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ARTICLE

Effects of Ripening Conditions on the '*Lomo embuchado*' Sausage Quality

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Abstract

The objective of this study was to investigate the effects of two different ripening durations, with, or without adding rosemary powder, on *Lomo embuchado* (LEO) sausage quality. All LEOs were ripened for two different durations, 45 or 60 d, with, or without the addition of rosemary powder, as follows: 1) LEO ripened for 45 d (LER45), 2) LEO ripened for 60 d (LER60), 3) rosemary LEO ripened for 45 d (RLE45), and 4) rosemary LEO ripened for 60 d (RLE60). Significant differences were observed in both moisture and ash content, with higher moisture and less ash content in LER45 ($p<0.05$). No trend was shown in the crude protein content of the four different treatments, but significantly low protein content was shown only in RLE45 ($p<0.05$). Ripening for 45 d improved the lightness, yellowness, and water activity of LEOs ($p<0.05$). However, ripening duration together with rosemary powder addition had no significant effects on redness ($p>0.05$). The LER45 generated significantly improved chewiness, gumminess, and hardness, as compared to both LER60 and RLE60 ($p<0.05$). In conclusion, the results suggest that ripening for 45 d seems to enhance LEO quality, but that rosemary powder addition may not be required to develop good LEO quality.

Key words: *Lomo embuchado*, sausage, ripening duration, rosemary powder, quality

Introduction

Dry-cured meat products can be classified into three different groups: *Lomo embuchado* (LEO), dry-cured ham, and dry-cured shoulder. '*Lomo embuchado*' is a typical Spanish dry-cured meat product, which does not use smoke for production (Fernanadez *et al*., 2007). Two main stages, including salt-seasoning and dry-maturation must be performed to prepare LEO, but only a short salting step is necessary compared to dry-cured ham and shoulder (Pérez-Alvarez *et al*., 1997). However, the salt added at the primary stage of the LEO process can effectively influence LEO quality by changing color and muscle protease activities (Jurado *et al*., 2007; Molinero *et al*., 2008; Pérez-Alvarez *et al*., 1997; Poligné *et al*., 2002). Due to salt supplementation on LED, only limited amount of free amino acids is released by reduced muscle protease activities and then the sweetness of LEO is able to be lowered (Jurado *et al*., 2007; Reina *et al*., 2014). Moreover, adding salt accelerates LEO oxidation (Aguirrezábal *et al*., 2000; Hernandez *et al*., 1999) and is not desirable for the LEO textual properties when they are ripened over longer periods of time.

Synthetic antioxidants, including butylated hydroxytoluene, butylated hydroxyanisole, and tertiary butylated hydroquinone are commonly added to meat products to scavenge reactive oxygen species (ROS) and to avoid rancidity (Hettiarachchy *et al*., 1996; Sallam *et al*., 2004; Shin, 2006). However, there may be a risk of generating toxicity and/or mutagenicity, when excessive synthetic antioxidants are included in meat products, and, health conscious consumers tend to avoid many categories of meat products (Li *et al*., 2009; Shin *et al*., 2011). ROS and microorganisms are generally formed during the ripening of meat products. They participate in increasing free amino acid and short chain fatty acids concentration resulting in tenderness and enhanced flavor. However, LEO can easily deteriorate due to ROS and microorganisms depending on the day of storage. Thus, ROS formation and

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microorganism should be regulated by natural antioxidants and antimicrobials including garlic, sorghum, and rosemary (Kil *et al*., 2009; Moreno *et al*., 2006; Sallam *et al*., 2004; Shin, 2006).

This study was conducted to evaluate the effects of ripening on LEO quality, as well as the effect of adding rosemary powder to LEO, as rosemary powder is a natural antioxidant and antimicrobial. The effects on LEO were estimated by assessing pH, color, microorganism population, and texture properties.

Materials and Methods

Lomo embuchado formulation and preparation

To manufacture LEOs, modified LEO formulation was tested in triplicate. Three kilogram of lean pork loin was purchased at a local slaughter house and trimmed to remove any visible fat and fascia. Every loin was salted and seasoned in accordance with the common technique of Spanish producers. Based on loin weight, four different mixtures of 2% sun-dried salt, 2% water, 0.02% nitrite, 4% Korean traditional red pepper paste, 1% onion powder, 1% garlic powder, and 1% ginger powder were prepared, and an additional 0.025% rosemary powder was added to two different mixtures. One of the four spice mixtures was homogeneously rubbed over the pork loin surface, and they were divided into four different groups: 1) LEO ripened for 45 d (LER45), 2) LEO ripened for 60 d (LER60), 3) rosemary LEO ripened for 45 d (RLE45), and 4) rosemary LEO ripened for 60 d (RLE60). All pork loins were stored at 4°C for 7 d. Each pork loin was then stuffed into cattle large intestines, which were washed, trimmed, and stored cleanly. Ripening in a drying chamber at 12°C and 65% relative humidity was applied for 45 or 60 d. LEOs derived from the four different groups were used to determine the effects of ripening time and rosemary powder on LEO.

Moisture, crude fat, crude protein and ash determination

Moisture, crude fat, crude protein and ash content were determined using an oven-drying, Soxhlet, Kjeldahl and dry ashing methods, as depicted in #934.01, #954.08, #984.13 and #900.02 of AOAC (1995), respectively. All data are reported as percentages (%).

pH, CIE color space values, and water activity determinations

Ten g of LEO and 90 mL of double distilled water were homogenized for 30 s at 13,500 rpm (T25B, IKA Sdn. Bhd., Malaysia). Duplicate readings of each sample were taken using a pH meter (Orion 420A+, Thermo Electron Co., USA), and the average pH value per treatment was reported.

A colorimeter (Minolta Chroma Meter CR-300, Minolta Co. Ltd, USA) was used to determine CIE L* (lightness), a* (redness) and b* (yellowness) color space values. The colorimeter was calibrated daily using a white tile (Y=92.8, x=0.3134, y=0.3193), and duplicate readings per sample were conducted daily. The average of each color space value was reported as CIE L*, a*, and b* color space values, respectively.

Individual water activity (a_w) of LEO was determined for each treatment using a Thermoconstanter a_w Sprint Novasina TH500 (Novasina, Axair Ltd., Switzerland) at 25°C, and the average of nine different LEO a_w readings per treatment was reported.

Microbiological analysis and identification

Each LEO sample was cut into 3 pieces, and the end pieces were discarded. The remaining piece was then sliced to 2 cm thickness, and approximately 0.1 cm of each LEO exterior was removed. A 25 g sample and 225 mL autoclaved peptone water were mixed together and homogenized using a mechanical stomacher (Stomacher®400, England) for 2 min. One mL of homogenized sample was collected, and a serial dilution with autoclaved peptone water was performed. A 200 or 100 μL aliquot of diluted sample was plated on plate count agar (Difco, USA), violet red bile agar (Difco, USA) or *de* Man Rogosa and Sharpe Agar (Difco, USA) with bromophenol blue staining for total plate counts, *E. coli* or lactic acid bacteria, respectively. All plates were then incubated for 24-72 h at 37°C, after which the colonies were counted and expressed as Log CFU/g.

All 25 g of homogenized sample and 225 mL of phosphate-buffered dilution water, *E. coli* broth, UVM-modified *Listeria*, peptone water or tryptone soy broth for *Bacillus cereus*, *E. coli* and *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus*, respectively, were mixed and homogenized. All homogenized samples were then incubated at 30-37°C for 24 h, after which the samples were marked on Mannitol-Egg Yolk Polymyxin Agar, Eosin Methylene Blue and SMAC, Oxford Agar, Xylose Lysine Desoxycholate or Mannitol Salt Agar plates, respectively. They were then re-incubated at 30-37°C for 24-48 h, depending on the microorganism. The *B. cereus*, *E. coli* and *E. coli* O157:

H7, *L. monocytogenes* and *Salmonella* spp. and *S. aureus* samples were then isolated and validated using the Vitek BCL (Biomerieux, Marcy I'Etoile, France) for *B. cereus*, Vitek GN+ (Biomerieux, Marcy I'Etoile, France) for *E. coli., Salmonella* spp. and *S. aureus*, or Vitek GP+ for *L. monocytogenes* (Biomerieux, Marcy I'Etoile, France). Additional analysis for *E. coli* O157:H7 in accordance with Vitek GN+ was confirmed using a serum test.

Texture property determination

All LEO samples were shaped into cubes (1×1×1 cm), and were warmed and placed on a texture analyzer (TA-XT Express, Stable Micro System, England) equipped with a cylindrically shaped plunger (5 mm diameter). The texture analysis for adhesiveness, chewiness, cohesiveness, gumminess, hardness, and springiness was established with a 2 mm/s pre-test speed, 1 mm/s test speed, and 2 mm/s post-test speed for the texture analyzer plunger. Each sample area was reported, averaged, and expressed as adhesiveness (N s), chewiness (N mm), cohesiveness (%), gumminess (N), hardness (N), and springiness (mm).

Statistical analysis

All data were analyzed using the General Linear Model (GLM) procedure of SAS ver. 6.12 software (SAS, 1998) (SAS Institute, USA), and differences were detected using Duncan's multiple range test with a $p<0.05$.

Results and Discussion

Proximate composition of *Lomo embuchado*

As shown in Table 1, both LER45 and RLE45 had higher levels of moisture (46.98 or 50.53%) and lower levels of ash (6.75 or 6.63%) than those of LER60 and RLE60. Pérez-Alvarez *et al*. (1999) reported that moisture decreases with increased duration of ripening and that most water loss occurs after 2 d of ripening. As the water content of LER60 and RLE60 declined in comparison to LER45 and RLE60, relatively high amount of raw sample instead of water was applied for protein and ash determination. Higher water loss due to ripening day of LEO may have been the cause of difference in ash and protein contents. LER60 had higher protein and ash contents than those of LER45.

pH, color and water holding capacity of *Lomo embuchado*

Neither ripening duration nor rosemary powder addition affected pH values or CIE a* color space values of the LEOs (Table 2); however, just in case of treatment added with rosemary, there was significant difference of lightness, yellowness and a_w between ripening duration. In general, pH accounts for CIE L* of sausages due to the denaturalization of protein resulting in water release (Fermández-López *et al*., 2004, 2008; Woelfel *et al*., 2002). Free surface water increases light refraction of sausages,

Table 1. Proximate composition of *Lomo embuchado* after 45 or 60 d ripening and/or adding rosemary powder

Treatment[1)]	Moisture	Crude fat	Crude protein	Ash
LER45	46.98^{ab}	3.30	42.97^{a}	6.75^{b}
LER60	41.55^{bc}	6.39	43.34^{a}	8.72^{a}
RLE45	50.53^{a}	6.50	36.34^{b}	6.63^{b}
RLE60	38.97^{c}	8.02	44.56^{a}	8.44^{a}
SEM[2)]	1.93	1.38	1.63	0.42

[1)]Treatments: LER45=*Lomo embuchado* ripened for 45 d, LER60=*Lomo embuchado* ripened for 60 d, RLE45=Rosemary *Lomo embuchado* ripened for 45 d, RLE60=Rosemary *Lomo embuchado* ripened for 60 d (n=3).
[2)]SEM=standard error of the mean.
[a,b,c]Mean values within a column followed by different letters indicate a significant difference ($p<0.05$).

Table 2. pH, color and water holding capacity of *Lomo embuchado* after 45 or 60 d ripening and/or adding rosemary powder

Treatment[1)]	pH	CIE Color space values			a_w[3)]
		L*(Lightness)	a*(Redness)	b*(Yellowness)	
LER45	6.00	37.33^{ab}	9.39	4.59^{a}	0.87^{ab}
LER60	6.10	35.85^{bc}	9.04	4.03^{ab}	0.83^{bc}
RLE45	5.94	38.25^{a}	10.02	5.26^{a}	0.88^{a}
RLE60	6.11	34.71^{c}	8.67	3.62^{b}	0.80^{c}
SEM[2)]	0.05	0.61	0.81	0.46	0.01

[1)]Treatments: LER45=*Lomo embuchado* ripened for 45 d, LER60=*Lomo embuchado* ripened for 60 d, RLE45=Rosemary *Lomo embuchado* ripened for 45 d, RLE60=Rosemary *Lomo embuchado* ripened for 60 d (n=3).
[2)]SEM=standard error of the mean. [3)]a_w=water activity.
[a-c]Mean values within a column followed by different letters indicate a significant difference ($p<0.05$).

which makes them lighter in color (Fermández-López *et al*., 2008; Susan *et al*., 1991). In this study, either LER45 or RLE45 contained high amount of moisture than others (Table 1), and only numerical pH changes of LER45 and RLE45 LEOs were made. Therefore, it is clear that lightness of LER45 and RLE45 was not motivated by moisture content and/or pH of LEOs. Ripening duration effectively influenced by eliminating water on the LEO surface, showing lower lightness in LEOs ripened for 60 d than others. Yellowness and a_w were numerically higher in LER45 as compared to those of LER60. However, changes in yellowness and a_w were completed when rosemary was added to LEOs ($p<0.05$). It seems that 45 and 60 d of ripening difference did not provide any alterations in yellowness and a_w, even though water content and/or activity can be influenced by adding salt and sugar to LEOs (Hutton, 2002; Ordóòez *et al*., 1999).

Microbiological analysis of *Lomo embuchado*

Only LER45 and RLE45 LEOs were examined to evaluate total plate count (TPC), *E. coli*., and LAB counts and to assess *B. cereus*, *E. coli*, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp and *S. aureu*s growth (Tables 3 and 4). TPC and LAB were observed in LEOs, but *E. coli* was not found in LER45 and RLE45. Both TPC and LAB did not significantly differ in LER45 and RLE45, respectively; however, a numerical distinction was observed. As LABs are predominant microorganisms in fermented sausage (Antara *et al*., 2002; Coppola *et al*., 2000), a common microorganism assessed in LEO was LAB. TPC values of LER45 and RLE45 were 7.85 and 6.64 CFU/g, respectively, and these were similar to LABs counted on both LER45 and RLE45. RLE45 LEOs, which included 0.025% rosemary powder, showed numerically low TPC and LAB values than those of LER45. Although the antimicrobial effects of rosemary are solvent dependent, both water and methanol rosemary extracts, containing carnosic acid, carnosol and/or rosmarinic acid, effectively influenced bacterial counts (Moreno *et al*., 2006), and hence, numerically low TPC and LAB numbers were determined for samples containing 0.025% rosemary extract when ripened for 45 d.

Powers *et al*. (1975) reported that about 53% of spices, generally added to sausage, have a large number of *B. cereus* spores; therefore, it was no surprise that *B. cereus* might be detected in all LEOs containing 10% added spice. However, *B. cereus* was only detected in RLE45, and this may be explained by the study of Ivanovic *et al*. (2012), which reported that rosemary was not able to influence the growth of *B. subtilis* but was effective for *Staphylococcus aureus* growth. This means that rosemary can easily be contaminated by *B. subtilis* than *Staphylococcus aureus*, and consecutive contamination of meat products may occur when rosemary is added. Therefore, rosemary may be a source of *B. subtilis* to LEO, as shown in Table 4.

Table 3. Quantitative evaluation of total plate, coliform and lactic acid bacteria counts of *Lomo embuchado* after 45 d ripening and/or adding rosemary powder

Treatment[1)]	Microorganisms (Log CFU/g)		
	Total plate count	*E. coli*	Lactic acid bacteria
LER45	7.85	ND[3)]	7.66
RLE45	6.64	ND	6.57
SEM[2)]	0.30	-	0.30

[1)]Treatment: LER45=*Lomo embuchado* ripened for 45 d, RLE45=Rosemary *Lomo embuchado* ripened for 45 d (n=3).
[2)]SEM=standard error of the mean. [3)]ND=not detected.

Texture properties of *Lomo embuchado*

The texture properties, including adhesiveness, cohesiveness, and springiness, were not affected by either ripening duration or rosemary powder ($p>0.05$). However, chewiness, gumminess, and hardness were influenced by ripening duration. The LEOs chewiness in Table 5 fluctuated, but high chewiness was determined in LER60 and RLE60 in comparison to that of LER45 and RLE45, respectively. A similar trend was determined for gumminess and hardness also, and LER60 and RLE60 treatments showed high gumminess and hardness than those of the others. As a longer ripening duration was applied to LEOs, both protein and lipid oxidation occurred, with conse-

Table 4. Qualitative evaluation for *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp. and *Salmonella aureu*s of *Lomo embuchado* after 45 d ripening and/or adding rosemary powder

Treatment[1)]	*Bacillus cereus*	*Escherichia coli*	*Escherichia coli* O157:H7	*Listeria monocytogenes*	*Salmonella* spp.	*Staphylococcus aureus*
LER45	−	−	−	−	−	−
RLE45	+	−	−	−	−	−

[1)]Treatments: LER45=*Lomo embuchado* ripened for 45 d, RLE45=Rosemary *Lomo embuchado* ripened for 45 d (n=3).
+=detected. −=not detected.

Table 5. Texture properties of *Lomo embuchado* after 45 or 60 d ripening and/or adding rosemary powder

Treatment[1)]	Adhesiveness (N s)	Chewiness (N mm)	Cohesiveness (%)	Gumminess (N)	Hardness (N)	Springiness (mm)
LER45	-5.36	9.84[bc]	0.40	11.66[bc]	28.39[b]	0.86
LER60	-5.86	15.09[a]	0.43	16.84[ab]	41.02[a]	0.90
RLE45	-3.37	9.41[c]	0.42	10.64[c]	25.41[b]	0.89
RLE60	-5.15	14.37[ab]	0.43	18.36[a]	48.61[a]	0.78
SEM[2)]	1.30	1.79	0.02	1.68	3.44	0.05

[1)]Treatments: LER45=*Lomo embuchado* ripened for 45 d, LER60=*Lomo embuchado* ripened for 60 d, RLE45=Rosemary *Lomo embuchado* ripened for 45 d, RLE60=Rosemary *Lomo embuchado* ripened for 60 d (n=3).
[2)]SEM=standard error of the mean.
[a-c]Mean values within a column followed by different letter indicate a significant difference ($p<0.05$).

quent water loss and fat degradation, which led to increased LEO hardness, with ripening (Hoz *et al*., 2004; Muguerza *et al*., 2004). Water loss and fat degradation seem to be the main factors determining the chewiness, gumminess and hardness of LEOs, leading to the distinction observed for LER60 compared to that of LER45.

In conclusion, this study was carried out to determine the effects of ripening duration and adding rosemary powder on LEO quality. Higher moisture and lower ash was observed for LER45 compared to those of LER60. LER 45 showed higher lightness, yellowness, and a_w than those of both LER60 and RLE60. Only *B. cereus* was recorded when rosemary powder was added, and samples were ripened for 45 d. LER45 exhibited improved chewiness, gumminess, and hardness, compared to those of LER60. Therefore, the results indicate that LER45 without adding rosemary enhanced the quality characteristics of LEO.

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