CHARACTERISATION AND TECHNOLOGICAL ASPECTS OF YEASTS ISOLATED FROM RAW MILK AND DIFFERENT TYPES OF CHEESES PRODUCED IN ARGENTINA

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Abstract

A total of 116 yeast strains were isolated from raw milks, whey of different cheeses manufactures and soft and hard argentinian cheeses. The different types of cheeses were sampled for yeasts isolation during all processing, before and after the ripening process and ready for marketing, and included both the inner part and the surfaces of the cheeses.

Yeasts were enumerated in cheeses samples after 1, 6, 10 and 15 days (soft cheeses), and 1, 3 and 6 months (hard cheeses) of ripening and in milks, whey, natural whey cultures and commercial lactic starter cultures at time of use for cheesemaking. The strains isolated were identified using standard conventional identification methods indicated by Kreger van Rij (1984) and Barnett et al. (2000). API tests were also applied for the identification.

In order to evaluate their potential incorporation at cheese starter cultures, some technological properties such as growth at different temperature and pH values, and tolerance to different concentrations of NaCl were tested.

The results obtained showed yeasts were present in all cheeses samples examined, at number ranging from 10 CFU/g to $4{,}5.10^5$ CFU/g of cheese, while the incidence levels in milks and whey were from 10 to 5.10^3 CFU/ml and from 10 to 2.10^4 CFU/ml respectively. In natural whey cultures and commercial lactic starters cultures yeasts were not detected.

Candida (50 %) and *Geotrichum* (50 %) prevailed in milks, *Candida* (42,2 %), *Cryptococcus* (21 %) and *Trichosporon* (36,8 %) were the genera found in whey, while *Candida* was the genus predominant in cheeses.

Less frequently isolated genera were *Brettanomyces* and *Schizosaccharomyces*.

Results from this study also showed association among species predominant in milks and that present in different cheese varieties. *Candida famata* was predominant in all raw milks and cheeses examined.

All studied strains were able to growth at different pH values and at 15 and 18°C, and some of them also exhibit psychrotrophic aptitude. All strains were also able to growth in the presence of 2,5 and 10% NaCl, while the ability to growth in presence of 15 and 20% NaCl was variable for each strain.

On the basis of these results and a further technological characterization, the selection of the yeast strains isolated from raw milks and cheeses for potential incorporation at cheese starter cultures is warranted.

Keywords: Yeasts; Raw milk; Dairy products; *Candida famata*

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1. Introduction

The yeasts represent an important part of the microflora of the surface-ripening cheeses (Fleet, 1990; Roostita and Fleet, 1996; Seiler and Busse, 1990 and Deàk, 1991). Yeasts, however, possess the ability to grow under conditions unfavorable to many bacteria in cheese and therefore play a significant role in the spoilage of dairy products as well as the ripening of some cheese varieties (Viljoen, 1998). The evolution of microbial population in the manufacture of cheese is dominated by lactic acid bacteria, but *Micrococcaceae, Enterobacteriaceae,* yeasts and moulds are also present (Coppola et al., 1990). The most important species of yeasts isolated from milks and cheeses reported by Corsetti et al. (2001), include those genera that can be considered as participating in the ripening, and they are: *Candida, Cryptococcus, Debaryomyces,* the yeast like *Geotrichum candidum* and its perfect form *Kluyveromyces, Pichia, Saccharomyces* and *Zygosaccharomyces.*

The ripening is characterized by succession largely undefined microbial communities on the surface of cheeses. At the beginning of the ripening process, yeasts metabolize the lactic acid produce by the lactic acid bacteria. In addition, the production of growth factors by the yeasts, such as vitamins, appears to promote growth and development of a salt tolerant bacterial microflora (Lenoir, 1984; Valdés-Stauber et al., 1997; Busse, 1989; Jones and Keddie, 1986; Hayashi et al., 1990; Jollivet et al., 1992; Besançon et al., 1992).

On the other hand, yeasts can also be responsible for defects in cheese (Rose and Harrison, 1970; Reed and Peppler, 1973). Excessive yeast growth will cause the rind to become soft and smeary, resulting in poor development of other members of the bacterial starters.

The aim of the current study was to characterize and identify the type of yeasts flora present in raw milks, cheese starters cultures, and argentinian soft and hard cheeses.

We will also report on the frequency of occurrence of yeasts associated with different types of cheeses manufactured and the results of the studies of some technological properties.

2. Materials and methods

2.1. Samples

Samples were obtained from different materials used in manufactures of cheeses (raw milk, natural whey cultures, commercial lactic starter cultures, whey and cheeses) from a Programa de Lactología Industrial (Fac. Ing. Química, Univ. Nac. Litoral). They were taken aseptically and immediately transported to the laboratory and kept at 4ºC until examined, within 24 hours after sampling.

2.2. Enumeration and isolation of yeasts

In order to follow the development of yeasts during maturation of different types of cheeses, samples from a particular batch were collected at 1, 6, 10 and 15 days for soft cheeses, and at 1, 3 and 6 months for hard cheeses. For enumeration of yeasts in raw milks, both types of starter cultures and whey, samples were collected at time of use for cheesmaking.

Yeasts were isolated from 10 ml or 10 g of sample. The samples of milk, whey and starter cultures were diluted in peptone water and the samples of cheeses were homogenized with sodium citrate 2% (w/v) in Stomacher Lab-Blender 400. Different dilutions were plated on YGCB agar (0,001 g bromophenol blue, 0,01 g cloramphenicol, 2 g glucose, 0,5 g yeast extract, 2 g NaCl, 1,5 g agar, 100 ml distillate water). Plates were incubated at 27ºC until fully developed. All samples were prepared and analyzed in duplicate. After incubation at 27ºC for 5 days, colonies were count and 5 to 10 colonies were randomly selected from YGCB plates. These colonies were purified on YM agar (0,3 g yeast extract, 0,3 g malt extract, 0,5 g casein peptone, 1 g glucose, 1,5 g agar, 100 ml distillate water) and stored on the same medium at 4ºC until their identification.

2.3. Yeast identification

The physiological and biochemical characterization of yeasts were investigated on media and under conditions described by Kreger-van Rij (1984) and the computerized identification system of Barnett et al. (2000). API 20 C AUX and API *Candida* (BioMèrieux, France) were also applied to complete the identification assays.

Each isolate was inoculated into six sugar fermentation media, 15 carbon source assimilation media and in vitamin-free medium. Additional test performed included growth at 37ºC, in 50% D-glucose medium, urea hydrolysis, splitting of arbutin, grown on 0.01% and 0.1% cycloheximide and staining of 4-week-old cultures with diazonium blue B salt reagent. Assimilation of nitrogen compounds, as performed by means of the auxanographic methods, was also included.

Ascospore formation was examined on Gorodkowa agar and malt extract agar. The inoculated media were incubated at 18ºC for 4 weeks and examined at 4-days intervals. Cell morphology and mode of reproduction were examined on malt extract agar. The formation of pseudomycelium and true mycelium was examined on cornmeal agar.

2.4. Yeast technological characterization

Growth assays under selected environmental conditions were carried out:

a) pH: growth at pH: 4,7 - 5,15 and 5,5 in YNB medium (0,67 % w/v) with glucose 0,5 % (Vivier et al., 1994) were studied. The cultures were incubated at 27ºC for 3 weeks.
b) Temperature: growth at temperatures of 5 - 10 and 18ºC were assayed in medium described by Vivier et al. (1994). The cultures were incubated for 3 weeks at the indicated temperatures.
c) NaCl: The ability of yeast isolates to growth in presence of different concentrations (2,5, 5, 10, 15, 20 % w/v) of NaCl was studied in medium described by Besançon et al. (1992). The cultures were incubated at 27ºC for 3 weeks.

3. Results and discussion

3.1 Evolution of yeast populations during cheese manufacture

The ability of yeasts to grow and compete with the lactic starter culture was evaluated in four cheesemaking. The evolution of yeasts during the ripening is shown in Figure 1 and 2 for soft and hard cheeses respectively.

Yeasts were always found and they were viable for a long period; however, a general decrease occurred during the ripening, specially in hard cheeses. The highest value ($4.5.10^5$ CFU/g) was found in soft cheeses. In these products the yeasts populations in general increased during the first day of production, and then they were maintain constant at the end of ripening.

As expected, in hard cheeses a high level of yeasts was detected from the first month of ripening, while the general trend observed for yeasts counts was a gradual decrease during the 3 months.

These results were in general coincident whit those obtained by other authors (FIL/IDF, 1998; Jakobsen and Narvhus, 1996); they also studied the yeasts possible role in cheese

ripening based on interactions between yeasts and bacterial starters cultures, proteolytic and lipolytic activities, aroma compounds formation and other metabolic activities.

The incidence levels of yeasts in raw milks were from 10 to 5.10^3 CFU/ml, and in assayed whey samples were from 10 to 2.10^4 CFU/ml.

In natural whey cultures and commercial lactic starters cultures yeasts were not detected.

3.2 Yeast genera and species identification

A total of 116 yeast strains were isolated from raw milk, whey and cheeses samples and identified accordingly. The results corresponding to taxonomical identification and genera and species distribution are presented in Table 1. *Candida* was the predominant species in all samples examined. *Candida* (50%) and *Geotrichum* (50%) were the genera present in raw milks. Strains of *Trichosporon* (36,8 %), *Candida* (42,2%) and *Cryptococcus* (21%) were found in the assayed whey samples.

Different species of *Candida* were largely predominant in soft and hard cheeses at different times of ripening, and another genera such us *Cryptococcus* and *Brettanomyces* also were found but with low incidence. The genera *Geotrichum* and *Schizosaccharomyces* were only present in lower percentages and in a limited number of cheese samples.

Candida famata was the predominant species in raw milk and cheese samples. The frequent occurrence of *C. famata* in dairy products has been reported by others authors (Devoyod and Sponem, 1970; Fleet and Mian, 1987; Seiler and Busse,1990; Kaminarides and Laskos, 1992) and is explained by its high tolerance to salt. The flora of the milk, starters and cheeses were rather similar; the occurrence of limited yeast species confirms that the cheese ecosystem is characterized by specific environmental conditions and composition, which promotes selection of a uniform and well-defined flora, at least for some technological characteristics (Viljoen, 2001; Fleet and Mian, 1987; Jakobsen and Narvhus, 1996).

3.3 Physiological and biochemical properties

Table 2 shows the capacity of yeast strains to growth on medium prepared at different pH (4,70 - 5,15 - 5,50), different concentration of NaCl (2,5 - 5 - 10 - 15 - 20%) and incubated at different temperatures (5 -15 - 18°C).

All the yeasts isolated in this study from dairy products growth at different assayed pH. In general, yeasts prefer a slightly acidic medium, with an optimum pH between 4,5 and 6,5. Despite the ability of yeasts to grow over a wide range of pH values, it must be remembered that the growth response is the result of other accompanying environmental factors (Deak, 1991; Fleet, 1990; Mossel and Ingraham, 1955; Deak and Beauchat, 1996). These factors include temperature, availability of water and nutrients, and the presence of other microorganisms and of course pH, which can have a profound effect on the growth of yeasts in dairy products.

Most yeasts tolerate the assayed range of temperature, and grow readily at values between 5ºC and 18ºC, although there are a considerable number of strains corresponding to all isolated genera (except *Schyzosaccharomyces*) which were restricted to growth at temperature values around 15ºC and 18ºC. The majority of yeasts live in different habitats, and their temperatures vary between 0ºC and 47ºC. Fleet (1990) reported that the species *Kluyveromyces marxianus*, *Candida diffluens* and *Saccharomyces cerevisiae* predominate at 20°C, while at 5ºC the cold tolerant yeasts species as Candida *diffluens*, *Candida famata* and *Cryptococcus albidus* exhibit the best growth in dairy products. Particular interest regarding the occurrence of yeasts in dairy products is that quite a member of yeasts are capable of growing at temperatures in the vicinity of 0ºC or even a few degrees below zero; for example, *Candida* and *Cryptococcus* species have optimum growth temperatures of less than 20ºC and survive at low temperatures (0 - 5ºC) (Davenport, 1980).

All the yeasts isolated in this study were grown in medium with 2,5 %, 5 % and 10 % of NaCl. Furthermore, a variable number of yeast strains can be able to growth with 15 % and 20 % of NaCl.

4. Conclusions

The results of our work shown that yeasts in high numbers are normally associated with raw milk and dairy products. *Candida* was found to dominate in all samples and it is assumed to make a significant contribution to the microbial ecology of cheese maturation according to the characteristics of the investigated isolates.

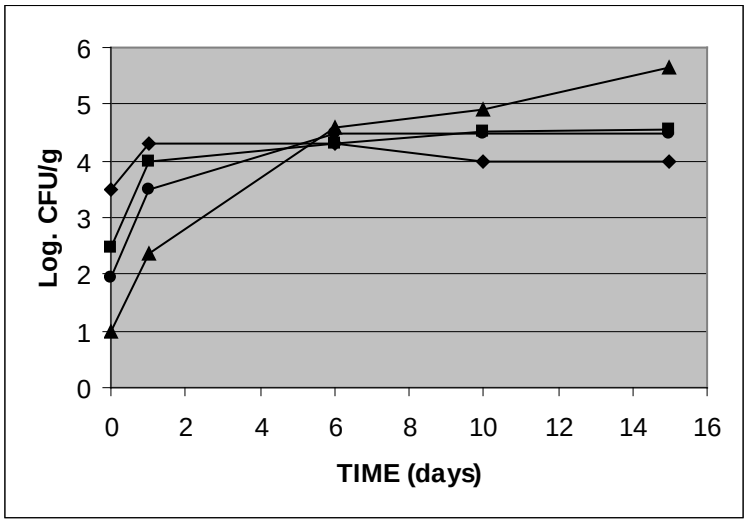
Yeasts can be assumed to be post-pasteurisation contaminants. Yeasts have a fundamental role in the ripening of surface-ripened cheeses. Further studies on the interactions between yeasts and lactic acid bacteria in the smear surface-ripened cheeses

are needed to optimise the application and the performances of such microorganisms in the dairy industry.

However, an excessive numbers of yeasts cells can cause holiness, bitterness and fruity flavours (Walker, 1988). Any cheese with levels of yeasts as high as 10^5 CFU/g is considered suspect with regard to off flavours. As yet, there is no proper explanation as to how these yeasts can be present in large numbers in one cheese and absent in another made under similar conditions. Perhaps it is the inherent variability of the cheese and the factory environmental which is responsible.

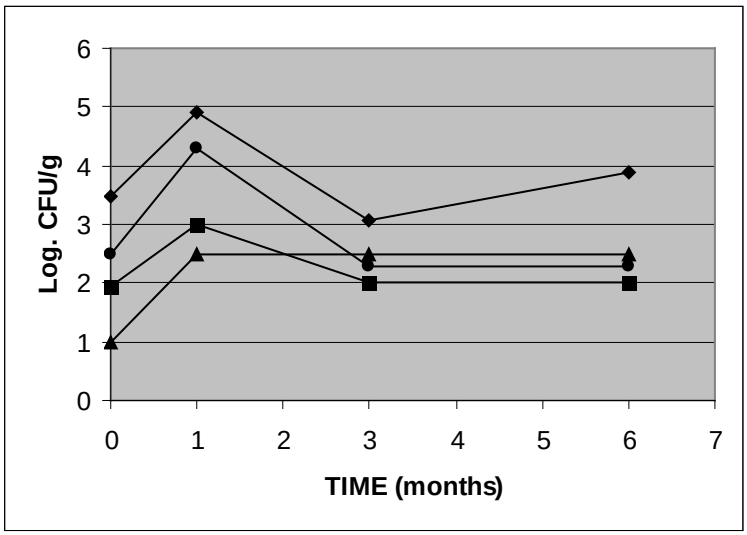
If it is necessary carried out complementary studies related with different aspects of yeast strains isolated from argentinian dairy products, specially a further technological characterization, the selection of strains isolated from raw milks and different types of cheeses for potential incorporation at cheese starter cultures is warranted.

Figure 1: Development of yeast population in soft cheese during maturation at 15ºC.



Date of manufacture: 06/15/02; 07/04/02; 08/01/02; • 08/09/02

Figure 2: Development of yeast population in hard cheese during maturation at 11ºC.



Date of manufacture: 06/15/02; 07/04/02; 08/01/02; • 08/09/02

Table 1: Percentile distribution of the isolates.

SAMPLES		GENUS	SPECIES	PARTTIALY %	TOTAL %
RAW MILK		*Candida*	*crusei*	25	50
			famata	25	
		Geotrichum	*sp.*	50	50
WHEY		*Candida*	*famata*	5,3	42,2
			parapsilosis	21,1	
			sp.	15,8	
		Cryptococcus	*albidus var. albidus*	10,5	21
			laurentii	10,5	
		Trichosporon	*cutaneum*	26,3	36,8
			sp.	10,5	
SOFT CHEESE (Ripening time:15 days)		*Brettanomyces*	*claussenii*	2,9	11,7
			custersii	2,9	
			intermedius	5,9	
		Candida	*famata*	53	61,8
			parapsilosis	5,9	
			tenuis	2,9	
		Cryptococcus	*albidus var. albidus*	2,9	23,6
			heveanensis	5,9	
			laurentii	14,8	
		Geotrichum	*sp.*	2,9	2,9
HARD CHEESE	Ripening time: 3 months	*Candida*	*famata*	85,7	100
			parapsilosis	14,3	
	Ripening time: 6 months	*Candida*	*famata*	45,5	81,9
			humicola	9,1	
			parapsilosis	27,3	
		Schizosaccharomyces	*japonicus*	18,1	18,1

Table 2: Technological characterization of the yeast strains.

GENUS	*SPECIES*	% positives strains						
		pH	Temperature (°C)			Sodium chloride (%)		
		4,7 - 5,5	5	15	18	2,5 - 10	15	20
Candida (74 strains)	*famata* (43 strains)	100	44	100	100	100	77	25.5
	crusei (1 strains)	100	0	100	100	100	100	0
	humicola (1 strain)	100	0	100	100	100	0	0
	parapsilosis (25 strains)	100	54	100	100	100	95	0
	tenuis (1 strain)	100	0	100	100	100	0	0
	sp. (3 strains)	100	33.3	100	100	100	100	100
Cryptococcus (12 strains)	*laurentii* (7strains)	100	100	100	100	100	100	28.6
	albidus var. *albidus* (3 strains)	100	100	100	100	100	66.6	66.6
	heveanensis (2 strains)	100	50	100	100	100	100	50
Trichosporon (9strains)	*cutaneum* (6 strains)	100	50	100	100	100	100	17
	sp. (3 strains)	100	100	100	100	100	100	100
Brettanomyces (4 strains)	*claussenii* (1 strain)	100	100	100	100	100	100	0
	custersii (1 strain)	100	0	100	100	100	100	100
	intermedius (2 strains)	100	50	100	100	100	50	50
Schizosaccharomyces (2 strains)	*japonicus* (2 strains)	100	100	100	100	100	100	100
Geotrichum (7 strains)	*penicilatum* (3 strains)	100	100	100	100	100	100	33.3
	sp. (4 strains)	100	0	100	100	100	50	0

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