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Study of the yeasts during the ripening process of Armada cheeses from raw goat's milk

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Abstract — The counts, species and characteristics of yeasts were studied throughout the manufacturing and ripening of four batches of Armada cheese, a craft variety made in the north of Spain from raw goat's milk. Counts in Oxytetracycline Glucose Yeast-Extract Agar ($1.82 \times 10^4 - 1.15 \times 10^5$ cfu·g^{-1} in milk) increased one log. unit in curd and another log. unit during the first week of ripening, remaining stable in two- and four-week-old cheeses and decreasing from this point to the end of the ripening process. The yeast percentage of the isolates carried out remained practically constant at around 75 % throughout the ripening process, dropping to 27 % at the last sampling point (16-week-old cheese). Of the 181 isolated yeast strains, 60 were identified as *Geotrichum candidum*, 58 as *Candida lambica*, 11 as *Candida krusei*, 4 as *Candida lipolytica*, 2 as *Candida ingens*, 1 as *Candida catenulata*, 1 as *Candida pintolopesii*, 1 as *Candida colliculosa*, 19 as *Kluyveromyces lactis*, 11 as *Kluyveromyces marxianus*, 10 as *Saccharomyces unisporus* and 3 as *Trichosporon beigelii*. *G. candidum* predominated in 1- and 2-week-old cheeses and *C. lambica* was the most abundant species in milk and in 4- and 8-week-old cheese while *C. krusei* dominated in 16-week-old cheese. Judging from their metabolic characteristics, the yeasts in Armada cheese seem to participate in the initial acidification of the curd and in the consumption of lactic acid which takes place throughout the ripening process, though, with the exception of *G. candidum*, they do not seem to play a relevant role in the lipolysis, either way its resistance to NaCl is not very high. However, they seem to play an important role in the degradation of the biogenic amines; of the 181 strains identified, 136 assimilated the four biogenic amines tested (cadaverine, histamine, putrescine and tyramine) and all the strains assimilated at least one of these amines. © Inra/Elsevier, Paris.

Armada cheese/ goat cheese/ yeast species/ yeast count / technological property

Résumé — Étude des levures pendant la fabrication et l'affinage des fromages de chèvre au lait cru de la variété Armada. L'étude a porté sur le dénombrement, l'identification et quelques carac-

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téristiques technologiques des levures au cours de l'élaboration et de l'affinage de quatre fabrications de fromage Armada, une variété artisanale élaborée au nord de l'Espagne à partir de lait cru de chèvre. Le nombre de microorganismes en milieu OGYEA ($1,82 \times 10^4 - 1,15 \times 10^5$ ufc·g^{-1} de lait) augmentait d'une unité logarithmique dans le caillé et d'une autre unité logarithmique pendant la première semaine de maturation, restait stable jusqu'à 4 semaines puis diminuait jusqu'à la fin du processus d'affinage. Le pourcentage de levures identifiées à chacun des stades d'échantillonnage représentait environ 75 % des isolements, excepté pour les fromages de 16 semaines (27 %). Des 181 souches de levures isolées, 60 étaient identifiées à *Geotrichum candidum*, 58 à *Candida lambica*, 11 à *Candida krusei*, 4 à *Candida lipolytica*, 2 à *Candida ingens*, 1 à *Candida catenulata*, 1 à *Candida pintolopesii*, 1 à *Candida colliculosa*, 19 à *Kluyveromyces lactis*, 11 à *Kluyveromyces marxianus*, 10 à *Saccharomyces unisporus* et 3 à *Trichosporon beigelii*. *G. candidum* était l'espèce majoritaire dans les fromages âgés de 1 et 2 semaines, *C. lambica* était l'espèce la plus abondante dans le lait et dans les fromages âgés de 4 et 8 semaines, tandis que *C. krusei* prédominait dans les fromages de 16 semaines. À en juger d'après leurs caractéristiques métaboliques, dans le fromage Armada les levures dont la résistance au sel n'est pas très élevée contribueraient à l'acidification initiale du caillé et à la consommation d'acide lactique tout au long du processus de maturation, mais, excepté *G. candidum*, elles interviendraient peu dans l'hydrolyse des lipides. En revanche, elles pourraient jouer un rôle important dans la dégradation des amines biogènes. Des 181 souches de levures identifiées, 136 assimilaient les quatre amines biogènes testées (cadaverine, histamine, putrescine et tiramine), et toutes les souches au moins l'une de ces amines. © Inra/Elsevier, Paris.

fromage Armada / fromage de chèvre / espèce de levure / nombre de levure / propriété technologique

1. INTRODUCTION

Armada cheese is a hard variety made in the north of Spain from raw goat's milk using artisanal procedures without addition of starter cultures, which means that the ripening process is performed only by the natural flora of the milk. This, together with the absence of any rigorous control of temperature and relative humidity during the ripening process, makes the final product very variable. This lack of uniformity limits its acceptance and distribution in the markets. The use of its own specific starters and the control of the ripening conditions would allow the making of a uniform product of constant quality capable of opening its way to markets both at home and abroad.

In order to elaborate an adequate starter culture for this cheese variety it is necessary to carry out an extensive microbiological study of the artisanal cheese, identifying the microbial species which are present and selecting those whose abundance and technological properties suggest an important intervention in the ripening process.

In previous works we have enumerated throughout the ripening process the microbial groups of technological interest identifying the lactic acid bacteria and enterococci [39] and we have also studied the microorganisms of the *Micrococcaceae* family [40]. The yeast and mould counts throughout the process (log cfu·g^{-1} about 5–6) [39] suggest that the microorganisms of this group could play an important role in the ripening of this cheese. This is why we have deemed it necessary to identify the mycoflora at species level and to study the main characteristics of the isolates throughout the ripening process.

The yeasts can have many functions throughout the ripening of the cheese. The yeasts which assimilate lactose contribute together with the lactic acid bacteria towards the drop in pH values which occur in the earlier stages of the ripening. The yeasts which also have fermentative ability, metab-

olize lactose to ethanol and CO_2 and from ethanol esters of fatty acids are originated, which are responsible for the development of flavour in certain types of cheese. Through the production of CO_2, the yeasts can also participate together with heterofermentative lactobacilli and leuconostoc on opening the curd. Some yeasts have proteolytic and lipolytic activities and can also play an important role in the development of the flavour in the cheese. Finally, other interesting activities of some yeasts are the influence on the elimination of biogenic amines and the ability to metabolize lactic acid, thus allowing for the stablishing of other microbial groups sensitive to acid pHs as micrococci.

Therefore, the yeasts actively partake in the transformations which occur during ripening as they have a great enzymatic potential and contribute to the development of flavour and texture [29]. They have also been the subject of many studies for being the most important contaminants in certain types of milk products in which low pH values offer a selective medium for their growth [34].

This article aims at identifying of yeast species present throughout the ripening process of Armada goat's cheese and the study of their possible role in the maturation.

2. MATERIALS AND METHODS

2.1. Cheese samples

Four batches of Armada cheese were elaborated by experienced cheesemakers according to the traditional methods as described by Tornadijo et al. [39]. From each batch, milk, curd, and 1-, 2-, 4-, 8- and 16-week-old cheese samples were taken. Each cheese sample was constituted of one whole cheese. Samples were transported to the laboratory under refrigeration (below 5 °C) and analyzed on arrival.

2.2. Microbiological analysis

Fifty g of each sample (after discarding the rind of the cheeses) were homogenized with 200 mL of a sterile solution of 2 % sodium citrate at 40–45 °C for 1 min in a Stomacher 400 Lab Blender (Seward Medical, London, England), thus making a 1/5 dilution. Consecutive decimal dilutions were prepared by mixing 10 mL of the previous dilution with 90 mL of 0.1 % sterile peptone water. One mL of each dilution was inoculated in duplicate in 20 mL of Oxytetracycline Glucose Yeast-Extract Agar (Oxoid, Unipath Ltd, Basingstoke, Hampshire, England) [28] and mixed before solidification. Plates were incubated for 5 d at 22 °C. After incubation, plates with 30 to 300 colonies were counted.

2.3. Isolation and identification of strains

From the count plates, 10 random colonies were taken from each sampling point with the aid of a Harrison disc [20]. The 274 isolates obtained were purified and maintained in Sabouraud dextrose agar (Oxoid) tubes under refrigeration, covered with sterile parafin in order to reduce their metabolic activity and to avoid the agar dehydration. Of the 274 isolates carried out throughout the manufacturing and ripening process, 188 were considered yeasts and were identified according to the methods and criteria proposed by Lodder [24] and Kreger-Van Rij [23]. The nomenclature of the species was made according to Barnett et al. [1]. Of each isolate the following characteristics were studied.

2.3.1. Morphological characteristics

Morphology of the cells and type of vegetative reproduction, presence of pseudomycelium and/or true mycelium, production of ballistospores, and arthroconidia formation.

2.3.2. Cultural characteristics

Growth on solid medium (2 % Glucose-yeast-peptone agar after 2–3 d of incubation at 25 °C). The aspect of the colonies (smooth, rough, level, dome shape, mucous), the colour and the pigment production were observed. Growth on liquid medium (2 % Glucose-yeast-peptone broth after 2–3 d of incubation at 25 °C). The film formation, aspect of the film, ring formation and sediment formation were observed.

2.3.3. Sexual characteristics

Asci and ascospores formation and interfertility of the yeasts. The presporulation media yeast malt broth and yeast malt agar were used, incubating at 25 °C for 1 or 2 d. From the cultures growth in the presporulation media, cultures in different sporulation media were made (the following media were tested before confirming that they did not produce ascospores: Gorodkowa agar, McClary's acetate agar, Kleyn's acetate agar, Vegetable juice agar, Corn meal agar, Potato glucose agar, and Starkey's ethanol agar), incubating at 25–28 °C and at 20 °C and observing the production of ascospores at 3, 7, 14, and 21 d of incubation. In order to investigate the interfertility of the yeasts and to prove that it did not deal with haploid types of heterothallic species, the strains studied which did not produce any ascospores in the previously mentioned media were used. To do so, strains were taken four by four (strains of a same previously identified species) and were inoculated in the aforementioned sporulation media in order to observe if there was ascospores formation.

2.3.4. Biochemical characteristics

Assimilation of glucose, lactose, maltose, raffinose, cellobiose, galactose, sucrose, trehalose, ribitol, L-sorbose, D-xylose, L-arabinose and inositol; fermentation of glucose, lactose, galactose, sucrose, maltose and melibiose; nitrate assimilation; urease. In order for a better identification, in some strains the growth in a medium without vitamins, and in presence of 100 ppm of cycloheximide were studied.

2.4. Study of some properties of technological interest

According to the methods described by Besançon et al. [3], on each isolate the following technological properties were studied: growth in 5, 10, 15 and 20 % of salt; assimilation of lactose at 0.5 %; assimilation of lactic acid at 0.5 %; assimilation of tributyrin; assimilation of cadaverine, histamine, putrescine and tyramine.

3. RESULTS

Table I shows the evolution of the counts in OGYE agar during the manufacturing and ripening, and the percentage of yeasts at the different sampling times. Counts in OGYE agar increased one log unit in curd and another log unit during the first week of ripening, remaining stable in cheese of two and four weeks of ripening. From this point onwards, counts decreased until the end of ripening. The percentage of yeasts in the isolates carried out remained fairly constant throughout the process, only falling at the last sampling time (16-week-old cheese).

Of the 188 isolated yeast strains, 60 were identified as *Geotrichum candidum* (*Galactomyces geotrichum*), 58 as *Candida lambica* (*Pichia fermentans*), 11 as *C. krusei* (*Issatchenkia orientalis*), 4 as *C. lipolytica* (*Yarrowia lipolytica*), 2 as *C. ingens* (*Dipodascus ingens*), 1 as *C. catenulata*, 1 as *C. pintolopesii* (*Arxiozyma telluris*), 1 as *C. colliculosa* (*Torulaspora delbrueckii*), 19 as *Kluyveromyces lactis*, 11 as *K. marxianus*, 10 as *Saccharomyces unisporus* and 3 as *Trichosporon beigelii*. Seven strains were not identified at species level in a reliable way. *Table II* shows the distribution of the isolated species in the sampling points. *G. candidum* (32 % of the isolated species) dominated in 1- and 2-week-old cheese. Among the typical yeasts, a clear predominance of anascosporogenous ones was observed; *C. lambica* (31 % of the isolated species) was the predominant species in milk and in 4- and 8-week-old cheese, being *C. krusei* dominant in 16-week-old cheese. Of the sporogenous yeasts, *K. lactis*, *K. marxianus* and *S. unisporus* predominated.

Table III shows the assimilation and the fermentation of tested sugars by the isolated species. *Table IV* shows the morphological, cultural and sexual characteristics of the strains. The properties of technological interest of these strains are shown in *table V*.

Table I. Changes in counts in OGYE agar ($\log_{10}$ cfu·g^{-1}) and in numbers and percentages of isolated strains identified as yeasts during manufacturing and ripening of Armada cheese (four batches).

Tableau I. Évolution des dénombrements ($\log_{10}$ ufc·g^{-1}) dans le milieu OGYEA et des nombres et des pourcentages de souches identifiées comme levures durant la fabrication et l'affinage du fromage Armada (quatre fabrications).

	Milk	Curd	Ripening time (weeks)				
			1	2	4	8	16
Counts in OGYE agar*	4.62 ± 0.33	5.40 ± 0.50	6.69 ± 0.18	6.40 ± 0.32	6.40 ± 0.54	5.66 ± 0.23	4.26 ± 1.84
No. of strains identified as yeasts	30	25	35	30	34	25	9
% of isolated strains identified as yeasts	75.0	62.5	87.5	75.0	85.0	62.5	27

* Mean ± standard deviation of four batches.
* Valeurs moyennes ± écart type des quatre fabrications.

4. DISCUSSION

Counts of moulds and yeasts throughout the ripening process of Armada cheese were similar to those observed in Valdeteja goat milk cheese [19]. However, they were higher than those observed in other Spanish goat milk cheese varieties as Majorero [12], Ibores [26], and Cendrat del Montsec [27].

According to our data, the oxytetracycline is not effective against bacteria at 100 %. Of the 274 strains isolated from OGYE agar during the manufacturing and ripening of Armada cheese, 188 were identified as yeasts, 30 as moulds, and 56 were bacteria. The moulds and yeasts represented only 80 % of the total strains isolated.

Geotrichum candidum was one of the main yeast species during the manufacturing and ripening of Armada cheese, being dominant in the earliest stages of the ripening. The majority of the strains of *G. candidum* isolated from Armada cheese and from the milk used in its making morphologically belong to the type 1 described by Guéguen and Jacquet [17], only 9 strains could be considered somewhat closer to type 2 of Guéguen and Jacquet as they showed a whitish and dusty aspect. *G. candidum* is present on the surface of many cheeses [16] where the high levels of fatty acids found are mainly due to the action of their enzymes. This species can exert a protection against the development of undesirable moulds of the *Mucor* genus [6, 9, 18].

In our study we isolated *Candida lambica* at high proportions in milk, curd and cheese at different stages of ripening which indicates that it can easily adapt to the Aw, pH and salt concentration conditions found in this cheese and it is highly competitive. Baroiller and Schmidt [2] isolated anascosporogenous yeasts at high levels from agricultural exploitations (silages, water, soil, and so on) with *C. lambica* being particularly important. However, these authors did not isolate this species in milk not even during the ripening of the cheeses.

Table II. Changes in yeast species isolated during the manufacturing and ripening of Armada cheese (four batches).
Tableau II. Distribution des espèces de levures isolées durant la fabrication et l'affinage du fromage Armada (quatre fabrications).

Species	Ripening time (weeks)													
	Milk		Curd		1		2		4		8		16	
	No. of isolates	(%)*	No. of isolates	(%)*	No. of isolates	(%)*	No. of isolates	(%)*	No. of isolates	(%)*	No. of isolates	(%)*	No. of isolates	(%)*
Geotrichum candidum	11	27.5	7	17.5	14	35.0	13	32.5	12	30.0	2	5.0	1	3.0
Candida lambica	12	30.0	7	17.5	3	7.5	8	20.0	15	37.5	12	30.0	1	3.0
Candida krusei	–	–	1	2.5	1	2.5	1	2.5	–	–	1	2.5	7	21.0
Candida catenulata	–	–	1	2.5	–	–	–	–	–	–	–	–	–	–
Candida ingens	–	–	–	–	–	–	–	–	–	–	2	5.0	–	–
Candida lipolytica	–	–	2	5.0	1	2.5	–	–	–	–	1	2.5	–	–
Candida pintolopesii	1	2.5	–	–	–	–	–	–	–	–	–	–	–	–
Candida colliculosa	–	–	–	–	–	–	–	–	–	–	1	2.5	–	–
Kluyveromyces marxianus	1	2.5	1	2.5	5	12.5	2	5.0	2	5.0	–	–	–	–
Kluyveromyces lactis	–	–	2	5.0	10	25.0	3	7.5	2	5.0	2	5.0	–	–
Saccharomyces unisporus	–	–	1	2.5	–	–	3	7.5	3	7.5	3	7.5	–	–
Trichosporon beigelii	1	2.5	2	5.0	–	–	–	–	–	–	–	–	–	–
Unidentified yeasts	4	10.0	1	2.5	1	2.5	–	–	–	–	1	2.5	–	–
TOTAL	30	75	25	62.5	35	87.5	30	75.0	34	85.0	25	62.5	9	27.0

* Percentage of the total isolates carried out at this sampling point.
* Pourcentage par rapport à l'ensemble des souches prélevées à ce stade d'échantillonnage.

Table III. Biochemical characteristics of yeast strains isolated from Armada cheese during manufacturing and ripening (four batches).

Tableau III. Caractères biochimiques des souches de levures présentes au cours de la fabrication et de l'affinage du fromage Armada (quatre fabrications).

	Geotrichum candidum	*Candida lambica*	*Candida krusei*	*Candida lipolytica*	*Candida ingens*	*Candida catenulata*	*Candida pintolopesii*	*Candida colliculosa*	*Kluyveromyces lactis*	*Kluyveromyces marxianus*	*Saccharomyces unisporus*	*Trichosporon beigelii*
Number of isolates	60	58	11	4	2	1	1	1	19	11	10	3
Assimilation of:												
Glucose	60	58	11	4	2	1	1	1	19	11	10	3
Lactose	0	0	0	0	0	0	0	0	19	11	0	3
Galactose	60	0	0	0	2	1	0	1	19	6	10	3
Maltose	0	0	0	0	0	0	0	0	16	0	1	3
L-sorbose	60	0	0	0	2	0	0	0	15	2	0	0
Trehalose	0	0	0	0	0	0	0	1	9	0	0	3
D-xylose	60	58	0	0	0	0	1	0	1	0	0	2
Cellobiose	0	0	0	0	0	0	0	0	2	0	1	2
L-arabinose	0	0	0	0	0	0	0	0	1	0	0	0
Sucrose	0	0	0	0	0	0	0	1	18	11	0	3
Raffinose	0	0	0	0	0	0	0	1	17	11	1	2
Ribitol	0	0	0	0	0	0	0	0	1	0	0	0
Nitrate	0	0	0	0	0	0	0	0	0	0	0	0
L-lysine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	10	N.D.
Fermentation of:												
Glucose	0	58	11	0	0	1	1	1	19	11	10	0
Galactose	0	0	0	0	0	1	0	1	19	0	10	0
Maltose	0	0	0	0	0	1	0	0	19	0	0	0
Sucrose	0	0	0	0	0	0	0	1	18	11	0	0
Melibiose	0	0	0	0	0	0	0	0	0	0	0	0
Lactose	0	0	0	0	0	0	0	0	19	11	0	0
Urease production	0	0	0	4	0	0	0	0	0	0	0	2

The numbers included in the table correspond to the number of positive isolates in each test. N.D.: not determined.
Les chiffres du tableau indiquent le nombre de souches positives pour chaque test. N.D. : non determiné.

Table IV. Morphological, cultural and sexual characteristics of yeast strains isolated from Armada cheese during manufacturing and ripening (four batches).

Tableau IV. Caractères morphologiques, culturaux et sexuels des souches de levures présentes au cours de la fabrication et de l'affinage du fromage Armada (quatre fabrications).

	Geotrichum candidum	*Candida lambica*	*Candida krusei*	*Candida lipolytica*	*Candida ingens*	*Candida catenulata*	*Candida pintolopesii*	*Candida colliculosa*	*Kluyveromyces lactis*	*Kluyveromyces marxianus*	*Saccharomyces unisporus*	*Trichosporon beigelii*
Number of isolates	60	58	11	4	2	1	1	1	19	11	10	3
Multipolar budding	–	58	11	4	2	1	1	1	19	11	10	0
Superficial ring	–	58	11	4	2	0	1	0	1	0	0	3
Soluble sediment	–	0	0	0	0	1	0	0	6	11	10	0
Clotted sediment	–	0	0	0	0	0	0	1	12	0	0	0
Ascospores	0	0	0	0	0	0	0	0	19	11	10	0
Conjugant cells	–	0	0	0	0	0	0	0	0	0	0	0
Ascospores which mature outside	–	0	0	0	0	0	0	0	0	0	0	0
Pseudomycelium	0	58	11	0	2	1	1	0	0	0	0	0
True mycelium	60	0	0	0	0	0	0	0	0	0	0	3
Growth at 20–25 °C	60	58	11	N.D.	N.D.	1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

The numbers included in the table correspond to the number of positive isolates in each test. N.D.: not determined.
Les chiffres du tableau indiquent le nombre de souches positives pour chaque test. N.D. : non determiné.

Table V. Technological characteristics of yeast strains isolated from Armada cheese during manufacturing and ripening (four batches).

Tableau V. Caractères technologiques des souches de levures présentes au cours de la fabrication et de l'affinage du fromage Armada (quatre fabrications).

	Geotrichum candidum	*Candida lambica*	*Candida krusei*	*Candida lipolytica*	*Candida ingens*	*Candida catenulata*	*Candida pintolopesii*	*Candida colliculosa*	*Kluyveromyces lactis*	*Kluyveromyces marxianus*	*Saccharomyces unisporus*	*Trichosporon beigelii*
Number of isolates	60	58	11	4	2	1	1	1	19	11	10	3
Growth at 5 % NaCl	60	58	11	4	2	1	1	1	19	11	10	3
Growth at 10 % NaCl	0	8	11	4	0	1	0	1	18	11	6	0
Growth at 15 % NaCl	0	0	2	0	0	0	0	0	12	4	0	0
Growth at 20 % NaCl	0	0	0	0	0	0	0	0	0	0	0	0
Tributyrin hydrolysis	60*	0	0	4	2	0	0	0	0	0	0	3
Assimilation of:												
Lactate at 0.5 %	60	58	11	4	0	1	1	0	18	11	0	0
Lactose at 0.5 %	0	0	0	0	0	0	0	0	17	11	0	3
Cadaverine	60	58	11	4	2	1	1	0	13	9	1	3
Histamine	60	58	11	4	2	1	1	0	18	11	4	3
Putrescine	60	58	11	4	2	1	1	0	18	11	0	3
Tyramine	60	58	11	4	2	0	1	1	4	5	10	3

The numbers included in the table correspond to the number of positive isolates in each test. * Weak hydrolysis.
Les chiffres du tableau indiquent le nombre de souches positives pour chaque test. * hydrolyse légère.

They regarded it as contaminant flora, which in cheese is seen as being displaced by other species better adapted to this food, such as *K. lactis* and its asexual form *Candida sphaerica*. In the evolution of *C. lambica* throughout the ripening process of Armada cheese only a slight drop in one- and two-week-old cheese was observed where *G. candidum* predominated, and in sixteen-week-old cheese where it is hardly present and is surpassed by *C. krusei*.

It is unusual to isolate *C. lambica* as the main yeast species from cheese, although it is usually found in not quite negligible percentages in some studies [7, 37]. *C. lambica* was also isolated in French goat's cheeses such as Crottin de Chavignol and Pouligny Saint-Pierre [29], although at not important levels. Moreover *C. lambica* is the imperfect state of *Pichia fermentans*, which was found in butter milk, kefyr and cheese [1]. In addition, in the review by Fleet [11] the perfect state *P. fermentans* is reported as a frequently found species in dairy products.

Other species of the genus *Candida* isolated in Armada cheese were *C. lipolytica*, *C. krusei*, *C. ingens*, *C. catenulata*, *C. pintolopesii* and *C. colliculosa*. *C. krusei* was also isolated by Sala Trepat and Burgos [35] in Cabrales cheese, by Deiana et al. [8] in goat's cheese from different places in Sardinia and by Cooke and Brazis [5] from raw milk, pasteurized milk and different milk products, cheese being included. Núñez et al. [32] also described this species (*Candida krusei* was previously denominated *Pichia membranaefaciens*) as being dominant in the inner of Cabrales cheese during ripening. *C. catenulata* was frequently observed in Pont-L'Evêque cheeses, representing 24 % of the isolates in this cheese varietie [4]; this species was also isolated by Nooitgedagt and Hargot [31] from Brie and Camembert cheeses. *C. pintolopesii* was also identified by Nahabieh and Schmidt [29] from goat's cheese at a percentage of 15 % of the total isolates, nevertheless these authors point out its presence to be unusual. *C. lipolytica* can play an important role in the aroma development of the cheese increasing the volatile fatty acid content [10, 33], its presence in different types of cheeses has been widely demonstrated [10, 25, 30]; Nahabieh and Schmidt [29] isolated this species in French goat's cheeses in its sexual and asexual forms in proportions of 13 % of isolations, being the dominant species in Crottin de Chavignol cheese throughout the ripening process.

K. lactis was isolated in important percentages in one-week-old cheese, representing approximately 28 % of the isolated yeasts at this sampling point. This species approximately made up 10 % of all isolated yeasts in Armada cheese. *K. marxianus* represented 6 % of all isolated yeasts in Armada cheese, with its presence being outstanding in one-week-old cheese. The importance of *K. lactis* and *K. marxianus* in dairy products, because they are able to metabolize lactose, is well known. The reported data confirm that point. Some authors [10] have attributed to *K. lactis* the ability to act on *Penicillium roqueforti* by stimulating the production of metilketones. Georgantas [15] also attributes it a stimulating effect on the production of acid through lactococci.

Saccharomyces unisporus was the only isolated species of the *Saccharomyces* genus and constituted 5 % of all the identified yeasts from Armada cheese. This species was also identified by Núñez et al. [32] from Cabrales cheese, making up 3 % of the isolates obtained in this cheese.

Trichosporon beigelii is a species which always comes from contaminations. In our study it represented 1.5 % of isolates carried out. Núñez et al. [32] isolated this species in milk destined for the making of Cabrales cheese. Nahabieh and Schmidt [29] isolated it in very low proportions, similar to ours, in goat's cheese. In some cheeses, however, it makes up the main species; in Kopanisti cheese, for example, Kaminarides and Anifantakis [22] identified as *T. beigelii* 61 of the 86 yeast strains isolated.

With regard to the biochemical and technological characteristics of the yeasts isolated from Armada cheese, only the *K. lactis* and *K. marxianus* strains were able to assimilate and ferment lactose which in agreement with Cooke and Brazis [5] leads us to conclude that the most part of the species we isolated are casual contaminants and not 'selfsame flora' of cheese and milk. The *K. lactis* and *K. marxianus* strains possibly contribute, together with the lactic acid bacteria, to the degradation of lactose which takes place in the early stages of the ripening process of this cheese [13]. The 90 % of the strains identified (the *G. candidum*, *K. lactis*, *K. marxianus*, *C. lambica*, *C. krusei*, *C. lipolytica*, *C. catenulata* and *C. pintolopesii* strains) showed the ability to assimilate lactic acid. These species could actively participate in the consumption of lactic acid and be responsible for the fall in D and L lactic acid contents observed in Armada cheese from the first week of ripening [13].

G. candidum seems to play a role in the lipolysis in Armada cheese. According to Sidebottom et al. [38] *G. candidum* produces 2 lipases: lipase A (not specific) and lipase B (specific for esters of cis-△-9 fatty acids) with a preference for $C_{18:1}$ (oleic acid) [21], although it also frees to a lower extent saturated fatty acids. In view to the profile of free fatty acids observed in Armada cheese throughout ripening [14], *G. candidum* seems to play an important role in the degradation of the lipids in the first stages of ripening. In this cheese, the ratio of the main free fatty acids $C_{16}/C_{18:1}$ is very low (0.5) and favourable to $C_{18:1}$ in the first weeks of ripening when *G. candidum* predominates, which agrees with the lipase B activity of *G. candidum*. This ratio increases up to 0.85 as *G. candidum* decreases from the second month of ripening.

The rest of yeasts from Armada cheese do not seem to take part in the lipid hydrolysis which takes place throughout the ripening process of this cheese. Of all the typical yeast strains isolated, only 9 (4 from *C. lipolytica*, 2 from *C. ingens* and 3 from *T. beigelii*) were able of hydrolyzing the tributyrin.

Of the 181 strains identified, 13 (60 from *G. candidum*, 58 from *C. lambica*, 11 from *C. krusei*, 4 from *C. lipolytica*, 2 from *C. ingens* and 1 from *C. pintolopesii*) assimilated the four biogenic amines tested. All the identified strains assimilated at least one of these amines.

All the *G. candidum* strains isolated showed a good growth at 5 % NaCl. *G. candidum* has been known for a long time to be particularly sensitive to salt with a total inhibition at concentrations of 5–6 % [36]. In this sense these strains have an exceptional behaviour.

The isolated yeasts do not seem to be very resistant to salt and only a few of them are capable of growing at NaCl concentrations higher than 5 %. Only 12 strains of *K. lactis* and 4 of *K. marxianus* were able to grow at a 15 % NaCl concentration. The resistance to high salt concentrations is regarded as being a very important characteristic in choosing starter cultures for cheese making [3].

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