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Yeast population during ripening of dry-cured Iberian ham

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Abstract

The relationship between the superficial yeast population and the ripening conditions of Iberian dry cured hams has been studied for three different locations. Tentative identifications were carried out for 836 isolates. *Candida zeylanoides* was the dominating yeast in early stages, whereas more than 99% of isolates from the surface of matured hams were identified as *Debaryomyces hansenii*. A great diversity of strains of *C. zeylanoides* and *D. hansenii* was found. The characteristic pattern of isolates from the various locations and the selection of various strains of *D. hansenii* during ripening make the study of the yeasts useful for estimating the progress of maturation.

Keywords: Raw ham; *Debaryomyces hansenii*; Ripening evaluation

1. Introduction

Iberian dry-cured ham is an uncooked fermented meat product obtained from highly marbled, Iberian pork. The hams are salted at refrigeration, dried during summer time and maturated in cellar in mountain sites for 12–24 months. During this time a_w decreases to 0.76 in the surface of the ham (Rodríguez et al., 1994). Only staphylococci, micrococci, yeasts, and moulds are found on the surface of fully ripened hams. Changes in lipids and nitrogen compounds during maturation have been described (García et al., 1991; Ventanas et al., 1992; Antequera et al., 1992; Córdoba et al., 1994a; Córdoba et

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al., 1994b). However, no test has been proposed to evaluate the stage of maturation. Given the limited types of microorganisms able to grow at the low a_w values reached on dry cured Iberian ham, the composition of the microbial population could serve as an indicator of the ripening process. In previous studies we have found that *Staphylococcus* and *Micrococcus* may be not recovered at the end of maturation of Iberian dry-cured ham, whereas yeasts can be the predominant microorganism (Rodríguez et al., 1994). Several yeasts have been detected during the early stages of ham maturation such as *Rhodotorula rubra* (Huerta et al., 1988) and *Hansenula sydowiorum*, *Hansenula holstii*, *Hansenula ciferrii*, *Rhodotorula glutinis* and *Cryptococcus albidus* (Molina et al., 1990). In fully matured hams *Debaryomyces* spp. dominate on Italian Parma (Comi and Cantoni, 1983) and Spanish (Monte et al., 1986; Huerta et al., 1988). Ten months of maturation under controlled conditions does not seem to be enough for this development of the yeast population (Molina et al., 1990). Other works focus on the possible contribution of yeasts to the maturation by proteolytic and lipolytic enzymes (Comi et al., 1983; Comi and Cantoni, 1983; Huerta et al., 1988; Molina et al., 1991).

The aim of this work has been to investigate the composition of the yeast population throughout the maturation of Iberian ham at different locations and the suitability of the yeasts profile as a characteristic of the maturation of dry-cured hams.

2. Materials and methods

2.1. Processing of the hams

Forty two hams were obtained from Iberian pigs with 130–150 kg live weight and frozen at −18°C for 15–20 days prior to further processing. After thawing for 48 h at 0°C, the hams were distributed in batches and brought to three different locations (I, II and III) for processing. At every processing location the hams were thoroughly rubbed with sea salt containing 1% potassium nitrate and trace amounts of nitrites and placed into piles of salt for 8 days at 2 ± 2°C. Next, the hams were brushed to remove unabsorbed salt from their surface and kept at 5 ± 1°C for 63–70 days. They were kept in a natural drying room for 3 months in location III, 4 months in I and 5 months in II, during spring and summer. To prevent the colonization by mites, outer muscular surfaces of the hams were covered with a thin layer of melted fat, right at the end of the drying stage. Finally, the hams were left to mature for 15–17 months in a cellar, under natural uncontrolled conditions.

Room temperature and relative humidity were measured daily at midday with a wet and dry bulb thermometer. Water activity was measured at 20°C with a thermoconstanter RTD-33 (Novasina, Zürich, Switzerland). Moisture were determined for 5 g samples after drying at 100–102°C in an air oven and cooling in a desiccator, according to the method described in ISO Method 1442 (1973). Measurement of pH was carried out, immediately after homogenizing 10 g samples with 10 ml distilled deionized water in a OMNI 5000 (OMNI International, Waterbury, CT, USA) for 30 s, using a micropH 2002 (Crison Instruments, Barcelona, Spain).

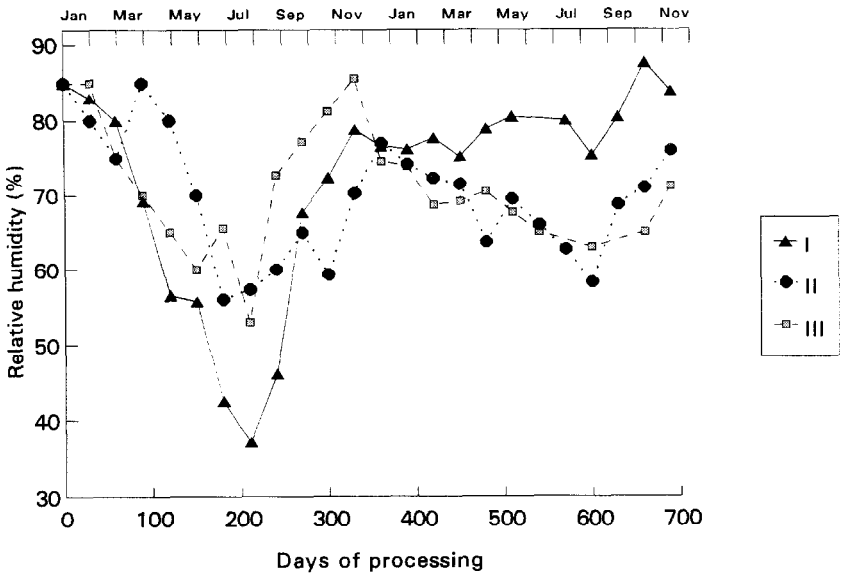

Fig. 1. Average monthly values of midday relative humidity in the process of the hams at locations I, II and III.

2.2. Sampling

Samples from hams were taken at fresh stage just before splitting the hams in batches (F), post-salting (PS), drying (D), and ripening in cellar (16MC). In addition, in one of

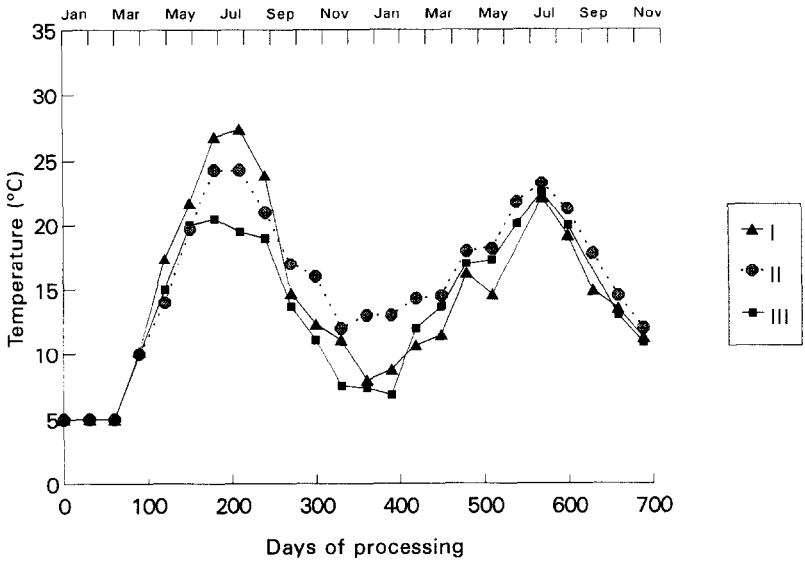

Fig. 2. Average monthly values of midday temperature in the process of the hams.

Table 1
Counts of yeasts (log CFU/g), number of isolates for each species examined and the number expressed as percentage of the yeast count.

	F [a]		PS [a]						D [a]					
			I		II		III [b]		I		II		III	
	RB [c]	DG [c]	RB	DG	RB	DG	RB	DG	RB	DG	RB	DG	RB	DG
Counts (log CFU/g)	4.44	3.74	7.56	7.63	6.05	6.25	4.17	5.63	4.15	7.11	4.82	5.10	6.21	6.16
Candida blankii (Percentage of CFU) [d]	–	–	–	17 (50)	–	3 (1)	–	–	–	–	–	–	–	–
Candida intermedia (Percentage of CFU)	–	–	–	18 (7)	–	–	–	–	–	–	–	–	–	–
Candida zeylanoides (Percentage of CFU)	52 (93)	45 (100)	–	3 (18)	25 (7)	18 (1)	5 (10)	13 (6)	12 (100)	3 (1)	9 (24)	2 (7)	2 (1)	–
Debaryomyces hansenii (Percentage of CFU)	–	–	–	9 (22)	34 (93)	31 (98)	48 (89)	79 (94)	–	54 (99)	28 (76)	47 (93)	8 (99)	7 (100)
Pichia carsonii (Percentage of CFU)	–	–	39 (93)	1 (3)	–	–	1 (1)	–	–	–	–	–	–	–
Rhodotorula rubra (Percentage of CFU)	2 (7)	–	1 (7)	–	–	–	–	–	–	–	–	–	–	–
TOTAL	54	45	40	48	59	52	54	92	12	57	37	49	10	7

[a] F, fresh stage; PS, end of post-salting; D, end of drying; 4MC, 4 months in cellar; 8MC, 8 months in cellar; 16MC, final product.
[b] I, II, III: locations of ripening.
[c] RB, Dichloran Rose Bengal Chloramphenicol Agar; DG, Dichloran-Glycerol Agar.
[d] Ratio of the species in the count, expressed as percentage of the average value for three hams.

	4MC [a]		8MC [a]		16MC [a]					
	I		I		I		II		III	
	RB	DG	RB	DG	RB	DG	RB	DG	RB	DG
Counts (log CFU/g)	4.74	5.29	6.24	6.29	4.48	4.79	5.51	5.59	4.79	4.49
Candida blankii (Percentage of CFU) [d]	–	–	–	–	–	–	–	–	–	–
Candida intermedia (Percentage of CFU)	–	–	–	–	–	–	–	–	–	–
Candida zeylanoides (Percentage of CFU)	–	–	–	–	–	–	1 (1)	–	–	–
Debaryomyces hansenii (Percentage of CFU)	34 (100)	31 (100)	47 (100)	41 (100)	16 (100)	18 (100)	12 (99)	13 (100)	4 (100)	1 (100)
Pichia carsonii (Percentage of CFU)	–	–	–	–	–	–	–	–	–	–
Rhodotorula rubra (Percentage of CFU)	–	–	–	–	–	–	–	–	–	–
TOTAL	34	31	47	41	16	18	13	13	4	1

the processing locations, intermediate samples were taken at 4 (4MC) and 8 (8MC) months in cellar.

Samples of approximately 10 g were taken aseptically from 25 cm^2, 1–4mm depth, *Gracilis* muscle, homogenized with 90 ml of peptone water (0.1%); serial dilutions were made in peptone water, 1ml fractions were plated on Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Oxoid, Unipath, Basingstoke, UK) and Dichloran-Glycerol Agar (DG18, Oxoid) that were incubated at 25°C for 5 days. All counts given are the average values from three hams.

2.3. Identification of isolates

Twenty per cent of the colonies randomly selected from the plates of both culture media were subcultured on Nutrient Agar (Difco, Detroit, MI, USA). Yeast colonies on Nutrient Agar were identified by the following tests proposed by Deák and Beuchat (1987): cellular morphology in Yeast Morphology Agar, formation of surface pellicle in liquid medium, urease activity on urea agar, glucose fermentation, nitrate assimilation in Yeast Carbon Base (Difco) and assimilation of D-cellobiose, erythritol, D-galactose, D-glucose, maltose, D-mannitol, raffinose, D-trehalose and D-xylose in Yeast Nitrogen Base (Difco). For further typing, selected isolates were characterized by the assimilation of L-arabinose, citric acid, inositol, lactose, L-rhamnose, ribitol, D-ribose, soluble starch, succinic acid and sucrose (Kreger-Van Rij, 1984). All incubations were performed at 25°C for 7 days.

2.4. Statistical analysis

Statistical analysis of data was carried out using the Statgraphics software package from Statistical Graphics Corp. (Rockville, MD, USA).

3. Results and discussion

The environmental conditions of ripening (Figs. 1 and 2) resulted in a pronounced reduction of moisture and a_w during the initial months. The average values of moisture, 18.5–25.9%, w/w, and a_w, 0.82–0.88 (results not shown), at the end of the drying stage were similar to other types of dry cured hams (Langlois et al., 1982; Huerta et al., 1988; Silla et al., 1989).

The relatively high counts (10^4 CFU/g) obtained for fresh ham (Table 1) can be explained by the exposure to freezing and thawing (Kemp et al., 1982). Maximum counts (close to 10^7 CFU/g) were reached at post-salting except at location III, where maximum numbers were reached at drying. The reason for these results at location III could be the higher pH observed during drying. Counts in the two culture media used were similar for most samplings, except for those of drying at location I. In this case counts in DG18 were 3 logarithmic units higher than those in DRBC.

Counts reported here are higher (ca. 2 log units) than those in other types of dry-cured ham, such as the American country-style hams (Langlois et al., 1982; Leak et

Table 2
Morphological and assimilation pattern determined by preliminary tests and identifications of yeasts found in Iberian ham

Pattern [a]	PSH	MAL	CEL	RAF	GAL	ERI	MAN	TRE	XIL	Species
L1	+	−	−	−	−	−	+	−	−	*Candida zeylanoides*
L2	+	−	−	−	−	−	+	+	−	*Candida zeylanoides*
L3	+	−	−	−	+	−	+	+	−	*Candida zeylanoides*
L4	+	−	−	+	−	−	+	+	−	*Candida zeylanoides*
L5	+	+	+	+	+	−	+	+	−	*Candida intermedia*
L6	+	+	+	+	+	−	+	+	+	*Pichia carsonii*
L7	+	+	+	+	+	+	+	+	+	*Candida blankii*
L8	−	+	−	+	−	+	−	+	−	Non identified
L9	−	+	−	−	+	+	−	−	+	Non identified
L10	−	+	−	+	+	−	+	+	+	*Rhodotorula rubra*
L11	−	−	+	−	+	+	+	+	+	*Debaryomyces hansenii*
L12	−	+	−	−	−	+	−	+	−	*Debaryomyces hansenii*
L13	−	+	−	−	+	+	+	+	−	*Debaryomyces hansenii*
L14	−	+	−	+	+	−	+	+	+	*Debaryomyces hansenii*
L15	−	+	−	+	+	+	+	+	+	*Debaryomyces hansenii*
L16	−	+	+	−	+	+	+	+	+	*Debaryomyces hansenii*
L17	−	+	+	+	+	−	+	−	+	*Debaryomyces hansenii*
L18	−	+	+	+	+	−	+	+	−	*Debaryomyces hansenii*
L19	−	+	+	+	+	−	+	+	+	*Debaryomyces hansenii*
L20	−	+	+	+	+	+	+	−	+	*Debaryomyces hansenii*
L21	−	+	+	+	+	+	+	+	−	*Debaryomyces hansenii*
L22	−	+	+	+	+	+	+	+	+	*Debaryomyces hansenii*
L23	−	+	+	+	+	+	+	−	−	*Debaryomyces hansenii*

[a] PSH, formation of pseudohyphae; and assimilation of: MAL, maltose; CEL, cellobiose; RAF, raffinose; GAL, galactose; ERY, erythritol; MAN, D-mannitol; TRE, trehalose; XYL, D-xylose.

al., 1987) and Spanish hams (Huerta et al., 1988; Silla et al., 1989) hams. On the other hand, our results are closer to those found in Iberian ham during the cellar stage (Monte et al., 1986).

The 836 yeast isolates examined were non-fermentative and non-nitrate assimilating organisms. According to the identification tests, they were grouped in 23 fenotype patterns as shown in Table 2. The profile of the yeast population greatly changed with the stage of processing (Table 1). *Candida zeylanoides* was the main species at fresh stage (more than 90% of isolates), but less than 20% of isolates at post-salting (results not shown). It was still recovered at drying. However, during the cellar stage, *C. zeylanoides* was isolated only from location II, where no significant reduction in a_w took place after post-salting (results not shown). Similarly, *Rhodotorula rubra* was not isolated after post-salting. Other yeasts, such as *Candida blankii*, *Candida intermedia* and *Pichia carsonii* were occasionally recovered from early stages. *Debaryomyces hansenii* dominated the yeast population after post-salting. At least 99% of isolates from the cellar sampling were identified as *D. hansenii*.

The two culture media used (DRBC and DG18) showed different efficiency to recover the various types of yeasts. *C. intermedia*, *C. blankii* and *R. rubra*, were

Table 3
Some characteristics and source of the main biotypes of yeasts obtained from the surface of Iberian ham

		Characterization tests [a]								Isolated from [b]					
Pattern	Biotypes	SUC	LAC	ARA	RIB	RHA	RIL	SCC	CIT	F	PS	D	4MC	8MC	I6MC
L2	Cz3	−	−	−	−	−	−	+	−		II,III [c]				
	Cz4	−	−	−	−	−	−	+	+		II,III				
	Cz7	+	−	−	−	−	−	+	−	I	II	II			II
	Cz8	+	−	−	−	−	−	+	+	I	II				
L18	Dh18	+	+	+	−	+	+	−	−		III		I		
L19	Dh19	+	−	+	−	−	+	−	−			I		I	
	Dh2O	+	−	+	−	−	+	+	−					I	II
	Dh21	+	−	+	−	+	+	−	−		II,III	III			
	Dh22	+	−	+	−	+	+	+	−			II		I	II
	Dh23	+	−	+	+	+	+	−	−			II			III
	Dh24	+	+	+	−	+	+	−	−		III	III			
	Dh25	+	+	+	−	+	+	+	−		III		I		
L22	Dh34	+	−	+	−	−	+	−	−		III				I
	Dh35	+	−	+	−	−	+	+	−			I			I
	Dh36	+	−	+	−	+	+	−	−		I,III	III			
	Dh37	+	−	+	−	+	+	+	−		II,III	I,II,III		I	I,II
	Dh39	+	−	+	+	−	+	+	−			I		I	
	Dh41	+	−	+	+	+	+	−	−		II	II,III		I	I, II, III
	Dh42	+	−	+	+	+	+	+	−			II I		I	
	Dh46	+	+	+	−	+	+	−	−		III				I

[a] Characteristics studied: assimilation of SUC, sucrose; LAC, lactose; ARA, L-arabinose; RIB, D-ribose; RHA, L-rhamnose; RIL, Ribitol; SCC, succinic acid; CIT, citric acid.
[b] F, fresh stage; PS, end of post-salting; D, end of drying; 4MC, 4 months in cellar; 8MC, 8 months in cellar; 16MC, final product.
[c] I, II, III: locations of ripening.

isolated only from one of the media (Table 1). On the other hand, *D. hansenii* was not isolated from DRBC at end of drying in location I, whilst 99% of isolates from DG18 were characterized as *D. hansenii*. The reason could be that the osmotic shock by exposure to high a_w levels in DRBC could damage the yeasts (Beuchat, 1983).

The complementary characterization tests of 191 representative strains revealed a remarkable diversity within some of the 23 fenotype patterns. Therefore, some of the fenotype patterns were further divided into biotypes, according to all the physiological properties tested. Seventy five different biotypes were obtained, including 12 *C. zeylanoides* and 52 *D. hansenii*. The strains isolated differed throughout the ripening process, which is particularly obvious for *D. hansenii*. Some 13 biotypes of this organism were detected during a period of 4 months in the cellar at location I. This can be related to the yeast population in the fat used to cover the surface of hams before taking them to cellar. Biotypes Dh19, Dh35 and Dh39 were found only in samples from location I, whilst Dh24 and Cz7 were only found at location III and II, respectively (Table 3). Therefore, all these biotypes can be associated to the location of ripening. In another investigation only *Debaryomyces marama* was isolated from 40 hams processed in a location different to the ones reported here (Monte et al., 1986). Biotypes Dh37 and

Dh41 were isolated at different sampling and from the three locations, increasing their proportion with the time of ripening. Therefore, the ratio of these biotypes to other strains could be used as an indicator of the ripening time.

In conclusion, the great diversity of yeasts found, together with the selective pressure of the ecological factors during ripening, makes the detection of certain yeasts useful to evaluate the stages of maturation and the effects of environmental conditions.

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