STUDY OF THE YEAST POPULATION THROUGHOUT THE MANUFACTURE OF DRY-CURED "LACÓN". EFFECT OF SALT LEVELS

[1]Purriños, L., [1]García, G., [2]Carballo, J., [1]Franco, D., [1]Lorenzo, J.M. and [1]García-Fontán, M.C. *

[1] Meat Technology Centre of Galicia, Rúa Galicia Nº4-Parque Tecnolóxico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain
[2] Food Technology Laboratory, University of Vigo, 32004 Ourense, Spain
*corresponding author to provide phone: 0034988548277, fax: 0034988548277, e-mail: caminogarcia@ceteca.net

Abstract- The influence of salt levels on yeast population during the manufacture of dry-cured "lacón" was studied. In this work, two batches with different salt levels were used in salting stage: 0.75 and 1.25 days/kg. The dominant species isolated from OGYEA throughout the manufacture of the two batches of dry-cured "lacón" were *C. famata* and *C. zeylanoides*. In the early processing stages (post-salting) the numbers of isolated species is higher that at the end of dry-ripening. This salting effect seems not to influence in the type of isolated yeasts. The salt content neither affect to the quantity of morphological yeasts isolated.

Keywords: **Dry-cured "lacón", yeasts population, *Candida famata*, Salt levels**

I. INTRODUCTION

"Lacón" is a dry–cured meat product made in the north-west of Spain (Galicia) from the fore extremity of the pig cut at shouldher blade-humerus joint, following a technological process very similar to those of dry-cured ham.

Surface layers of dry cured hams harbour a typical microbial flora composed of bacteria, yeasts and filamentous fungi (Huerta, T., Querol, A. and Hernández, E., 1988).

During ripening of dry-cured products a_w decreases and given the limited types of microorganisms able to grow at low a_w values reached, the composition of the microbial population could serve as an indicator of ripening process. Previous studies reported that staphylococci and micrococci may not recovered at the end of maturation of the Iberian dry-cured ham, whereas yeasts can be the predominant microorganisms (Núñez, F., Rodríguez, M.M., Córdoba, J.J., Bermúdez, M.E. and Asensio, M.A., 1996). Studies on the Spanish raw ham and "lacón" evidenced that the profile of yeast population greatly changed during processing (Núñez, F., Rodríguez, M.M., Córdoba, J.J., Bermúdez, M.E. and Asensio, M.A., 1996; Lorenzo, J.M., García-Fontán, M.C., Franco, I. and Carballo, J., 2005).

Yeast colonization on the surface of dry-cured meat products could also play an important role against pathogenic microorganisms which may cause health problems to the consumer (Metaxopoulos, J., Stavropoulos, S., Kakouri, A. and Samelis, J., 1996). The aim of this work was to quantify and characterize the yeast wild population on the surface of dry-cured "lacón" during the ripening period, to evaluate the effect of salt levels over the dynamics of dry-cured "lacón" yeasts.

II. MATERIAL AND METHODS

II.1 Samples

In order to carry out this study two batches of "lacón" were manufactured in our centre. Each batch consisted of seven "lacón" pieces that in the fresh stage weighted 4 kg each. The first batch was salted during 3 days (0.75 days/kg) and the second during 5 days (1.25 days/kg) being the temperature of the salting room between 2 and 5 ºC and the relative humidity (RH) between 80 and 90 %. After the salting stage, the pieces were taken from the pile, brushed, washed and transferred to a post-salting room where they stayed for 14 days at a 2-5 ºC and 85-90% RH. After the post-salting stage, the pieces were transferred to a room at 12 ºC and 74-78% RH where a drying ripening process took place for 84 days. For microbial analysis, in each batch, samples were taken from after 7 and 14 days of post-salting and after 7, 14, 28, 56 and 84 days of drying ripening.

II.2. Microbial analysis

Surface samples, approximately 2 mm thickness, were aseptically removed from different areas of the lean surface, and collected together in a sterile bag. The samples were weighed and diluted 1:3 (w/w) with peptone physiological solution (PPS) composed of 8.5 g NaCl (Merck); 1 g trytone (Oxoid) and 1000 mL distilled water (Simoncini, N., Rotelli, D., Virgili, R. and Quintavalla, S., 2007). They were homogenized using a IUL-INSTRUMENT mod. Masticator for 2 min. For each sample, serial dilutions were made in PPS and 0.1 mL of the appropriate dilution was spread-plate inoculated onto Oxytetracycline Glucose Yeast Extract agar plates (OGYEA, Merck).

II.3. Isolation and identification of strains

For two batches, from OGYEA plates, colonies morphologically different were taken from each sampling point and each batch. The isolates were purified by 4 alternate subcultures on Glucose Yeast Extract (GYE, Merck) broth. The purified strains were maintained at -80ºC with 20% glycerol as a cryoprotective agent.

The isolated yeast were identified with the aid of the API® 20 C AUX system (Biomerieux). The following tests were carried out on each isolate: glycerol and calcium 2-keto-gluconate utilization, fermentation of D-glucose, L-arabinose, D-xylose, adonitol, xylitol, D-sorbitol, methyl-αD-glucopyranoside, N-acetyl-glucosamine, D-cellobiose, D-lactose, D-saccharose, D-trehalose, D-melezitose, D-rafinose and presence of Hyphae/Pseudo-Hyphae. Additional tests included the study of colony and cell morphology on MEA (Kreger-Van Rij, 1987).

III. RESULTS AND DISCUSSION

Table 1 and 2 shows the distribution of the species of yeasts throughout the manufacture of two batches of dry-cured "lacón" (0.75 and 1.25 days/kg).

Table 1: Distribution of the species of yeast throuhhout manufacture of dry-cured "lacón" (0.75 days/kg).

Species	**Post-salting (days)**		**Dry-ripening (days)**					**TOTAL**	
	7	14	7	14	28	56	84		
	Nº isolates	**Nº isolates**	**Nº isolates**	**Nº isolates**	**Nº isolates**	**Nº isolates**	**Nº isolates**	**Nº isolates**	**%[a]**
Rodothorula glutinis	2	1						3	6.8
Candida famata	2	3	3	3	2	2	1	16	36.3
Candida krusei/inconspicua	1							1	2.3
Candida boidinii	1							1	2.3
Candida zeylanoides	1	2					2	5	11.4
Candida parapsilopsis	1							1	2.3
Candida glutinis		1						1	2.3
Candida guilliermondii				1	1			2	4.5
Cryptococcus laurenti	2			2				4	9.1
Trichosporum mucoides		1		1				2	4.5
Bacterias	7		1					8	18.2
TOTAL OF ISOLATES	17	8	4	7	3	2	3	44	

[a]The % was calculated relative to the total of isolated strains in OGYEA (44 strains).

Table 2: Distribution of the species of yeast throuhhout manufacture of dry-cured "lacón" (1.25 days/kg).

Species	**Post-salting (days)**		**Dry-ripening (days)**					**TOTAL**	
	7	14	7	14	28	56	84		
	Nº isolates	**Nº isolates**	**Nº isolates**	**Nº isolates**	**Nº isolates**	**Nº isolates**	**Nº isolates**	**Nº isolates**	**%[a]**
Rodothorula glutinis	2	2		4	2	2	1	4	9.3
Rodothorula mucilaginosa								1	2.3
Candida famata	2	4	2					18	41.9
Candida lusitaneae			1		1	1	1	1	2.3
Candida zeylanoides	1	2			1		1	7	16.3
Candida parapsilopsis	1							1	2.3
Candida pelliculosa						1		1	2.3
Cryptococcus laurenti	1			1				2	4.6
Kodamaea ohmeri							1	1	2.3
Bacterias	6	1		1				7	16.3
TOTAL OF ISOLATES	13	9	3	7	5	4	5	43	

[a]The % was calculated relative to the total of isolated strains in OGYEA (43 strains).

From OGYEA, 10 different species were isolated from the dry- cured "lacón" with 0.75 days of salted and 9 with 1.25 days of salt. In our study, 72 of the 87 strains isolated from OGYEA throughout the manufacture of the two batches of dry-cured "lacón" were identified as yeast (82.7% of the strains isolated from this culture medium), which indicates that the selectivity of the OGYEA for the isolation of yeast was good. Similar results were found for Lorenzo, J.M., García-Fontán, M.C., Franco, I. and Carballo, J. (2005). In accordance with other other authors (Huerta, T., Querol, A. and Hernández, E., 1988; Núñez, F., Rodríguez, M.M., Córdoba, J.J., Bermúdez, M.E. and Asensio, M.A., 1996; Simoncini, N., Rotelli, D., Virgili, R. and Quintavalla, S., 2007) the second predominant species was *C. zeylanoides*. The dominant species was *C. famata*. Comi and Cantoni (1983) and Lorenzo, J.M., García-Fontán, M.C., Franco, I. and Carballo, J. (2005) isolated this strain as the main species from Parma ham and dry-cured "lacón". *C. famata* has been described as species characteristic of the Parma ham and Comi and Cantoni (1983) isolated this strain in 100% of the studied hams. The profile of yeast populations changed during processing as shown in table1 and 2. *C. famata* was isolated throughout the whole processing. In the early processing stages (post-salting) the numbers of isolated species is higher that at the end of dry-ripening. This salting effect seems not to influence in the type of isolated yeasts. The salt content neither affect to the quantity of morphological yeasts isolated (10 different species in 0.75 days/kg and 9 in 1.25 days/kg).

IV. CONCLUSIONS

The dominant species isolated isolated from OGYEA throughout the manufacture of the two batches of dry-cured "lacón" (0.75 and 1.25 days of salted/kg) were *C. famata* (36.3% and 41.9% respectively) and *C. zeylanoides* (11.4% and 16.3% respectively).

In the early processing stages (post-salting) the numbers of isolated species is higher that at the end of dry-ripening. The salting effect seems not to influence in the type of isolated yeasts. The salt content neither affect to the quantity of morphological yeasts isolated.

ACKNOWLEDGEMENTS

Authors are grateful to Xunta de Galicia (the Regional Government) for its financial support (07TAL003CT). Special thanks to Elena Carballo for her help in this work.

REFERENCES:

Comi, G. and Cantoni, C. (1983). Presenza di lieviti nei prosciutti crudi stagionati. *Industrie Alimentari*, 22: 102-104.

Huerta, T., Querol, A. and Hernández, E. (1988). Yeasts of dry-cured hams: quantitative and qualitative aspects. *Microbiologie Aliments Nutrition*, 6: 227-231.

Lorenzo, J.M., García-Fontán, M.C., Franco, I. and Carballo, J. (2005). Estudio de la población de levaduras durante el proceso de elaboración del lacón crudo-curado. *Alimentaria*, 367: 46-55.

Metaxopoulos, J., Stavropoulos, S., Kakouri, A. and Samelis, J. (1996). Yeasts isolated from traditional Greek dry salami. *International Journal of Food Science*, 1: 25-32.

Núñez, F., Rodríguez, M.M., Córdoba, J.J., Bermúdez, M.E. and Asensio, M.A. (1996). Yeast population during ripening of dry-cured Iberian ham. *International of Journal of Food Microbiology*, 29: 271-280.

Simoncini, N., Rotelli, D., Virgili, R. and Quintavalla, S. (2007). Dynamics and characterization of yeasts during ripening of typical Italian dry-cured ham. *Food Microbiology*, 24: 577-584.*lied Microbiology,* 89, 1018-1026.