# Food safety in products ready to eat of suckling lamb "Lechazo de Castilla y León"

Diez, A.M., Osés, S.M., Melero, B., Gómez, E., Wilches, D., Jaime, I., Rovira, J.

Facultad de Ciencias/Departamento de Biotecnología y Ciencia de los Alimentos, Universidad de Burgos, Burgos, España.

*Abstract*—**Consumers are increasingly demanding products which can be prepared easily and quickly (convenience foods concept). So, the aim of this study was to assess the microbial safety in suckling lamb ready to eat products, after a short roasting treatment (250 ºC/20min) and a microwave treatment (5 min at full power) on previously roasted samples (200 ºC/2h) inoculated with *Listeria monocytogenes* and *Escherichia coli* ($10^6$ cfu/g) in order to simulate a cross-contamination situation. Microbiological tests were performed with culture-dependent methods: counts of thermophiles, mesophiles and psychrophiles, lactic acid bacteria (LAB), enterobacteria, *Brochotrix thermosphacta*, heat-resistant microorganisms (*Bacillus cereus* and *Clostridium perfringens*) and pathogens such as *E. coli* and *L. monocytogenes*.**

***Brochotrix thermosphacta and Clostridium perfringens* were not detected in any of the samples studied, whereas *Bacillus cereus* was found in a small number of roasted samples (6/15) before the final cooking treatment, although further roasting or microwave treatment resulted in its complete disappearance. The only resistant microorganisms to the previous roasting treatment were BAL (2.65±1.43). The results obtained with the short roasting and microwave treatments were similar, being able to reduce the inoculated *E. coli* and *L. monocytogenes* counts by more than 5 log units, the psychrophiles, mesophiles, thermophiles and enterobacteria counts between 4 and 5 log units and the BAL count more than 2 log units.**

**Concluding, BAL may be the principal responsible of spoilage of these products but the short roasting treatment and a microwave treatment can eliminate these BAL, heat-resistant and reduce pathogens microorganism obtaining a safe product.**

*Keywords*— **"Lechazo de Castilla y León", "*Listeria monocytogenes*", "*Escherichia coli*".**

## **I.** INTRODUCTION

Spain is one of the largest EU sheep producers (157 mil ton in 2008) with a per capita consumption of 5.2 kg in 2007 (including lamb and goat meat consumption) [1, 2]. Among the types of sheep meat, the suckling lamb (fed exclusively with maternal milk) is the most appreciate by consumers in Spain due to its paler and more delicate flavour [3, 4]. For that reason, in Spain, there are various Protected Geographical Indications (PGI) that guarantee the quality of suckling lamb meat. Since 1999, in "Castilla y León", the EU has granted this meat as PGI "Lechazo de Castilla y León" (Commission Regulation EEC No 2107/1999).

The food industry should be well aware of customers' needs and expectations, one of the most important of which is undoubtedly to guarantee the quality and safety of any foodstuff. Modern society's consumption model has changed in response to a series of factors such as market demand, consumers' purchasing power, tastes, needs, lifestyles, perception and ideology, information and advertising, and the availability of foodstuffs, etc. [5]. Indeed, consumers are increasingly demanding more natural foodstuffs free from artificial preservatives which have been submitted to preservation treatments that do not affect their nutritional and/or sensory properties, but which can be stored for long periods of time and can be prepared easily and quickly (convenience foods concept).

The aim of this study was to assess the microbial safety in products ready to eat of suckling lamb after a short roasting treatment (250 ºC/20min) and a microwave treatment (5 min at full power) on previously roasted samples (200 ºC/2h) inoculated with *Listeria monocytogenes* and *Escherichia coli* ($10^6$ cfu/g) in order to simulate a cross-contamination situation.

## **II.** MATERIALS AND METHODS INTRODUCTION

### *A. Preparation of the bacterial suspension*

Three strains of *L. monocytogenes* (one from lamb, CECT 4032 and CECT 5366) and three of *E. coli*

(CECT 434, CECT 4783 and NCTC 12079) were used for inoculation. Each strain was grown in 10 ml of BHI (Oxoid, Basingstoke, UK) overnight (at 37 ºC for *L. monocytogenes* strains and at 42 ºC for *E. coli* strains), then centrifuged (Eppendorf, Hamburg, Germany) at 7000 rpm for 15 min at 4 ºC, washed three times and re-suspended in 10 ml of Ringer's solution (Oxoid) to achieve cell concentrations of $10^8$ cfu/ml. Each bacterial suspension was diluted 1:10 in Ringer's solution (Oxoid) prior to sample inoculation to achieve a cellular concentration of $10^7$ cfu/ml.

*B. Sample preparation*

Ten portions weighed between 250 and 350 g, (5 fore and 5 hind) were roasted at 200 ºC for 2 h. Samples were roasted directly in an oven and then, where applicable, vacuum-packed individually as hygienically as possible in bags impermeable to oxygen and water (Cryovac Sealed Air). Then four of them were inoculated with *Escherichia coli* and four with *Listeria monocytogenes*. The remaining two samples were used as controls. All samples were stored overnight at 4ºC, then half were roasted at 250ºC for 20 min and the other half microwaved for 5 min at full power. Samples for microbiological analysis were taken before and after the roasting and microwave treatments. A sample of raw meat was also taken from each control sample prior to roasting for subsequent analysis.

*C. Sample preparation*

Both bacteria were spiked by spraying to fresh lamb meat cuts to reach a final product concentration of around $10^6$ cfu/g, using a 1:10 dilution of each bacterial suspension.

*D. pH measurements*

Potentiometric pH measurements were made by inserting the pin electrode of a pH meter (micropH2001, CRISON, Barcelona, Spain) directly into the sample. Three independent measurements were obtained for each sample.

*E. Microbiological analysis*

A slice of 25 g of suckling lamb was sterile-weighed, diluted in 225 ml of Ringer solution (Oxoid, Basingstoke, UK), and homogenized for 120 s in a laboratory blender (Stomacher 400, Seward, London, UK) prior to the preparation of 1/10 serial dilutions for microbiological analysis. The following microbial parameters were determined: total viable count (TVC): plated on PCA agar plates (Oxoid) and incubated at 30 ºC for 48 hours; psychrophilic bacteria: plated on PCA agar plates (Oxoid) and incubated at 5 ºC for 10 days; thermophile bacteria: plated on PCA agar plates (Oxoid) and incubated at 42 ºC for 48 hours; lactic acid bacteria (LAB): grown in MRS agar (Biokar Diagnostics, Beauvais, France) and incubated anaerobically in 6 % CO2 at 30 ºC for 48 hours; *Enterobacteriaceae*: tested in VRBG agar (Oxoid) and incubated at 37 ºC for 48h; *Escherichia coli*: plated on TBX (Biolife, Milan, Italy) and incubated at 42 ºC for 24h; *Listeria monocytogenes*: according to ISO 11290-1:1996/A1, using demi-Fraser and Fraser-like pre-enrichment or enrichment media (Oxoid) prior to plating on chromogenic listeria agar base (Oxoid) at 37 ºC for 48h; *Bacillus cereus*: spread over the surface of MYP agar and incubated at 30 °C for 18-40 h; *Brochothrix thermosphacta*: grown in STAA agar and incubated at 22 ºC for 48 h; *Clostridium perfring*ens: plated on TSN agar (Oxoid) and incubated at 45 ºC for 24-48 hours under anaerobic conditions.

## **III.** RESULTS

The microbial counts and pH values obtained for the various parameters studies are listed in Table 1.

*Brochotrix thermosphacta* and *Clostridium perfringens* were not detected in any of the samples studied (detection limit: 2 and 1 log cfu/g, respectively), whereas *Bacillus cereus* was found in a small number of roasted samples (6/15) which did not undergo further treatment, although further roasting or microwave treatment resulted in its complete disappearance. The only microorganisms studied found to be resistant to the roasting treatment were LAB (2.65±1.43). The results obtained with the short roasting and microwave treatments were similar, with both being able to reduce the inoculated *E. coli* and *L.*

*monocytogenes* counts by more than 5 log units, the psychrophile, mesophile, thermophile and enterobacteria counts by between 4 and 5 log units and the BAL count by more than 2 log units.

Table 1: Microbial counts (log cfu/g) and pH values.

| SAMPLES | pH | PCAT | PCAM | PCAP |
|---|---|---|---|---|
| A | 6.35±0.20 | 1.94±1.36 | 1.82±1.28 | 1.62±1.42 |
| AEA | 6.16±0.13 | 6.17±0.17 | 5.88±0.71 | 5.15±0.25 |
| EA | 6.31±0.19 | 1.33±0.52 | 1.37±0.58 | ND |
| AEM | 6.32±0.06 | 6.17±0.20 | 6.69±0.20 | 5.43±0.11 |
| EM | 6.37±0.05 | ND | 2.07±1.22 | 1.41±0.65 |
| ALA | 6.17±0.11 | 6.52±0.45 | 7.26±0.20 | 6.06±0.40 |
| LA | 6.22±0.09 | ND | 2.16±1.12 | 3.29±0.08 |
| ALM | 6.20±0.16 | 6.69±0.04 | 7.59±0.13 | 6.52±0.73 |
| LM | 6.45±0.19 | 1.56±0.87 | 2.07±1.01 | 4.12±1.48 |
| SAMPLES | MRS | TBX | ALOA | VRBG |
| A | 2.65±1.43 | ND | ND | ND |
| AEA | ND | 6.42±0.23 | ND | 5.91±0.14 |
| EA | ND | ND | ND | ND |
| AEM | ND | 6.88±0.15 | ND | 6.00±0.15 |
| EM | ND | ND | ND | ND |
| ALA | ND | ND | 6.39±0.25 | ND |
| LA | ND | ND | 1.00±0.00 | ND |
| ALM | ND | ND | 6.18±0.24 | ND |
| LM | ND | ND | 1.00±0.00 | ND |

ND: no detected. A: roasted; AEA: roasted inoculated with *E. coli* before brief roasted; EA: roasted inoculated with *E. coli* after brief roasted; AEM: roasted inoculated with *E. coli* before microwave; EM: roasted inoculated with *E. coli* after microwave; ALA: roasted inoculated with *L. monocytogenes* before brief roasted; LA: roasted inoculated with *L. monocytogenes* after brief roasted; ALM: roasted inoculated with *L. monocytogenes* before microwave; LM: roasted inoculated with *L. monocytogenes* after microwave.
PCAT: for termophile total count; PCAM: for mesophile total count; PCAP: for psychrophile total count; MRS: for lactic acid bacteria count; TBX: for *E. coli* count; ALOA: for *L. monocytogenes* and VRBG for *Enterobacteriaceae* count.

## **IV.** DISCUSSION

Pathogens may contaminate ready-to-eat meat products after cooking but before packaging. *Listeria monocytogenes* and *E. coli* are formidable contaminants in the food processing environment and are relatively heat resistant compared with other non-spore forming pathogens [6].

Lactic acid bacteria may be the principal responsible of spoilage of these products packaged in oven-proof bags and preserved at chilling temperature. LAB have been reported as the predominant spoilage microorganisms in vacuum packaged meat products, as the vacuum produces an ecosystem that encourages their growth [7].

Other authors have found similar results for *L. monocytogenes* and *E. coli* in different meat products. Frankfurters should be microwave reheated for 75 s at high power (1100 W) to reduce *L. monocytogenes* counts of up to 3.7 log cfu/cm$^2$. Longer times are needed when the product has supported growth of the pathogen to levels > 3.7 log cfu/cm$^2$, due to prolonged storage time [8]. In beef hamburger inoculated with $10^7$ MPN/g of *E. coli* O157:H7, after microwave treatment (60 s at full power) counts were not found [9]. Reductions between 2.0 to 4.2 log cfu/g were found in coarsely ground beef inoculated with *E. coli* O157:H7 (eight-strain composite, 6 to 7 log cfu/g) and roasted (Oster toaster oven and Magic Chef standard kitchen oven) to a geometric center temperature of 65 °C [10].

However, short time exposure (of up to 30 s) of microorganisms on chicken meat to microwaves (at a frequency of 2450 MHz) have not been significant bactericidal effect on growth of *Escherichia coli* K12 (5-6 log cfu/cm$^2$) [11].

## **V.** CONCLUSIONS

Concluding, LAB may be the principal responsible of spoilage of suckling lamb ready to eat products but the short roasting treatment and a microwave treatment can eliminate these microorganisms and also some heat-resistant bacteria, and reduce pathogen microorganisms obtaining a safe product.

## **VI.** ACKNOWLEDGEMENTS

The authors thank to the European Union the financial support by Pathogen Combat project. Contract nº 007081.

## **VII.** REFERENCES


1. Eurostat. (2008) Food. From farm to fork statistics. European Communities. ISBN 978-92-79-08736-3.
2. Eurostat.(2009)http://epp.eurostat.ec.europa.eu/tgm/table.do?tab=table&init=1&language=en&pcode=tag00017&plugin=0.
3. Miguélez E., Zumalacárregui J.M., Osorio M.T., Beteta O., Mateo J. (2006) Carcass characteristics of suckling lamb protected by the PGI "Lechazo de Castilla y León" European quality label: Effect of breed, sex and carcass weight. Meat Science 73:82-89.
4. Sañudo C., Alfonso M., San Julián R., Thorkelsson G., Valdimardottir T., Zygoyiannis D., Stamataris C., Piasentier E., Mills C., Berge P., Dransfield E., Nute G.R., Enser M., & Fisher A.V. (2007) Regional variation in the hedonic evaluation of lamb meat from diverse production systems by consumers in six European countries. Meat Science 75:610-621.
5. Tapia M.S., Roa V. (1998) Tecnologías emergentes en el procesamiento de alimentos. Anales Venezolanos de Nutrición 11 (1):21–27.
6. Houben J.H., Eckenhausen F. (2006) Surface pasteurization of vacuum-sealed precooked ready-to-eat meat products. Journal of Food Protection 69 (2):459-468.
7. Jeya Shakila R., Jeyasekaran G., Vijayakumar A., Sukumar D. (2009) Microbiological quality of sous-vide cook chill fish cakes during chilled storage (3 °C). International Journal of Food Science and Technology 44 (11):2120-2126.
8. Rodríguez-Marval, M., Geornaras I., Kendall P.A., Scanga J.A., Belk K.E., Sofos J.N. (2009) Microwave oven heating for inactivation of *Listeria monocytogenes* on frankfurters before consumption. Journal of Food Science 74 (8):M453-M460.
9. Quesada O., Laura Arias M., Chaves C. (2003) Effect of microwave oven over the growth and survival of *Escherichia coli* O157:H7 inoculated in bovine minced meat. Archivos Latinoamericanos de Nutrición 53 (1):65-69.
10. Shen C., Adler J.M., Geornaras I., Belk K.E., Smith G.C., Sofos, J.N. (2010) Inactivation of *Escherichia coli* O157:H7 in non intact beefsteaks of different thicknesses cooked by pan broiling, double pan broiling, or roasting by using five types of cooking appliances. Journal of Food Protection 73 (3):461-469.
11. Göksoy E.O., James C., Corry J.E.L. (2000) Effect of short-time microwave exposures on inoculated pathogens on chicken and the shelf-life of uninoculated chicken meat. Journal of Food Engineering 45 (3):153-160.