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PREVALENCE OF LACTIC ACID BACTERIA IN SLICED COOKED HAM AS AN INDICATOR OF ITS SHELF LIFE

PREVALENCIA DE BACTERIAS DE ÁCIDO LÁCTICO EN JAMÓN COCIDO FRICCIONADO COMO INDICADOR DE SU VIDA ÚTIL

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ABSTRACT

Background: Ready to eat sliced-cooked-meat-products (RTE) are popular convenience foods. Slicing of such products at retail point is a common practice in supermarkets. Due to handling, as well as the supermarket environment, it has been suggested that the counts and presence of specific lactic acid bacteria (LAB) in these products may be associated with their rapid deterioration and short shelf life. **Objectives**: The aim was to quantify and identify LAB in commercial sliced cooked ham. **Methods:** Thirty samples of sliced cooked ham were collected from five supermarkets. Each sample was analyzed in terms of: *Lactobacillus* on De Man Rogosa, Sharpe agar (30°C), mesophilic (30°C) and thermophilic (37°C) *Streptococcus* on M17 agar containing 1% lactose, *Lactococcus* on LM17 agar containing 100 μg per liter of cycloheximide (25°C) and *Enterococci* on Kanamicin Aesculin Azide agar containing kanamycin (37°C), all under anaerobic conditions (Gas-Pack System®, BBL) for 48-72h. Twenty-one colonies were randomly picked and physiologically and biochemically characterized. **Results:** No difference was observed in genuses between supermarkets; however, significant differences were observed between microbial genuses. Enterococci showed the lowest count (2.34 ± 0.05 $\log_{10}$ CFU/g) and Lactobacilli had the highest counts (5.98 ± 0.04 $\log_{10}$ CFU/g). Of the strains isolated 23.8% were identified as thermophillic *Lactobacillus*, 23.8% mesophilic *Lactobacillus,* 28.6% *Enterococcus*, 14.3% *Lactococcus* and 9.5% *Streptococcus*. From these, only six could be characterized to species level; one was *L.lactis* subsp. *lactis* and five were *L. amylolyticus*. **Conclusions:** High LAB counts are a common cause of spoilage in RTE meat products, since these are commonly found in meat environments. Therefore a study on the processing, distribution and in-site handling of RTE meat products at supermarkets may be necessary in order to increase its shelf life.

Keywords: Cooked ham, lactic acid bacteria, shelf life, *Lactobacillus* spp.

RESUMEN

Antecedentes: Los productos cárnicos rebanados cocidos listos para el consumo (RTE) son alimentos populares de conveniencia. El rebanado de este tipo de productos es una práctica común en los supermercados. Debido a la manipulación, así como al entorno del supermercado, se ha sugerido que los recuentos y la presencia de bacterias ácido lácticas (LAB) en estos productos pueden estar asociados con su rápido deterioro y corta vida en anaquel. **Objetivo:** El objetivo de este trabajo fue cuantificar e identificar LAB en jamón cocido rebanado en el supermercado. **Métodos:** se recolectaron 30 muestras de

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jamón cocido rebanado de cinco supermercados. Cada muestra se analizó en términos de: *Lactobacillus* en agar De Man Rogosa, Sharpe (30°C), *Streptococcus* mesofílicos (30°C) y termofílicos (37°C) en agar M17 conteniendo 1% de lactosa, *Lactococcus* en agar LM17 contiendo 100 μg por litro de ciclohexamida (25°C), y *Enterococci* en agar Kanamicin Aesculin Azide conteniendo kanamicina (37°C), todos bajo condiciones anaeróbicas (Gas-Pack System®, BBL) por 48-72h. Veintiún colonias seleccionadas al azar fueron fisiológica y bioquímicamente caracterizadas. **Resultados**: No se observaron diferencias en el género entre los supermercados; sin embargo, se observaron diferencias significativas entre género microbiano. Los enterococos mostraron los recuentos más bajos (2,34 ± 0,05 $\log_{10}$ CFU/g) y los lactobacilos los más altos (5,98 ± 0,04 $\log_{10}$ CFU/g). De las cepas aisladas 23,8% fueron identificados como *Lactobacillus* termófilos, 23,8% *Lactobacillus* mesófilos, 28,6% *Enterococcus*, 14,3% *Lactococcus* y 9,5% *Streptococcus*. De éstos, sólo seis pudieron caracterizarse a nivel de especie; uno fue *L.lactis* subsp *lactis* y cinco fueron *L. amylolyticus.* **Conclusiones:** Conteos altos de LAB son una causa común del deterioro de los productos cárnicos listos para el consumo, ya que estos se encuentran comúnmente en los entornos donde se maneja carne. Por lo tanto, es necesario llevar a cabo un estudio sobre el procesamiento, distribución y manejo de productos cárnicos listos para el consumo en los supermercados con el fin de aumentar su vida en anaquel.

Palabras clave: Jamón cocido, bacterias ácido lácticas, vida en anaquel, *Lactobacillus* spp.

INTRODUCTION

Ready to eat (RTE) meat products are popular convenience foods. In recent years, the popularity of them have increased as they are a quick, easy and accessible option for consumers (1). They are retailed chilled with a shelf life depending on their formulation, and handling. During handling, which includes slicing and packing, these products might be contaminated with microorganisms from the supermarket environment (e.g. slicer, counter, refrigerator, staff, etc)(1). In addition, if the cold supply chain is abused and the products are not handled as stated by the regulations, they might be rapidly spoiled. Lactic acid bacteria (LAB) are widespread in nature and consistently exist on mucous membranes and body. Hence, they are part of the initial flora on raw meats after slaughter and in sliced cooked meats they are considered contaminants.. LAB may inhibit the growth of pathogenic microorganisms as a result of the production of organic acids and other antagonist substances (2); however, organic acids might also accelerate the decomposition of meat products (3). It has been reported that LAB cause acidification, increase viscosity, excessive drip and discoloration (greening) in meat products (4 -7). Kreyenschmidt *et al.* (2010) reported that the shelf life of meat products ends when LAB reach 10^7 CFU/g, as the product is no longer sensorially acceptable because of the existance of signs of spoilage such as off-odours and viscosity (6). Mexican legislation for meat products does not required analysis of LAB; although they are of great importance since they cause economic losses during shelf life. In Mexico, most RTE meat products are sliced at supermarkets, this practice may increases the degree of contamination since one slicer is used for different products. For these reasons the aims of this study were to quantify and presumptive identify LAB strains in sliced ham as an indicator of its shelf life.

MATERIALS AND METHODS

Ham samples

Six samples of 250 g commercial cooked ham sliced at the retail point and elaborate without starter cultures were obtained on six consecutive Mondays at the same time from five different supermarkets of the same retail chain in the City of Chihuahua, Mexico. The study was conducted from April to December 2012. Microbiological analyses were done within the first 24h, all samples were kept under refrigeration until analyses.

Microbiological analyses

Ten grams of cooked ham were taken aseptically, placed in a stomacher bag (Lab-blender type 80 Modelo: BA 6020, USA) with ninety ml of buffered peptone water (BD Difco®, Sparks, MO, USA) for 1 minute. After decimal dilutions, samples were surface-plated (100 μl) onto appropriate media. *Lactobacillus* were plated on de Man Rogosa, Sharpe agar

(MRS; Oxoid®, Basingstoke, Hampshire, England), mesophilic and thermophilic *Streptococcus* on M17 agar (Oxoid®, Basingstoke, Hampshire, England) containing 1% lactose (Sigma Aldrich, Basingstoke, Hampshire, England), *Lactococcus* on LM17 (BD®, Sparks Glencoe, MD, USA) containing 100 μg per liter of cycloheximide (Fluka Chemica®, Milan, Italy) and Enterococci on Kanamicin Aesculin Azide agar containing kanamycin (KAA; Oxoid, Basingstoke, Hampshire, England). Incubation was at 30°C for *Lactobacillus* and mesofilic *Streptococcus*, at 25°C for *Lactococcus* and 37°C for thermophilic *Streptococcus* and *Enterococcus*, all under anaerobic conditions (Gas-Pack System®, BBL) for 48-72h.

In order to multiply the cultures, twenty-one colonies were randomly picked and inoculated in MRS broth at 30°C for presumptive *Lactococcus*, *Lactobacillus* and *Leuconostoc*, and in Brain Heart Infusion broth (BHI, Lab M®, Bury, Lancashire, UK) for presumptive *Enterococcus* and *Streptococcus* at 37°C. Then colonies were transferred to nutrient agar (Oxoid®, Basingstoke, Hampshire, England) to assure purity. All strains were Gram stained and tested by the KOH method, catalase formation directly onto each plate, and morphology by phase contrast microscopy. The strains were cryopreserved in vials (Nalgene®) with liquid medium (60% glycerol (Fermont®, Monterrey, Nuevo León, México) and 40% of the strain in a liquid medium) at -20°C until they were used for characterization.

Working cultures were grown in MRS and M17 broth media for rods and cocci respectively. Overnight Gram-positive and catalase-negative strains were further tested for their ability to produce CO_2 from glucose in MRS broth containing Durham tubes, growth after incubation at 10°C for 7 days, at 45°C for 2 days, and in the presence of 20, 40 and 65 g NaCl/L after incubation at 30°C for 4 days. Based on these results, all strains were presumptively classified to genus level (8), as shown in.

Table 1. Morphological and physiological criteria used in presumptive identification of the isolates in this study

Shape	Heterofermentation	Growth in NaCl(g/l)			Growth at		Identification
		20	40	65	10°C	45°C	
Cocci	−	+	+/−	−	+	−	Lactococcus
Cocci	+	+	+/−	ND	+	−	Leuconostoc
Cocci	−	+	+	+	+	+	Enterococcus
Cocci	ND	+	−	−	−	+	Streptococcus thermophilus
Rods	ND	ND	ND	ND	ND	+	Thermophilic Lactobacillus
Rods	+/−	+	+	+	ND	−	Mesophilic Lactobacillus

+, Positive; −, negative; +/−, positive or negative; ND, not determined.

The fermentation of carbohydrates was determined on a standard liquid media using phenol red (BD, Sparks Glencoe, MD, USA) as pH indicator. The carbon sources were Arabinose, Cellobiose, Fructose, Galactose, Glicerol, Lactose, Maltose, Mannose, Mannitol, Melibiose, Melezitose, Raffinose, Rhamnose, Ribose, Sorbitol, Sucrose, Trehalose, Xylose added to the medium to a final concentration of 1% (w/v), and Salicin, added to a final concentration of 0.5% (w/v), (all from Sigma Aldrich, Basingstoke, Hampshire, England). Aliquots of 250 μL of each media were added to a microplate (Costar, Cole-Parmer, USA) and inoculated. Incubation was microaerophyically at 35°C for 24 h. Microaerophilic conditions were generated by sealing the microplate with parafilm. The carbohydrate fermentation was considered positive when the color of the media changed to yellow. The strains were presumptively classified to genus and species level by matching results with Bergey's Manual (8).

Statistical Analysis

Bacterial counts were transformed from CFU/g to $\log_{10}$ CFU/g. Data were analyzed by ANOVA with significant differences ($p< 0.05$). Subsequently

the data were subjected to analysis of variance by fitting a model that included the fixed effects of supermarket, species and their interaction using PROC GLM® (SAS, 2002). The comparison of means was performed by the Tukey-Kramer test (SAS). The isolation and identification of LAB species was reported in the number and percent of isolations and the species identified.

RESULTS

Table 2 shows LAB counts of sliced cooked ham analyzed in this study. Differences ($p<0.05$) between microbial genuses were found. Enterococci showed the lowest count (2.34 ± 0.05 $\log_{10}$ CFU/g) and *Lactobacillus* the highest (5.98 ± 0.04 $\log_{10}$ CFU/g). No difference ($p>0.05$) was observed in genus between supermarkets.

Table 2. Numbers of lactic acid bacteria from cooked ham sliced at retail point estimated in different microbial media

Supermarket	Microorganism counts (log10 CFU/g)1				
	Lb	Lc	Sm	St	E
1	5.98±0.04a	5.92±0.04[a]	5.97±0.04[a]	5.96±0.04[a]	2.36±0.05b
2	5.88±0.04[a]	6.06±0.04[a]	5.86±0.04[a]	5.97±0.04[a]	2.39±0.05b
3	6.01±0.04[a]	5.96±0.04[a]	5.87±0.04[a]	6.04±0.04[a]	2.53±0.05b
4	6.00±0.04[a]	5.97±0.04[a]	5.90±0.04[a]	5.97±0.04[a]	2.22±0.05b
5	6.06±0.04[a]	5.95±0.04[a]	5.92±0.04[a]	5.91±0.04[a]	2.19±0.05b
Total	5.98±0.04[a]	5.97±0.04[a]	5.90±0.04[a]	5.97±0.04[a]	2.34±0.05b

[ab] Means with different superscripts in the same row differ ($P<0.05$). Lb=*Lactobacillus*, Lc=*Lactococcus*, Sm=*Streptococcus* mesophiles, St= *Streptococcus* thermophiles and E= *Enterococcus*. CFU/g = colony forming units per gram. 1 *Lactococcus* plated onto LM17 + 100 μg/lt cycloheximide (25°C); *Streptococcus* onto M17 + 1% lactose (30 and 37, for mesophiles and thermophiles, respectively), *Enterococcus* onto KAA agar (37°C), all incubated under anaerobic conditions

Table 3. Morphological, physiological and biochemical characteristics of bacteria isolated from cooked ham sliced at retail point

Characteristics	Strains code																					
	01	02	03	04	05	09	11	13	14	15	17	18	19	22	23	24	25	28	29	33	34
Morphology	C	C	C	C	C	C	C	R	R	R	R	Cc	R	R	R	R	C	Cc	R	C	R
Gram stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gas production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at																					
10°C	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+	-	+	-	+	+	+
45°C	-	-	+	+	+	-	-	+	+	-	-	-	+	+	-	+	+	-	-	+	-
Growth in NaCl																					
2%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6.5%	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
Presumptive Genera	Lactococcus	Lactococcus	Enterococcus	Enterococcus	Enterococcus	Lactococcus	Enterococcus	Thermophilic Lb.	Thermophilic Lb.	Mesophilic Lb.	Mesophilic Lb.	Streptococcus	Thermophilic Lb.	Thermophilic Lb.	Mesophilic Lb.	Thermophilic Lb.	Streptococcus	Enterococcus	Mesophilic Lb.	Enterococcus	Mesophilic Lb.

Morphology C = cocci, R = rods, Cc = cocci chains, + = positive, - = negative. Note: sugar fermentation is not shown.

Table 3 shows the results of the 21 isolates that were randomly picked and presumptively identified as: thermophilic *Lactobacillus* (23.8%), mesophilic *Lactobacillus* (23.8%), *Enterococcus* (28.6%), *Lactococcus* (14.3%), and *Streptococcus* (9.5%). Of these, only six could be characterized to species level; one was *L.lactis* subsp. *lactis* (01) and five were *L. amylolyticus* (isolates numbers 13, 14, 19, 22 and 24).

DISCUSSION

Lactobacillus had the highest counts (5.98 ± 0.04 $\log_{10}$ CFU/g) followed by *Lactococcus* and *Streptococcus* thermophiles (5.97±0.04 $\log_{10}$ CFU/g), *Streptococcus* mesophiles (5.90±0.04 $\log_{10}$ CFU/g) and *Enterococcus* (2.34±0.05 $\log_{10}$ CFU/g). Meanwhile, *L.lactis* subsp. *lactis* (1 isolate) and *L. amylolyticus* (5 isolate) were identified. These suggest that the products have consistent quality, in terms of BAL counts, regardless of the supermarket where they were purchased. Although different supermarkets were sampled, these were from the same retail chain, so it can be assume that the cleaning and disinfection protocols are the same in all sampled supermarkets. As reported by Kreyenschmidt *et al*. the shelf life of RTE meat products ends when LAB counts reached 10^7 CFU/g (8 $\log_{10}$ CFU/g) (6). The results found in this study were similar to those found by Pérez-Rodríguez *et al*. who reported LAB counts of 5.92 ± 1.06 $\log_{10}$ CFU/g in cooked ham (1). Moreover, Samelis *et al*., at the day of purchase, reported LAB counts of 3.48 $\log^{10}$ CFU/g; then, at day 15, counts exceeded 8 $\log_{10}$ UFC/g (9). This suggests that the samples in this study might have a shelf life shorter than 15 days since at the day of purchase counts were higher than 5 $\log_{10}$ for all microbial genuses but Enterococci. Borch *et al*. found that during the aerobic storage of cooked and sliced meat products the dominant LAB were *Bacillus*, *Lactobacillus* and *Micrococcus* (4). In cooked sausages it was reported the presence of *Pediococus*, *Lactobacillus*, *Enterococcus* and *Aerococcus* (3). Gibson *et al*. studied cross contamination using a melamine copolymer resin (fluorescent compound) as a contaminant, demonstrating how easily the resin was distributed. It was identified that the most likely areas of contamination were; gloves, the slicing machine, the collection tray and the blade (10). Slicing is of vital importance, as the higher the contamination of cooked meats at this stage, the shorter the shelf life, independent of the storage conditions (7,11,12).

Respect to the strains presumptive identified *L.lactis* subsp. *lactis* and *L. amylolyticus*, Rodríguez *et al*. analyzed Spanish fermented sausages samples reporting the presence of *Lactococcus* and isolated two strains of *Lc. Lactis* (13). While Hamasaki *et al*. identified strains of *Lc. lactis* subsp *lactis* in cooked sliced ham (14). The lack of reports on the presence of *Lc. lactis* in meat products may be attributed to the research being addressed to dairy products given the supposition that they are found only in them, so isolates are classified as "atypical" strains. Meanwhile, *L. amyloliticus* have been also isolated from fresh meats (15), so that its presence in cooked ham may be due to contamination. Although phenotypic characterization, based on sugar fermentation pattern and conventional phenotypic properties, might not always provide sufficient information for the reliable identification of LAB, this is a useful tool for presumptive characterization (16). The findings in this study may reflect the reality in Mexico, since all supermarkets are handled similarly in the deli area. However, these should be viewed with caution, since the samples were taken from one supermarket chain. Therefore, it is recommended that in future works, samples of sliced cooked ham are obtained from several supermarket chains. Likewise, Mexican legislation for cooked meat products does not require analysis of LAB, just some pathogens such as *Escherichia coli*, *Staphylococcus*, and *Salmonella*. The results of this study show the importance of LAB in cooked ham quality and its shelf life, suggesting that it is important to regulate their content and presence, to improve food quality by regulating the handling and storage.

CONCLUSIONS

The LAB initially present in the samples showed counts between 5.98±0.04 and 2.34±0.05 at the day of purchase, being Lactobacillus the most numerous group. *L.lactis* subsp. *lactis* and *L. amylolyticus* were identified as a spoilage bacteria of sliced cooked ham. Meat products are popular convenience foods that are highly perishable and rapidly spoiled due to defective handling. High LAB counts are a common cause of spoilage in meat products since these are commonly found in meat environments. The quantification and phenotyphic methodologies based on sugar fermentation are helpful to better understand the growth and presumptive identification of LAB as a spoilage microorganism of sliced cooked ham.

The study of the evolution of the LAB microbiota in sliced cooked ham may be important for selecting the main deteriorating LAB aiming to decrease their presence in this food product. Therefore a study on the processing, distribution and in-site handling of RTE meat products may be necessary in order to increase their shelf life. Also, the evaluation might include enumeration and growth rate of LAB and the presence and quantification of organic acids and other chemical compounds.

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