REX group and the highest in control burgers with intermediate values in the rest of the batches. At the end of the study, ROL and RP samples presented the highest values for LAB, while REX ones showed the lowest, in the Control and REX groups values were intermediates.

3.3. Lipid oxidation

All samples showed an increase (p < .001) of the TBARS substances with the storage period and significant differences (p < .001) were observed among groups at all analysis times (Table 3). Control raw burgers showed significantly higher values than seasoned samples. During all study period, the lowest values were found in RP samples with values below 0.25 mg MDA/kg meat. In ROL burgers values ranged from 0.67 to 1.09 mg MDA/Kg of meat. REX and REO batches showed similar values and ranged from ≈1.3 to values close to 2 mg MDA/Kg meat with time.

4. Discussion

4.1. Physicochemical parameters

pH is one of the main factors that affect quality parameters such as color and the shelf-life of meat products. Our study showed an initial pH close to 5.8 (in all batches) and this value decreased with time, amid a significant microorganism count increase. This agrees with that found by Jalosińska and Wilczak (2009) in meat balls, which indicated that for storage purposes it is important that meat reaches the lowest possible pH level to ensure meat shelf-life stability. In addition, pH during storage time was in accordance with the highest counts of lactic acid bacteria. This agrees with Karakaya et al. (2011) and Fernández-López et al. (2005) who indicated that the decrease in pH of vacuum-packed meat could be due to the presence of lactic acid through the growth of lactic acid bacteria and the dissolution of CO₂ into the meat aqueous phase. Curiously, the decrease was especially strong in the samples seasoned with oleoresin, which reached the lowest values (<5.5). These results contrast with those found in our previous paper (Vergara et al., 2020), in which different forms of oregano were compared in this same meat matrix, since the addition of oregano oleoresin on lamb burgers caused the highest pH. For this reason, it could be stated that the effect caused by the format of a spice depends on the spice itself.

Color is a critical quality factor of meat and meat products since it affects consumer acceptance and shelf life of products (Grebitus et al., 2013). Throughout the study, the a* and L* values were higher than 14.5 and 44, respectively, which according to Khliji et al. (2010) it is necessary for a consumer, selected at random, to consider acceptable a sample with high (>95%) probability. In general, there was a high stability of the color coordinates, including the control group, so it could be associated to the vacuum preservation method, in agreement with Berruga et al. (2005). Rosemary is considered one of the most effective of all spices in retaining the red color of processed meats by inhibiting the flavor and color degradation of fats and oils in them (Raghavan, 2006). In the present study REX and REO did not show variation in any coordinate. Semenova et al. (2019) indicated that the effect of essential oils on meat color can be explained by their minimizing myoglobin and lipid oxidation. These same authors, by comparing the effects of essential oils on minced meat, concluded that rosemary oil showed the greatest effect on color stability. Cayuela et al. (2004) in pork loin, De Paula Paseto Fernandes et al. (2013) in lamb loin and Souza et al. (2005) in pork sausages, with rosemary extracts, reported a high stability of L*, a*, b* coordinates with the storage time. Our results showed that the rosemary, particularly extracts and essential oil, can be used to stabilize color in refrigerated-vacuum packaged lamb burgers. However, the addition of ground rosemary provides the highest red value to the samples.

4.2. Microbiology

The indicator microorganism count is widely used to verify the effectiveness of efforts to ensure microbiological quality and food safety (Buchanan & Oni, 2012). Consumers demand, more and more frequently, food with a long shelf life, no risk of causing foodborne illness and free of synthetic substances. Thus, the control of microbial contamination has been a major concern in the meat industry (Ahn et al., 2007), increasingly interested in using natural substances to solve this problem. According to Zaika (1988), rosemary is a spice with medium antimicrobial activity. This effect was studied by Hashemi et al., (2017) in frozen beef burger; Jalosińska and Wilczak (2009) in meat-ball products; Martínez et al. (2019) in fish patties; Ržinar et al. (2006) in chicken
| Parameter               | Storage time(days) | C (n = 96)  | RP (n = 96)  | REX (n = 96) | REO (n = 96) | ROL (n = 96) | Sig-RF |
|------------------------|--------------------|-------------|-------------|-------------|-------------|-------------|--------|
| pH                     | 0                  | 5.81 ± 0.06  | 5.86 ± 0.05  | 5.81 ± 0.03  | 5.81 ± 0.04  | 5.79 ± 0.03  | *      |
|                        | 7                  | 5.83 ± 0.02  | 5.85 ± 0.02  | 5.85 ± 0.03  | 5.83 ± 0.02  | 5.82 ± 0.04  | *      |
|                        | 10                 | 5.79 ± 0.05  | 5.59 ± 0.22  | 5.71 ± 0.22  | 5.85 ± 0.03  | 5.69 ± 0.20  | **     |
|                        | 14                 | 5.67 ± 0.14  | 5.62 ± 0.14  | 5.69 ± 0.13  | 5.63 ± 0.14  | 5.47 ± 0.20  | **     |
| Sig-ST                 |                    |             |             |             |             |             |        |
| L*                     | 0                  | 45.72 ± 2.59 | 46.79 ± 2.49 | 45.50 ± 3.08 | 45.92 ± 2.69 | 44.17 ± 2.48 | NS     |
|                        | 7                  | 44.16 ± 2.40 | 45.17 ± 2.37 | 44.44 ± 4.43 | 45.72 ± 2.44 | 45.04 ± 3.86 | NS     |
|                        | 10                 | 45.19 ± 2.64 | 47.28 ± 2.22 | 44.22 ± 2.50 | 45.62 ± 1.98 | 44.78 ± 2.51 | NS     |
|                        | 14                 | 43.79 ± 1.52 | 45.99 ± 2.40  | 45.05 ± 1.94  | 45.61 ± 2.50  | 47.06 ± 2.63  | *      |
| Sig-ST                 |                    |             |             |             |             |             |        |
| a*                    | 0                  | 17.09 ± 1.10 | 15.49 ± 0.75  | 15.32 ± 1.10  | 15.00 ± 1.16  | 15.49 ± 0.89  | ***    |
|                        | 7                  | 17.28 ± 1.15 | 16.57 ± 1.42  | 15.80 ± 1.36  | 15.83 ± 1.47  | 15.95 ± 1.36  | NS     |
|                        | 10                 | 16.56 ± 0.63  | 17.12 ± 1.34  | 16.05 ± 1.00  | 15.65 ± 1.15  | 15.50 ± 1.64  | *      |
|                        | 14                 | 17.25 ± 0.91  | 17.33 ± 1.12  | 16.21 ± 1.31  | 16.23 ± 1.38  | 15.70 ± 1.32  | *      |
| Sig-ST                 |                    |             |             |             |             |             |        |
| b*                    | 0                  | 8.35 ± 1.05  | 9.00 ± 0.78  | 7.46 ± 1.54  | 7.60 ± 1.13  | 7.40 ± 0.61  | **      |
|                        | 7                  | 7.44 ± 1.66  | 7.66 ± 0.97  | 7.25 ± 1.09  | 6.87 ± 0.99  | 6.85 ± 0.74  | NS     |
|                        | 10                 | 6.92 ± 0.99  | 8.91 ± 0.86  | 6.52 ± 1.30  | 6.74 ± 1.42  | 6.14 ± 0.97  | **      |
|                        | 14                 | 7.43 ± 0.49  | 7.99 ± 0.67  | 6.63 ± 1.12  | 6.98 ± 0.91  | 6.60 ± 0.77  | **      |
| Sig-ST                 |                    |             |             |             |             |             |        |

Type of burger: C: Burgers without spice; RP: Burgers with rosemary; REX: Burgers with rosemary extract; REO: Burgers with rosemary-oleoresin; Sig-RF: Effect of rosemary form (ANOVA test); Sig-ST: Effect of storage time (ANOVA test); 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) due to the effect of storage time (0, 7, 10 and 14 days). a, b: Values in the same row with different superscripts are significantly different (p < 0.05) due to the added rosemary form.

N = 96 per batch (Three replicates were made on each batch and 8 samples were analyzed per batch type and per sampling time).

Table 1. Effect of added rosemary on pH and colour coordinates (L*a*b*) values (mean ± standard error) on vacuum-packaged lamb burgers.

Tabla 1. Efecto del formato de romero añadido en los valores (Medias: E.S.) de pH y coordenadas de color (L*a*b*) de hamburguesas de cordero envasadas al vacío.
Table 2. Effect of added rosemary form on microbial growth (media ± standard error) on vacuum-packaged lamb burgers.

| Microorganism          | Storage time(days) | C (n = 96) | RP (n = 96) | REX (n = 96) | REO (n = 96) | ROL (n = 96) | Sig-RF |
|------------------------|--------------------|------------|-------------|--------------|--------------|--------------|--------|
|                        |                    | 0          | 7           | 14           |              |              |        |
| TVC                    | 5.11 ± 0.04        | 5.09 ± 0.06 | 5.09 ± 0.12 | 5.16 ± 0.07   | 5.18 ± 0.14   | NS           |        |
|                        |                    | 5.99 ± 0.40 | 6.30 ± 0.38 | 5.90 ± 0.57   | 5.89 ± 0.35   | 6.17 ± 0.49   | NS     |
|                        |                    | 6.44 ± 0.36 | 6.88 ± 0.27 | 6.46 ± 0.69   | 6.29 ± 0.31   | 6.67 ± 0.86   | NS     |
|                        |                    | 6.98 ± 0.35 | 7.24 ± 0.23 | 6.87 ± 0.51   | 6.75 ± 0.41   | 7.05 ± 0.29   |        |
| Sig-ST Enterobacteriaceae | 1.66 ± 0.40   | 1.93 ± 0.12 | 1.97 ± 0.23 | 1.98 ± 0.42   | 1.94 ± 0.17   | NS           |        |
|                        |                    | 2.50 ± 0.48 | 2.60 ± 0.44 | 2.20 ± 0.44   | 2.69 ± 0.53   | 2.92 ± 0.67   | NS     |
|                        |                    | 3.39 ± 0.81 | 3.66 ± 0.98 | 3.08 ± 1.05   | 2.58 ± 0.54   | 3.37 ± 1.14   | NS     |
|                        |                    | 3.51 ± 0.21 | 4.60 ± 0.44 | 3.09 ± 0.69   | 3.39 ± 0.78   | 3.60 ± 1.02   |        |
| Sig-ST Pseudomonas spp.  | 4.87 ± 0.07       | 4.92 ± 0.07 | 4.49 ± 0.42 | 4.59 ± 0.40   | 4.52 ± 0.43   |        |        |
|                        |                    | 5.48 ± 0.43 | 6.11 ± 0.55 | 5.04 ± 0.78   | 5.29 ± 0.59   | 5.34 ± 0.72   |        |
|                        |                    | 5.44 ± 0.35 | 5.56 ± 0.44 | 5.10 ± 0.86   | 5.82 ± 0.70   | 5.66 ± 0.65   | NS     |
|                        |                    | 6.35 ± 0.28 | 6.60 ± 0.28 | 5.90 ± 0.63   | 5.96 ± 0.63   | 6.11 ± 0.47   |        |
| Sig-ST LAB              | 3.22 ± 0.21       | 3.35 ± 0.26 | 3.46 ± 0.30 | 3.13 ± 0.60   | 3.54 ± 0.41   | NS           |        |
|                        |                    | 3.76 ± 0.42 | 3.74 ± 0.37 | 4.12 ± 0.42   | 3.85 ± 0.32   | 4.26 ± 0.51   |        |
|                        |                    | 4.47 ± 0.66 | 4.85 ± 0.67 | 5.38 ± 0.44   | 4.67 ± 0.48   | 5.37 ± 0.47   |        |
|                        |                    | 5.42 ± 0.30 | 5.66 ± 0.53 | 5.00 ± 0.66   | 5.57 ± 0.39   | 5.77 ± 0.45   |        |

Type of burger: C: Burgers without spice; RP: Burgers with powder rosemary; REX: Burgers with rosemary extract; REO: Burgers with rosemary essential oil; ROL: Burgers with rosemary-oleoresin; Sig-RF: Effect of rosemary form (ANOVA test); Sig-ST: Effect of storage time (ANOVA test): NS: Not significant. **, ***: Indicates significance levels at 0.05, 0.01 and 0.001, respectively. *, **: Values in the same column with different superscripts are significantly different (P < 0.05) due to the effect of storage time (0,7,10 and 14 days); **, ***: Values in the same row with different superscripts are significantly different (P < 0.05) due to the added rosemary form.

N = 96 per batch (Three replicates were made with each batch and 8 samples were analyzed per batch type and per sampling time).

Table 3. Effect of added rosemary form on lipid oxidation (mg MDA/kg of meat; media ± standard error) on vacuum-packaged lamb burgers.

| Parameter                  | Storage time (days) | C (n = 96) | RP (n = 96) | REX (n = 96) | REO (n = 96) | ROL (n = 96) | Sig-RF |
|----------------------------|---------------------|------------|-------------|--------------|--------------|--------------|--------|
| Lipid oxidation (mg MDA/ Kg of meat) | 0                   | 2.27 ± 0.25 | 0.24 ± 0.03 | 1.14 ± 0.29   | 1.23 ± 0.32   | 0.67 ± 0.16   | ***    |
|                           | 7                   | 2.95 ± 0.22 | 0.21 ± 0.01 | 1.45 ± 0.33   | 1.89 ± 0.56   | 0.94 ± 0.38   | ***    |
|                           | 10                  | 2.93 ± 0.33 | 0.12 ± 0.02 | 1.99 ± 0.63   | 2.15 ± 0.44   | 1.10 ± 0.39   | ***    |
| Effect of storage time    |                     | 2.56 ± 0.30 | 0.14 ± 0.01 | 1.93 ± 0.30   | 2.17 ± 0.31   | 1.09 ± 0.21   | ***    |

Type of burger: C: Burgers without spice; RP: Burgers with powder rosemary; REX: Burgers with rosemary extract; REO: Burgers with rosemary essential oil; ROL: Burgers with rosemary-oleoresin; Sig-RF: Effect of rosemary form (ANOVA test); Sig-ST: Effect of storage time (ANOVA test): NS: Not significant. **, ***: Indicates significance levels at 0.05, 0.01 and 0.001, respectively. *, **: Values in the same column with different superscripts are significantly different (P < 0.05) due to the effect of storage time (0,7,10 and 14 days); **, ***: Values in the same row with different superscripts are significantly different (P < 0.05) due to the added rosemary form.

N = 96 per batch (Three replicates were made with each batch and 8 samples were analyzed per batch type and per sampling time).

frankfurters. However, our study showed that none of the forms of rosemary studied were able to inhibit the TVC count, an indicator of the deterioration of the meat, reaching, at the end of the experiment, values close (in REX, REO) or highest (in RP, ROL groups) to those considered unsatisfactory, 7 log CFU/g, from a hygienic point of view in minced meat (Regulation EC 2073/05 (I)).

*Enterobacteriaceae* counts are considered a hygiene indicator for food and environment (Zeitoun et al., 1994) and have been used to indicate fecal contamination since the primary pathogens of concern are members of the *Enterobacteriaceae* family (Buchanan & Oni, 2012). According to Sani et al. (2017) the initial count of this microorganism found in our study close to 2 log CFU/g indicated a good quality of samples. During storage, there was a significant increase (p < .001) within all groups and the values at the end of the study (ranged from 3.08 to 4.60 log CFU/g) could indicate an antimicrobial effect against this microorganism in the next order: REX ≥ REO ≥ RP. The highest increase of *Pseudomonas spp.* was found in samples without spice or with powdered rosemary. In both batches, at 14 days, values were close to 7 log CFU/g, considered the limit to the slime presence and bad odor (Nychas et al., 2008), and rosemary was effective in a similar order as for *Enterobacteriaceae*. It is evident that, despite of some authors (Jones, 2004) indicated that the growth of organisms such as *Pseudomonas spp.* is limited
under vacuum conditions, in our study this preservation system did not inhibit the increase of this microorganism with time. However, since the statistical differences, the addition of rosemary in extract form, and to a lesser extent essential oil and oleoresin, could have a synergistic effect with the vacuum method. Therefore, these differences among groups could indicate that the antimicrobial effectiveness of rosemary to inhibit these microorganisms depend on the format added.

LAB constitute a significant part of natural microflora of meat (Sani et al., 2017), the dominant spoilage microflora in vacuum or modified atmospheres packaged meat (Yost & Mattress, 2000) with the formation of slime and off-odors when 7Log cfu/g meat is reached (Feiner, 2006). In agreement with our result, an increase in the number of LAB with the storage was reported by Vasilatos and Savvidais (2013) in turkey meat packages under vacuum. In addition, the effectiveness of rosemary against this microorganism group was the following: REX > REO > RP = ROL, and no batch exceeded the value mentioned above.

Many factors can affect antimicrobial activity of spice such as, concentration, storage conditions, substrate composition (Souza et al., 2005), all this would explain the low antimicrobial capacity of the various forms of rosemary studied in our study. Consistent with this, Sirocchi et al. (2017) found that the scarce antimicrobial activity and inhibition in meat could be due to a low concentration of spice added. Comparing with the results obtained in our previous work (Vergara et al., 2020), it could be concluded that (1) rosemary, in any of its forms, is more effective than oregano in inhibiting the growth of the microorganisms analyzed and (2) that the powdered form is the least effective. Based on the microbial count, and the cited values considered safe for these microbial indicators, the shelf life of the hamburgers was 10 days for the RP and control batches and 14 days for the REX, REO and ROL samples.

### 4.3. Lipid oxidation

The susceptibility to oxidative changes is the major disadvantage in hamburgers (Borella et al., 2019). Shahidi (2016) described the factors that influence lipid oxidation of meat products and among them, the content of unsaturated fatty acids is considered a relevant element. Precisely, our previous study (Linares et al., 2020) showed the richness of lamb burgers in these constituents. Lipid oxidation involves the formation of toxic compounds such as peroxides, aldehydes and ketones, which decrease the shelf life of food and turn products unsatisfactory for consumers (Özcan & Arslan., 2011). To solve this problem, several lines of research are emerging, mainly focused on obtaining natural antioxidants to replace synthetic ones (Domínguez et al., 2019). After Rac and Ostric (1995) reported the antioxidant effect of rosmarin leaves extract, there have been many studies concerning the antioxidant properties of isolated rosemary compounds (Yanishlieva-Maslarova & Heinonen, 2000). However, there are no studies that compare the effect of the various forms of rosemary on a meat matrix. In our study, the changes in TBARS values of the control samples were higher than in the spiced batches. Rosemary was effective for preventing rancidity in lamb burgers, but the antioxidant effectiveness of this spice depends on the added form and in the next order: powder > oleoresin > extract ≥ essential oil. According to Amaral et al. (2018) the antioxidant potential of this spice has been attributed to its phenolic acids content (caffeic, ferulic, and rosmarinic acid) and phenolic diterpenes (carnosic acid and camosol). Shahidi et al. (1995) studied the antioxidant efficacy of several ground spices in minced pork and observed that rosemary ranked third after cloves and sage. In our study, TBARS values reached in samples with ground rosemary were similar to those found in our previous papers in lamb burgers spiced with powder of thyme or sage (Cózar et al., 2018) or with oregano (Vergara et al., 2020).

Antioxidant efficacy of oleoresin rosemary has been studied in several meat matrices: in roast beef slices (Murphy et al., 1998); in ground chicken meat (Keokamnerr et al. 2008); in pork steaks (Liu et al., 1992). However, there are no reported data on the effect of this rosemary form on lamb meat products. Our results showed that ROL exhibit better antioxidant effect than their essential oil or that extract form, whose reaching values close to 2 mg MDA/g at 14 days, which compromised consumer acceptability (Campo et al., 2006).

### 5. Conclusions

From the results obtained in this study we can conclude that:
1. The combination of vacuum packaging with rosemary, particularly extracts or essential oil, achieves high color stability in lamb burgers during storage. (2) The highest antioxidant effect, measured by TBARS values, were found in the batches seasoned with the ground rosemary form (RP). (3) Microbial growth was the factor that limited shelf life, since none of the forms of rosemary studied were able to inhibit the microorganisms analyzed. (4) Considering both, the microbial and lipid oxidation values, shelf life was two weeks for burgers with REX, REO and ROL but only 10 d for RP. Finally, our results could be of interest to the meat industry to offer to consumers a meat product without synthetic additives and to extend meat shelf life. A consumer test would be very useful to measure the sensory consequences of the inclusion of different forms of rosemary studied.

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