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International Journal of
Food Microbiology 28 (1996) 393–404

International Journal of Food Microbiology

The occurrence and growth of yeasts in Camembert and Blue-veined cheeses

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Accepted 16 March 1995

Abstract

Yeast populations greater than 10^6cfu/g were found in approximately 54% and 36%, respectively in surface samples of retail Camembert (85 samples) and Blue-veined (45 samples) cheeses. The most predominant species isolated were *Debaryomyces hansenii*, *Candida catenulata*, *C. lipolytica*, *C. kefyr*, *C. intermedia*, *Saccharomyces cerevisiae*, *Cryptococcus albidus* and *Kluyveromyces marxianus*. The salt concentration of the surface samples of the cheeses varied between 2.5–5.5% (w/w) (Camembert) and 7.5–8.3 (Blue-veined), depending upon brand, and influenced the yeast ecology, especially the presence of *S. cerevisiae*. Yeasts grew to populations of 10^6–10^8 cfu/g when cheeses were stored at either 25°C or 10°C. These populations decreased on continued storage at 25°C, but such decreases were not so evident on storage at 10°C. The properties of yeasts influencing their occurrence and growth in cheeses were: fermentation/assimilation of lactose; production of extracellular lipolytic and proteolytic enzymes, utilisation of lactic and citric acids; and growth at 10°C.

Keywords: Yeasts; Cheese; Dairy products; *Debaryomyces hansenii*; *Candida* spp.; *Kluyveromyces marxianus*; *Saccharomyces cerevisiae*

1. Introduction

Yeasts are frequently found within the microflora of Camembert and Blue-veined cheeses (Fleet, 1990; Devoyod, 1990). They originate as natural contaminants of the cheesemaking process and, depending on the species, grow to

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Elsevier Science B.V.
SSDI 0168-1605(95)00018-6

populations as high as 10^6–10^9 cfu/g during the maturation phase of production. Yeast growth in these cheeses probably continues during retailing but this possibility has not been studied. The main species found during maturation and retailing include *Debaryomyces hansenii*, *Kluyveromyces marxianus*, *Yarrowia lipolytica* and various species of *Candida* (Lenoir, 1984; de Boer and Kuik, 1987; Nooitgedagt and Hartog, 1988; Besançon et al., 1992). These yeasts impact on cheese quality through their production of lipolytic and proteolytic enzymes, fermentation of residual lactose, utilisation of lactic acid and their autolysis (Choisy et al., 1987a,b; Fleet, 1990; Devoyod, 1990).

Although production of Camembert and Blue-veined cheeses is well established in Europe, their production in Australia is a relatively new enterprise, mainly developing during the last 10–15 years. In Australia, these cheeses are produced from pasteurised milks whereas in Europe there has been a tradition of producing such cheeses from non-pasteurised milk. To date, most of the yeast studies on these cheeses have been done on products made from non-pasteurised milks. Given this difference and relative newness of the Australian industry, it was of interest to determine what yeasts, if any, are associated with Australian produced cheeses.

This paper reports: (1) the occurrence of yeast species in Australian produced Camembert and Blue-veined cheeses; and (2) the potential for yeast growth in these cheeses during retail storage.

2. Materials and methods

2.1. Cheese samples

Cheese samples (500 g) were purchased from a range of retail outlets in Sydney, New South Wales and immediately analysed for microbiological status. Sub-samples were frozen and stored for subsequent analysis of pH and salt content. Only Australian produced Camembert cheeses were examined as imported. Camembert cheeses were pasteurised and contained no viable yeasts. At the time of study, only two brands of Blue-veined cheese were regularly available at local outlets. These were: brand A, Australian Blue-veined; and brand B which was imported Camembert cheeses from four different producers were examined. These were: brand A, Tasmanian Camembert; brand B, Unicorn Camembert; brand C, Warwick Camembert; and brand D, Farm-House Camembert. All cheeses were produced from milk that had been pasteurized at 72°C for 15 s.

2.2. Enumeration and isolation of yeasts

Preliminary studies showed that greatest yeast counts were obtained by analysing outer sections of cheese samples, compared with sections taken from inner locations. This finding is well established in the literature (Fleet, 1990; Devoyod,

1990). Consequently, sub-samples from the outer surfaces (but penetrating 5–10 mm into the curd) were analysed. Sub-samples (10 g) were aseptically removed, and blended with 90 ml of 0.1% (w/v) peptone water for 60 s in a Stomacher (Lab Blender 400 Seward Medical, London, UK). Decimal dilutions of the homogenate were made in 0.1% peptone water and 0.1 ml spread inoculated in duplicate over the surface of plates of Malt Extract Agar (MEA) (Oxoid, Melbourne, Australia) pH 5.4, supplemented with 100 μg per ml of filter-sterilised oxytetracyclin (Sigma Chemical Co. Missouri, USA) to inhibit bacterial growth. Plates were incubated at 25°C for 2–4 days after which the total number of yeast colonies were counted. Colonies were differentiated on the basis of morphological character and counts for the different colony types were also recorded. Duplicate samples (10 g) of each cheese were examined.

Representatives (usually three colonies) of each colony type were isolated and purified by streaking onto MEA plates without oxytetracycline and subsequently maintained on slants of MEA until they were identified.

2.3. Identification of yeasts

Yeast isolates were identified by conducting the full range of morphological, sporulation, biochemical and physiological tests as described in Kreger-van Rij (1984) and Barnett et al. (1983). In addition, each isolate was examined in ATB-32C strips (BioMerieux, Marcy – l'Etoile, France). Data were interpreted using the keys in Kreger-van Rij (1984) and Barnett et al. (1983) and the computer program of Barnett et al. (1987).

Ability of the yeasts to hydrolyse protein was examined by culture on plates of both skim milk agar and malt extract agar containing 12% (w/v) added gelatin (Harrigan and McCance, 1976). Production of extracellular lipases was determined on Tributyrin agar (Oxoid) and butter fat agar (Harrigan and McCance, 1976; Shelley et al., 1987).

2.4. pH

Cheese samples (1.0 g) were homogenised with 10 ml of distilled water and the pH of the homogenate was measured using a pH meter and standard buffers. Data presented are the means of 10 samples for each brand.

2.5. Sodium chloride

The concentration of sodium chloride in cheese samples (2.0 g taken as described for microbiological analyses) was determined by Volhard titration (Association of Official Analytical Chemists, 1984). Data presented are the means of 10 samples for each brand.

3. Results and discussion

3.1. Salt concentration and pH of cheeses

The mean concentrations of sodium chloride for the surface samples of the Camembert cheese were: brand A 2.8% (w/w); brand B 5.5% (w/w); brand C 5.0% (w/w); and brand D 5.1% (w/w). Generally, Camembert cheeses have salt contents in the range 1.5–2.5% (w/w) (Choisy et al., 1987a; Guinee and Fox, 1987; Hardy, 1987) which means that many of the Australian cheeses tend to have values higher than expected. The mean concentrations of sodium chloride for the two brands of Blue-veined cheeses were 8.3% (w/w) for brand A and 8.1% (w/w) for brand B which agree with published values (Fernández-Salguero et al., 1986; Hardy, 1987).

The mean pH values for the Camembert cheeses were 5.9 (brand A), 6.3 (brand B), 6.0 (brand C, and 5.9 (brand D). For the Blue-veined cheeses, the mean pH values were 6.4 (brand A) and 6.5 (brand B). These data agree with published values (Gripon, 1987; Choisy et al., 1987a). For samples within the one brand, pH values could vary by as much as 1.0 unit. Such variation is not unexpected because the pH of the cheese sample at the time of analysis will depend on its age and the activity of the maturation flora in causing proteolysis and utilisation of organic acids during retailing.

3.2. Yeast populations

Of the 85 samples of Camembert cheese examined, 36% exhibited yeast populations exceeding 10^7 cfu/g. Eighteen percent of the samples exhibited populations in the range 10^6–10^7 cfu/g and 6% had populations of 10^5–10^6 cfu/g. For 28% of the samples, the populations were less than 10^3 cfu/g. Similar trends were found in all four brands of Camembert. Of the 45 samples of Blue-veined cheeses examined, 26% exhibited populations greater than 10^7 cfu/g, 10% were in the range 10^6–10^7 cfu/g. 13% were in the range 10^5–10^6 cfu/g and 35% had populations less than 10^3 cfu/g. These population statistics are similar to those reported for European cheeses (Nakasake and Komagata, 1977; de Boer and Kuik, 1987; Nooitgedagt and Hartog, 1988) and reinforce the general conclusion that yeasts can be an important part of the natural flora of these products (Lenoir, 1984; Choisy et al., 1987b; Fleet 1990; Devoyod, 1990).

Within the one brand of cheese, there was notable variation in yeast counts for different samples. While some samples gave counts greater than 10^6 cfu/g, others gave counts less than 10^3 cfu/g. Such variation could reflect the age of the cheese at the time of examination. Because yeasts continue to grow in these cheeses during retail storage (see later section), older samples would be expected to give greater populations. For most of the cheeses, the age of the cheese was not known at the time of sampling. Also, the variation in yeast populations could reflect the casual, accidental nature of yeast contamination during processing of the cheese and variations in process conditions (e.g. salt concentration, temperature) that

Table 1
Populations of most prevalent yeast species in Camembert cheeses

Yeast species	Percentage of samples in population range (cfu/g)					
	$<10^3$	10^3–10^4	10^4–10^5	10^5–10^6	10^6–10^7	$>10^7$
Brand A						
Candida famata	30	27	11	–	20	12
Debaryomyces hansenii	20	11	15	25	22	7
Saccharomyces cerevisiae	26	34	6	10	13	9
Candida intermedia	35	56	–	–	–	9
Brand B						
Debaryomyces hansenii	20	14	5	16	30	15
Candida lipolytica	21	39	–	10	10	20
Candida kefyr	20	45	–	3	6	18
Candida famata	25	22	–	32	16	5
Candida catenulata	31	15	20	16	6	12
Candida intermedia	19	30	13	23	11	4
Cryptococcus albidus	40	49	3	3	5	–
Brand C						
Candida kefyr	28	6	5	20	16	25
Debaryomyces hansenii	13	29	8	10	30	10
Candida lipolytica	11	28	15	15	9	22
Candida catenulata	19	21	11	30	10	18
Candida tropicalis	21	27	5	40	7	–
Brand D						
Debaryomyces hansenii	9	15	6	23	17	30
Candida catenulata	13	22	8	7	32	18
Candida lipolytica	7	17	8	20	33	15
Candida kefyr	22	30	16	17	15	–

Number of samples examined for each brand. A = 20; B = 30; C = 20; D = 15.

would affect yeast growth. Such influences could vary with the production batch. Samples within the one brand of cheese were purchased at different times and represented different production batches.

3.3. Yeast species

A total of 240 isolates from the cheeses were identified and these included 40 strains of *D. hansenii*, 35 strains of *Candida catenulata*, 30 strains of *Candida lipolytica*, 20 strains of *Candida kefyr*, 20 strains of *Candida famata*, 22 strains of *K. marxianus* and 11 strains of *Saccharomyces cerevisiae*.

The most predominant species in Camembert cheeses were *D. hansenii, Candida lipolytica, Candida kefyr, Candida catenulata* and *Candida famata* and they were frequently found at populations exceeding 10^6–10^7 cfu/g (Table 1). There was some variation in species that were isolated from the different brands of cheese. *D. hansenii* was isolated from cheeses in all four brands. *Candida catenulata* and *Candida lipolytica* were not found in samples of brand A, but were found

Table 2
Populations of most predominant yeast species in Blue-veined cheeses

Yeast species	Percentage of samples in population range (cfu/g)					
	$< 10^3$	10^3–10^4	10^4–10^5	10^5–10^6	10^6–10^7	$> 10^7$
Brand A						
Debaryomyces hansenii	30	22	16	–	20	12
Candida catenulata	40	10	12	18	15	5
Cryptococcus albidus	13	35	14	20	16	2
Candida famata	36	16	20	12	11	5
Candida lipolytica	31	41	13	15	–	–
Brand B						
Candida catenulata	10	18	–	–	48	24
Kluyveromyces marxianus	11	30	2	16	24	17
Candida lipolytica	17	27	–	24	20	12
Debaryomyces hansenii	40	33	–	4	14	9
Cryptococcus albidus	15	50	6	9	20	–
Candida colliculosa	25	49	4	8	14	–
Candida tropicalis	36	32	18	2	12	–
Candida kefyr	42	30	8	14	6	–
Hansenula anomala	38	33	10	14	5	–
Candida intermedia	29	31	20	17	3	–

Number of samples examined for each brand. A = 20; B = 25.

in samples of the other three brands. *Candida famata*, the asporogenous form of *D. hansenii*, and *Candida intermedia* were only isolated from cheese samples within brands A and B. *S. cerevisiae* was only found in cheeses of brand A which were characterised by their lower salt concentration (mean 2.8%) compared with cheeses of the other brands. Generally, these data agree with the species most frequently found in French Camembert cheeses (Lenoir, 1984; Gripon, 1987; Devoyod, 1990) with the exceptions that we did not find *K. marxianus* and there was a lesser occurrence of *S. cerevisiae* in the Australian cheeses. Also, there appears to be a greater incidence of *Candida lipolytica* and *Candida catenulata* in Australian produced Camembert cheeses. *Candida catenulata* has not been reported in European Camembert cheeses, but was isolated by Rohm et al. (1992) from other styles of European cheeses. These differences could be due to the higher salt concentration found in most of the Australian produced Camembert cheeses. As noted already, *Candida kefyr* was frequently isolated and this species is the non-sporogenous or imperfect counterpart of *K. marxianus*.

The predominant yeasts in the Australian produced blue-veined cheese (brand A) were *D. hansenii / Candida famata*, *Candida catenulata*, *Cryptococcus albidus* and *Candida lipolytica* (Table 2). These same species as well as *K. marxianus* also dominated in the imported blue-veined cheese (brand B) but with a greater incidence at the higher populations. The latter observation probably reflects the greater age of the imported product. Again, except for the absence of *K. marxianus*, our data on the Australian cheese agree with findings for European pro-

duced cheeses (Devoyod and Sponem, 1970; de Boer and Kuik, 1987; Besançon et al., 1992; Kaminarides and Anifantakis, 1989). At the time of our study, only one brand of Australian produced Blue-veined cheese was commercially available; now there are many more locally produced brands, and it would be interesting to study their yeast flora.

3.4. Relevant properties of yeasts isolated from cheeses

Camembert and Blue-veined cheeses have unique physical and chemical properties that are likely to select for the growth and prevalence of particular species of yeasts. These properties include high concentrations of fat (23–30% w/w) and protein (19–24% w/w), residual unfermented lactose (0.02–0.5% w/w), high concentration of lactic acid (1–1.6% w/w), significant concentrations of salt and small amounts of citric and acetic acids (Choisy et al., 1987a,b). Moreover, they are generally stored at temperatures less than 10°C.

Table 3 shows the response of several yeast species to properties likely to govern their growth in cheeses. Very strong growth in the presence of salt, growth at low, temperature and ability to utilise lactic and citric acids would be key determinants that encourage the predominance of *D. hansenii* in the cheeses (Table 3; Besançon et al., 1992). None of the 35 strains of this species that were examined in this study exhibited extracellular proteolytic or lipolytic activities according to the methods used, suggesting that these properties were not essential for its growth and predominance in cheeses. Absence of such activities in this species has been noted by Ahearn et al. (1968) and Carini et al. (1975), although Besançon et al. (1992) found a small proportion of strains to be positive for these reactions. High intracellular protease activities occur in *D. hansenii* (Nunez et al., 1981; Lenoir, 1984). These enzymes would be significant in affecting cheese proteins after release by cell autolysis, provided they were active at the pH and salt content of the cheese (Choisy et al., 1987a).

The frequent occurrence of *Candida lipolytica* and *Candida catenulata* in the cheeses correlated with their very strong extracellular lipolytic and proteolytic properties, ability to utilise lactic and citric acids, and, especially for *Candida lipolytica*, its strong growth at 5 and 10°C (Table 3). The proteolytic and lipolytic properties of *Candida lipolytica* are well reported (Comi et al., 1981; Lenoir, 1984; Ratledge and Tan, 1990; Ogrydziak, 1993). Federici (1983) has described proteolytic activity in *Candida catenulata*. Apart from a few substrate assimilation tests, the taxonomic distinction between *Candida lipolytica* and *Candida catenulata* is not strong (Kreger-van Rij, 1984). Noteworthy, however, is the better growth of *Candida lipolytica* than *Candida catenulata* at low temperature (Table 3).

The very strong ability of *K. marxianus* to assimilate and ferment lactose (Table 3) is considered to be a key property contributing to its growth in cheeses and dairy products (Lenoir, 1984; Fleet, 1990; Devoyod, 1990). Also significant would be its weaker utilisation of lactic acid, citric acid, proteins and fats. The extracellular proteolytic and lipolytic properties of *K. marxianus* have been reported elsewhere (Lenoir, 1984; Fleet and Mian, 1987; Besançon et al., 1992), but were not strong

Table 3
Properties of yeast species that affect their growth in cheese

Property	*Debaryomyces hansenii*	*Candida catenulata*	*Candida lipolytica*	*Candida kefyr*	*Kluyveromyces marxianus*	*Cryptococcus albidus*	*Saccharomyces cerevisiae*
Fermentation of lactose [b]	0/35 [a]	0/20	0/15	6/12	12/12 (vs)	0/15	0/8
Assimilation [c] of: lactic acid	30/35	19/20	14/15	10/12 (w)	10/12	9/15 (w)	4/8
citric acid	32/35	20/20	12/15	6/12 (w)	6/12 (w)	12/15 (w)	0/8
Hydrolysis [d] of: protein (skim milk, gelatin)	0/35	15/15 (vs)	15/15 (vs)	6/12	12/12 (w)	15/15 (w)	0/8
fat (tributyrin, butterfat)	0/35	15/15 (vs	15/15 (vs)	0/12	12/12 (w)	15/15 (w)	0/8
Growth [e] in: 6% (w/w) sod. chloride	35/35 (vs)	20/20	15/15	12/12	12/12	15/15	4/8(w)
Growth [e] at: 5°C	20/20	15/20	15/15 (vs)	12/12	12/12	15/15	8/8
10°C	20/20	20/20	15/15 (vs)	12/12	12/12	15/15	8/8

[a] 0/35; means 35 strains tested; 0 strains positive.
[b] vs; very strong gas production within 2 days.
[c] w; weak turbidity and sediment. vs; very strong and rapid development of turbidity and sediment.
[d] vs; very strong 10–25 mm clear zones on plate. w; weak 2–5 mm clear zones.
[e] vs; very strong growth after 2 days; otherwise growth after 5 days.

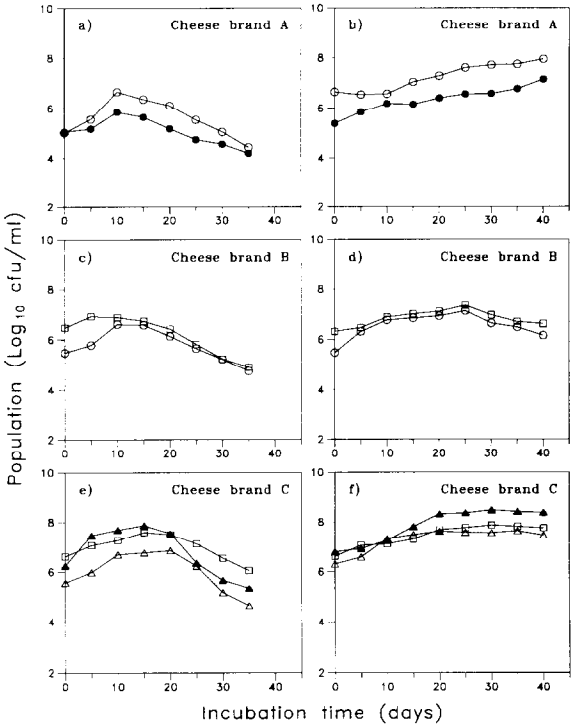

Fig. 1. Growth of yeasts in Camembert cheeses, brands A, B and C stored at 25°C (a, c, e) and 10°C (b, d, f). *Debaryomyces hansenii*, ○; *Saccharomyces cerevisiae*, ●; *Candida lipolytica*, □; *Candida catenulata*, △; *Candida kefyr*, ▲.

for the strains examined in Table 3. This species also has relatively strong intracellular proteolytic activity (Chang et al., 1972; Devoyod, 1990).

The infrequent association of *S. cerevisiae* with high salt cheeses will be related to its weak ability or inability to tolerate NaCl at concentrations exceeding 5% (w/w). However, this species is capable of good growth in cheeses of low salt concentration (Fig. 1, brand A) and must utilise some cheese components as growth substrates. According to Table 3, it would not be able to utilise cheese lactose, fat, protein or citric acid. It is a weak and variable utiliser of lactic acid and this is a possible growth substrate. It is more likely, however, that growth of *S. cerevisiae* in cheese is related to utilisation of the protein and fat breakdown products of other species.

3.5. Growth of yeasts in cheeses during storage

Cheese samples were selected for storage to illustrate the growth of a range of yeast species. These species included *D. hansenii*, *S. cerevisiae*, *Candida lipolytica*, *Candida catenulata*, *Candida kefyr* and *Cryptococccus albidus* and were present as

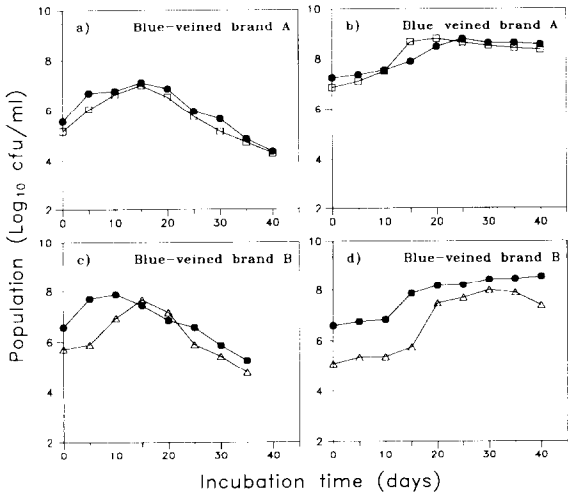

Fig. 2. Growth of yeasts in Blue-veined cheeses, brands A and B stored at 25°C (a, c) and 10°C (b, d). *Debaryomyces hansenii*, ●; *Cryptococcus albidus*, □; and *Candida kefyr*, △.

natural contaminants in the cheeses before they were stored. The populations of these species increased by approximately 100-fold during storage of the cheeses at either 25°C or 10°C (Figs. 1 and 2). Although yeast growth was faster during storage at 25°C, the cells started to die off after reaching maximum populations of 10^6–10^8 cfu/g. Growth was slower but was sustained over longer periods during storage at 10°C and in some cases (Fig. 1b,d and Fig. 2b,d) gave higher maximum populations than growth in corresponding samples stored at 25°C. The decreased survival of the yeasts at the higher storage temperature could reflect stronger interactive stresses of the salt and low water activity.

The cheeses exhibited decreased sensory appeal on storage, becoming softer in texture and developing a less attractive odour. While many other biochemical and microbiological factors contribute to these reactions, it is evident from Figs. 1 and 2 that growth of yeasts will continue to occur during retail storage of these cheeses and will contribute to the spoilage process and decreased shelf-life of the product.

4. Conclusion

In conclusion, our study has shown that yeasts make a significant contribution to the microbial ecology of Camembert and Blue-veined cheeses which are produced from pasteurised milk. Apart from the frequent occurrence of *Candida catenulata* and less frequent incidence of *K. marxianus*, the yeast ecology was comparable to that reported for cheeses produced in Europe. Yeasts exhibited strong growth in the cheeses during storage, impacting on their sensory quality and shelf-life.

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