Acta Alimentaria, Vol. 32 (3), pp. 317–322 (2003)

MONITORING YEAST SPECIES IN QUARG, QUARG PRODUCTS AND THEIR PRODUCTION ENVIRONMENT DURING THE MANUFACTURING PROCESS

J. ŠALOMSKIENĖ[a]*, A. PAŠKEVIČIUS[b] and I. MAČIONIENĖ[a]

[a] Lithuanian Food Institute, LT-3031 Kaunas, Taikos pr. 92. Lithuania
[b] Lithuanian Institute of Botany, LT-2021 Vilnius, Žaliųjų ežerų 49. Lithuania

(Received: 7 July 2002; accepted: 3 March 2003)

A total of 65 yeast cultures were isolated from quarg and its products, from the air of the production facilities and from rinses of equipment. The yeast isolates were identified and their proportion was determined.

Of these isolates, 8 species were identified in the rinses of equipment, 2 from the air of the production facilities, and 9 from the final product, i.e., quarg and quarg products. Four species of the yeasts isolated from quarg, namely *Debaryomyces hansenii*, *Trichosporon cutaneum*, *Kluyveromyces marxianus* var. *marxianus* and *Candida zeylanoides* were also present in the rinses of equipment. *Debaryomyces hansenii* and *Trichosporon cutaneum* were the predominant species both in the rinses of equipment and in the final product.

Keywords: yeasts, species, quarg, environmental sources, manufacturing process

Much attention has been given lately to studies on yeasts, which cause the spoilage of foods, particularly milk products. Literature references provide sufficient data on the occurrence of yeasts in milk products, including the description of their properties and enzymatic activity (WALKER & AYRES, 1970; ROHM, 1991; FLEET, 1992; JAKOBSEN et al., 1998).

The identification of yeasts at the species or genus level is not always necessary in studying the microbial contamination of food products by yeasts. In most cases, it is sufficient to enumerate the total yeast counts in the product and confirm their presence by microscopic observation. The identification of yeasts, however, also provides a great deal of relevant information on the microbial ecology of the product and the properties of yeast species present (FLEET, 1989). This helps to understand the growth conditions of these microorganisms and find ways and means of inhibiting the development of undesirable species.

In pasteurised milk the predominant yeast species are *Candida famata*, *Kluyveromyces marxianus* and *Cryptococcus flavus*, in cream these are *Candida famata*, *Rhodotorula glutinis* and *Candida diffluens*, and in butter the prevailing species

* To whom correspondence should be addressed.
Fax: +370 37 312393; e-mail: mikrobjs@lmai.lt

are *Rhodotorula glutinis*, *Rhodotorula rubra* and *Saccharomycopsis lipolytica* (FLEET & MIAN, 1987). In rennet cheese the most commonly occurring species are *Debaryomyces hansenii*, *Kluyveromyces marxianus* var. *lactis*, *Kluyveromyces marxianus* var. *marxianus* and *Saccharomyces cerevisiae* (TUDOR & BOARD, 1993). The latter two species, together with *Candida famata*, *Candida krusei* and *Candida lusitaniae*, are predominant in yoghurt. Similar species have been reported in Gouda (WELTHAGEN & VILJOEN, 1998), Camembert and blue-veined cheeses (ROOSTITA & FLEET, 1996).

According to ENGEL (1988), in 174 samples of quarg tested, *Geotrichum candidum* accounted for 30% of the total number of yeast isolates. The respective figures for the remaining species were: *Kluyveromyces marxianus* var. *marxianus* 18%, *Candida valida* 14% and *Candida kefyr* 10%. The critical quantities of predominant yeasts which can cause the spoilage of quarg were the following: *Geotrichum candidum* and *Candida kefyr* 10^4 CFU g^{-1}, *Candida lipolytica* and *Candida curvata* 10^6 CFU g^{-1} and *Candida valida* 10^5 CFU g^{-1}.

Quarg and quarg products are the important part of the traditional diet in Lithuania. The aim of this study was to isolate and identify different species of yeasts in these products.

1. Materials and methods

1.1. Sampling and isolation

Quarg is manufactured from pasteurised milk by the addition of 2–5% of mesophylic lactococci starter. Milk fermentation at 24–28 °C is allowed to proceed till the acidity of the clot reaches 70–75 °T. For a better removal of whey the clot is heated at 60 °C for 20–25 min. The whey is then separated and the curd is poured into spontaneous pressing vats. The ready made quarg is cooled till 6–15 °C and packed up or it is used for making quarg products. Quarg products (sweetened creamed quarg, in Lithuania so-called "Varškės sūreliai") are made from quarg by adding some flavour and aroma (sugar, vanilla, raisins). The additives are mixed up in a ready made quarg, the product is packed up and cooled to 6 °C.

The samples (n=320) of quarg and quarg products were taken from three Lithuanian dairies during the manufacturing process at the following selected points: pasteurised milk and milk mix (milk mixed with starter), milk clot and quarg after spontaneous pressing, cooled quarg and the final product. All the samples were analysed immediately after manufacture. Liquid samples (1 ml) were diluted with 9 ml of sterile peptone-salt solution. Solid samples were macerated by weighing 10 g into 90 ml of peptone-salt solution and homogenizing under aseptic conditions in a peristaltic-type blender for 30 s. Further decimal dilutions of the suspension were then prepared as required. Aliquots (0.1 ml) of the dilutions were spread inoculated, in duplicate, over the surface of oxytetracycline-glucose yeast extract agar (OGYEA, Oxoid Ltd) plates. Yeast colonies were isolated from the highest dilutions on plates

incubated at 25 °C for 4 days. Air was sampled by settle plates (standard 90 mm Petri dishes) containing OGYEA with an exposure time of 5 min. The rinses of equipment were taken from surfaces of approx. 100 cm^2 by using sterile cotton swabs moistened in sterile salt solution.

1.2. Identification

After isolation, the yeast colonies were purified and then stored on OGYEA slants at 4 °C during the period of investigation until characterisation. The isolates were identified using the methods described by BARNETT and co-workers (1983), KREGER-VAN RIJ (1984) and KURTZMAN and FELL (1998) including cellular morphology and type of budding, formation of pseudo-mycelium and true mycelium, sporulation, carbohydrate fermentation, carbohydrate assimilation, nitrogen assimilation, growth in vitamin free medium, growth at 25–42 °C, hydrolysis of urea, casein hydrolysis, starch production, cycloheximide resistance and growth in a medium containing 50% and 60% glucose.

2. Results and discussion

A total of 65 yeast cultures were isolated from environmental sources and during the manufacturing process of quarg and its products. Sources of yeast contamination representing the environmental samples included rinses of equipment and the air of production facilities. There were 22 yeast cultures isolated from the rinses of equipment and 2 yeast cultures isolated from the air of production facilities. The identified species are given in Table 1.

As shown in Table 1, 8 yeast species were identified in the rinses of the equipment. These included *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij (28%), *Trichosporon cutaneum* (De Beurm, Gougerot et Vaucher) Ota (23%), *Candida parapsilosis* (Ashford) Langeron et Talice (14%), *Rhodotorula rubra* (Demme) Lodder (14%), *Candida zeylanoides* (Castellani) Langeron et Guerra (4%), *Kluyveromyces marxianus* var. *marxianus* van der Walt (9%), *Pichia kluyveri* Bedford ex Kudriavzev (4%) and *Pichia fermentans* Lodder (4%).

Two species of yeasts, namely *Candida scottii* Diddens et Lodder and *Rhodotorula rubra* were found in the air of the production facilities. The same species were also identified in the rinses of equipment and in the quarg during the technological process.

There were 41 yeast cultures isolated during the manufacturing process of quarg and its products. The identified species are shown in Table 2.

Eight species of yeasts and one species of yeast-like fungi were identified in the final product (quarg and its products). These included: *Debaryomyces hansenii*, *Trichosporon cutaneum*, *Kluyveromyces marxianus* var. *marxianus*, *Candida zeylanoides, Kluyveromyces marxianus* var. *lactis* van der Walt, *Saccharomyces cerevisiae* Meyen ex Hansen, *Candida kefyr* (Beijerinck) van Uden et Buckley, *Torulaspora delbrueckii* (Lindner) Lindner and *Geotrichum candidum* Link ex Persoon.

The occurrence of *Geotrichum candidum*, *Kluyveromyces marxianus* var. *marxianus* and *Candida kefyr* in quarg has been reported previously (ENGEL, 1988). *Debaryomyces hansenii* and *Trichosporon cutaneum*, which predominated in the final product (32% and 20%, resp.), also prevailed in the rinses of equipment (28% and 23%, resp.). *Debaryomyces hansenii*, the perfect form of *Candida famata*, predominated in most studies of yeasts associated with dairy products (FLEET & MIAN, 1987). The remaining species accounted for 4% to 9% of the total count.

Table 1. Species of yeasts isolated from environmental sources

Yeast species	Air of production facilities	Rinses of equipment				
		Clotting vat	Pressing vat	Packing machine	Pipe to the clotting vat	Cooler
Candida scottii	+					
Candida parapsilosis		+	+	+		
Candida zeylanoides			+			
Debaryomyces hansenii		+	+	+		
Kluyveromyces marxianus var. *marxianus*					+	+
Pichia kluyveri			+			
Pichia fermentans				+		
Rhodotorula rubra	+		+			+
Trichosporon cutaneum		+	+	+		+

Table 2. Species of yeasts and yeast-like fungi isolated from the environment of quarg production lines

Yeast and yeast-like fungi species	Rinses of equipment	Air of production facilities	Technological process					
			Pasteurised milk	Milk mix	Milk clot	Quarg after spontaneous pressing	Cooled quarg	Final product
Candida scottii		+	+					
Candida famata				+			+	
Candida parapsilosis	+			+		+	+	
Candida kefyr								+
Candida zeylanoides	+							+
Debaryomyces hansenii	+		+		+	+		+
Kluyveromyces marxianus var. *marxianus*	+				+			+
Kluyveromyces marxianus var. *lactis*						+		+
Rhodotorula rubra	+	+	+					
Pichia membranaefaciens							+	
Pichia fermentans	+							
Pichia kluyveri	+							
Saccharomyces cerevisiae			+	+				+
Trichosporon cutaneum	+						+	+
Torulaspora delbrueckii								+
Geotrichum candidum								+

The sources of the 65 isolates and the identified species are given in Table 2.

As shown in Table 2, 75% of the yeast species isolated from the rinses of equipment were present in the technological process. These included *C. parapsilosis*, *C. zeylanoides*, *R. rubra*, *D. hansenii*, *K. marxianus* var. *marxianus* and *T. cutaneum*. The species identified in the air of the production facilities, namely *Candida scottii* and *Rhodotorula rubra* were also found in the rinses of equipment (*R. rubra*) and in the pasteurised milk during the technological process (*C. scottii* and *R. rubra*).

The results of this study suggest that the occurrence of yeast species undergo slight changes during the manufacturing process of quarg and its products. Of the eight species found in the rinses of equipment, four were identified in the final product. Besides, four additional species of yeasts and one species of yeast-like fungi were found in the quarg and its products. Some of these yeasts might have got into the quarg from the environment. Other species identified in the rinses of equipment may have been affected or destroyed by various factors, including the starter bacteria, the heating temperature of the milk clot (20 to 25 min at 60 °C), the acidity of the medium (pH 4.5–4.7) or other technological factors.

3. Conclusions

The 65 yeast isolates from quarg and its products, the air of the production facilities and the rinses of equipment were found to belong to 16 species from *Candida*, *Debaryomyces*, *Kluyveromyces*, *Rhodotorula*, *Pichia*, *Saccharomyces*, *Trichosporon*, *Torulaspora* and *Geotrichum* genera.

The occurrence of yeast species has been found to undergo insignificant changes during the technological process. The results of this research showed that 75% of the yeast species isolated from the rinses of equipment were present in the technological process.

The species *Debaryomyces hansenii* and *Trichosporon cutaneum* were predominant both in the rinses of equipment and in the final product.

References

BARNETT, J.A., PAYNE, R.W. & YARROW, D. (1983): *Yeasts: characteristics and identification*. Cambridge University Press, Cambridge, pp. 17–288.

ENGEL, V.G. (1988): Hefen in Quark. *Deut. Milchw.*, *39* (16), 512–514.

FLEET, G.H. (1989): Food spoilage yeasts. -in: SPENCER, J.F.T. & SPENCER, D.M. (Eds) *Yeast technology*. Springer-Verlag, Berlin, Heidelberg, pp. 124–152.

FLEET, G.H. (1990): Yeasts in dairy products – a review. *J. appl. Bacteriol.*, *68*, 199–211.

FLEET, G.H. (1992): Spoilage yeasts. *Crit. Rev. Biotechnol.*, *12* (1/2), 1–44.

FLEET, G.H. & MIAN, M.A. (1987): The occurrence and growth of yeasts in dairy products. *Int. J. Fd Microbiol.*, *4*, 145–155.

JAKOBSEN, M., NARVHUS, J. & VILJOEN, B.C. (1998): *Yeasts in the dairy industry: positive and negative aspects*. IDF, Brussels, Belgium, pp. 7–160.

KREGER-VAN RIJ, N.J.W (1984): *The yeast, a taxonomic study*. 3rd ed., Elsevier, Amsterdam, pp. 45–103.

KURTZMAN, C.P. & FELL, J.W. (1998): *The yeasts, a taxonomic study*. 4th ed., Elsevier, Amsterdam, pp. 75–107; 891–945.

ROHM, H. (1991): Zur Bedeutung von Hefen und Schimmelpilzen für die Milchwirtschaft. *Mikrobiol.*, *14*, 404–406.

ROOSTITA, R. & FLEET, G.H. (1996): The occurrence and growth of yeast in Camembert and blue-veined cheeses. *Int. J. Fd Microbiol.*, *28*, 393–404.

TUDOR, D.A. & BOARD, R.G. (1993): Food spoilage yeasts. -in: ROSE, A.H. & HARRISON, J.S. (Eds) *The yeasts*. Vol. 5, *Yeast technology*. 2nd ed., Academic Press, London, pp. 436–516.

WALKER, H.W. & AYRES, J.C. (1970): Yeasts as spoilage organisms. -in: ROSE, A.H. & HARRISON, J.S. (Eds) *The yeasts*. Vol. 3, *Yeast technology*. Academic Press, London, pp. 463–527.

WELTHAGEN, J.J. & VILJOEN, B.C. (1998): Yeast profile in Gouda cheese during processing and ripening. *Int. J. Fd Microbiol*., *41*, 185–194.