UDC 663.12:666.013.8
ISSN 1330-9862

professional paper

The Resistance of Dairy Yeasts Against Commercially Available Cleaning Compounds and Sanitizers

*Piet J. Laubsher and Bennie C. Viljoen**

Department of Microbiology and Biochemistry, P.O. Box 339,
University of the Orange Free State, Bloemfontein, 9300, South Africa

Received: March 17, 1999
Accepted: October 20, 1999

Summary

Seven yeasts, isolated from a cheese factory were screened for their resistance against normal sanitary practices in the industry. Nine commercial cleaning compounds and sanitizers, incorporated individually, were tested for their inhibitory effect against the yeast isolates at varying temperatures.

Candida rugosa exhibited the highest resistance to all the compounds. The »Peroxide Based Acid Sanitizer« proved to be the most effective inhibitor against most of the isolates causing the least growth after 45–60 min contact. None of the compounds used, however, was able to kill all the yeasts. The variation in temperature caused no significant differences in the compounds inhibitory effect on the yeast isolates.

Key words: resistance, yeasts, sanitizers, cleaning compounds, dairy

Introduction

Yeasts developing as natural contaminants play a substantial role in the manufacturing processes of cheese due to their ability to grow under environmental conditions unfavourable to many bacteria (*1–5*). Their progression is governed by the ability to grow at low temperatures, low water activity, high salt concentrations, fermentation/assimilation of lactose, production of lipolytic and proteolytic enzymes and utilisation of lactic and citric acid (*3–5*).

High numbers of yeasts, exceeding counts of 10^4 cfu/g and even as high as 10^8 cfu/g are responsible for the spoilage of dairy products causing yeasty or fruity flavours, gassiness, slime formation and discolouration of products (*5–8*). Under poor hygienic conditions, an increase in the number of yeasts may result in early blowing and off-flavours during ripening of certain cheeses (*9*).

Typical dairy associated yeasts isolated from cheeses are dominated by *Kluyveromyces marxianus, Yarrowia lipolytica, Debaryomyces hansenii, Candida* spp., *Rhodotorula* and *Cryptococcus* species (*2,5–9*). All of these yeast species are capable to survive under the selective environmental stresses associated with dairy products and may cause spoilage or contribute positively to the final product (*3*). Despite the high occurrences of yeasts, no studies attempted to elucidate the reasons for the high number of yeasts other than blaming it on post-pasteurisation contamination, development as secondary microflora or improper sanitation practices.

Proper disinfection of production facilities in the dairy industry is therefore imperative to minimise not only bacterial contamination but also yeast contamination to secure quality products. This requires that cleaning compounds and sanitizers must be active against the spoilage yeasts.

This study reports the inactivation of seven species of yeasts, isolated from Cheddar cheese, by several cleaning compounds and sanitisers used in dairy processing. The effects of these compounds on the yeasts were examined at two different temperatures, 10 and 25 °C.

* Corresponding author; Tel. ++27 514 012 621; Fax: +27 514 443 219; E-mail: ViljoenBC@micro.nw.uovs.ac.za

Materials and Methods

Microorganisms

Candida versatilis, C. rugosa, Debaryomyces hansenii, Dekkera custersiana, Rhodotorula mucilaginosa, Trichosporon beigelii and *Torulaspora delbrueckii* were strains isolated from matured Cheddar and the immediate environment. Isolates were identified by using the methods described by Kreger-van Rij (*10*) and the computerized identification system of Barnett *et al.* (*11*). These cultures were maintained on yeast extract malt extract (YM) (*12*) and checked for purity by streak plating onto this medium before use in experiments. The sources of origin are listed in Table 1.

Cultivation of yeast isolates

Sample preparation of the yeasts was conducted with UHT-treated milk purchased from local supermarkets. The composition of such milk is similar to that of raw milk (*13*). Milk (200 mL) was aseptically dispensed into sterilised Erlenmeyer flasks (500 mL), inoculated with the relevant yeast cultures and incubated at 25 °C on a rotary shaker at 160 rpm (throw = 50 mm) for 48 h.

The yeast cells were harvested by centrifugation at 10 000 g for 5 min at 4 °C. The final cell pellet was resuspended in sterile water to achieve a concentration of 1 g/100 mL (1 %).

Cleaning compounds and sanitizers

The cleaning compounds and sanitizers comprised nine commercial products normally used for cleaning/sanitation in the dairy industry. Cleaning compounds and sanitizers were mixed with distilled water according to the manufactures instructions and concentration. The cleaning compounds and sanitizers used are listed in Table 2.

Test procedures

The experimental yeast cultures (1 %) were inoculated (1 mL) into 10 mL of the individual cleaning compounds and sanitizers solution. Contact times of 0, 10, 20, 45 and 60 min were used at 10 and 25 °C. During incubation, samples (1 mL) of the culture were taken and analysed for viable yeast counts. Aliquots (1 mL) were transferred into 9 mL of Ringer solution and thoroughly mixed. Further dilutions were carried out as required for microbiological assays and plated by the spread plate technique onto duplicate plates of YM agar. YM plates were incubated for 5 days at 25 °C and the surviving colonies counted.

Results and Discussion

The microbial spoilage of dairy products is generally associated with the growth of bacteria (*14*). Yeasts are, however, a frequent cause of spoilage of a wide range of dairy products. Despite the ability to cause spoilage, and references indicating post-pasteurisation contamination originating from the environment (*1*), and the yeasts greater resistance compared to bacteria (*15*), little consideration has been given to the effect of cleaning compounds and sanitizers on these organisms. Although the heat-tolerance (*16*), activity of biocides (*17*), resistance against food preservatives, *etc.* (*18*) of vegetative cells and the ascospores are known, the authors mainly focussed on *Saccharomyces cerevisiae* as the challenge organism, and little is known of the non-*Saccharomyces* species present in dairy products. It is probable that, many yeasts may survive cleaning and disinfection procedures as ascospores (*17*).

When vegetative cells (from mid-exponential phase) of *Candida versatilis, C. rugosa, Debaryomyces hansenii, Dekkera custersiana, Rhodotorula mucilaginosa, Trichosporon beigelii* and *Torulaspora delbrueckii* were exposed to different cleaning compounds and sanitizers currently applied in the dairy industry, their growth were inhibited and some of the yeast types was completely killed (Tables 3–9). No substantial differences in the inhibition of the growth of the yeasts were observed when the cleaning compounds and sanitizers were applied at 10 or 25 °C. *Candida rugosa* was the most resistant yeast species against the cleaning compounds and sanitizers, while *Debaryomyces hansenii* also proved to be resistant to some extent. This corresponds with results obtained by Bundgaard-Nielsen and Nielsen (*19*) indicating that *D. hansenii* is more resistant to disinfectants like chlorine dioxide, compared to related yeasts. The resistance of *D. hansenii* against the compounds may be a cause for concern, as the species proved to be dominant in various dairy products (*1*), being frequently recovered from salt brines (*2,5*),

Table 1. Identity and sources of origin of the dairy associated yeasts

Organisms	Source
Rhodotorula mucilaginosa	Raw milk, equipment, air, hands, cheese curd and cheese
Candida versatiles	Raw milk, equipment, rennet, cheese curd and cheese
Candida rugosa	Raw milk, equipment and cheese
Trichosporon beigelii	Raw milk, equipment, rennet, hands, cheese curd, cheese and aprons
Debaryomyces hansenii	Raw milk, equipment, air, rennet, hands, aprons, cheese curd and cheese
Dekkera custersiana	Raw milk, hands, cheese air, equipment and cheese curd
Torulaspora delbrueckii	Cheese, raw milk, hands and rennet

Table 2. Cleaning compounds and sanitizers used in the present study

Products	Description	Class
Chlorinated general cleaner	High foaming powder detergent designed specifically for cleaning of handwash equipment	Slightly alkaline
Concentrated acid detergent	A concentrated acid detergent for treating process equipment after cleaning with a caustic detergent	Strong acid
Germicidal hand soap	A synthetic germicidal hand soap, used in the dairy industry	Neutral detergent
Heavy duty caustic detergent powder (cold wash)	A foaming detergent powder which is designed specifically for heavy duty cleaning of processing equipment in dairy industry	Alkaline
Iodophor sanitizer	A broad spectrum, fast acting sanitizer used to control all types of microorganisms	Iodine acids
Pasteuriser detergent	A unique blend of alkalies, sufactants and several sequestering agents	Alkaline
Peroxide based acid sanitizer	An advanced technological sanitizer which supercedes conventional halogen sanitizers. It is stabilized and the hydrogen peroxide constituent causes rapid disinfection to occur to all Cacteria, yeasts, moulds and viruses	Peracids
Heavy duty chlorinated alkaline detergent	Higly active, non foaming chlorinated alkaline detergent designed for dairy equipment	Chlorines
Heavy duty caustic detergent powder (hot wash)	Foaming caustic detergent powder designed for heavy duty cleaning in the dairy industry (evaporation)	Alkaline

Tables 3–9. Cleaning compounds and sanitizers activity against isolated yeasts. Results are the means of three repetitions.

Table 3. *Rhodotorula mucilaginosa*

	cfu/mL									
	10 °C					25 °C				
Detergent	0 min	10 min	20 min	45 min	60 min	0 min	10 min	20 min	45 min	60 min
W1	$156 \cdot 10^5$				19 020	$156 \cdot 10^5$				18 270
W2	$156 \cdot 10^5$			23 200	8 620	$156 \cdot 10^5$			20 120	7 930
W3	$156 \cdot 10^5$			18 260	9 200	$156 \cdot 10^5$				10 270
W4	$156 \cdot 10^5$	128 000	3 140	720	460	$156 \cdot 10^5$	119 000	2 980	690	390
W5	$156 \cdot 10^5$		1 920	640	520	$156 \cdot 10^5$		1 810	720	490
W6	$156 \cdot 10^5$		2 810	730	280	$156 \cdot 10^5$		3 120	690	270
W7	$156 \cdot 10^5$	3 000	80	0	0	$156 \cdot 10^5$	2 000	90	0	0
W8	$156 \cdot 10^5$			9 370	4 920	$156 \cdot 10^5$			10 210	5 370
W9	$156 \cdot 10^5$				8 320	$156 \cdot 10^5$				7 980

Table 4. *Candida versatilis*

	cfu/mL									
	10 °C					25 °C				
Detergent	0 min	10 min	20 min	45 min	60 min	0 min	10 min	20 min	45 min	60 min
W1	$384 \cdot 10^5$				14 800	$384 \cdot 10^5$				13 910
W2	$384 \cdot 10^5$				12 100	$384 \cdot 10^5$				14 200
W3	$384 \cdot 10^5$				9 780	$384 \cdot 10^5$				11 310
W4	$384 \cdot 10^5$		14 080	1 590	580	$384 \cdot 10^5$		15 080	1 620	620
W5	$384 \cdot 10^5$	132 000	2 320	840	560	$384 \cdot 10^5$	148 000	2 460	600	380
W6	$384 \cdot 10^5$		9 600	980	490	$384 \cdot 10^5$		10 200	1 070	380
W7	$384 \cdot 10^5$	1 900	1 989	860	80	$384 \cdot 10^5$	920	840	620	70
W8	$384 \cdot 10^5$				5 310	$384 \cdot 10^5$				6 120
W9	$384 \cdot 10^5$		2 480	1 000	190	$384 \cdot 10^5$		3 280	1 260	160

Table 5. *Candida rugosa*

	cfu/mL									
	10 °C					25 °C				
Detergent	0 min	10 min	20 min	45 min	60 min	0 min	10 min	20 min	45 min	60 min
W1	$216 \cdot 10^5$				29 890	$216 \cdot 10^5$				31 800
W2	$216 \cdot 10^5$				19 630	$216 \cdot 10^5$				17 190
W3	$216 \cdot 10^5$				23 840	$216 \cdot 10^5$				24 310
W4	$216 \cdot 10^5$		14 248	1 260	580	$216 \cdot 10^5$	598 000		980	490
W5	$216 \cdot 10^5$	642 000		804	180	$216 \cdot 10^5$	683 000	10 200	980	160
W6	$216 \cdot 10^5$		7 260	620	480	$216 \cdot 10^5$		8 320	710	510
W7	$216 \cdot 10^5$	620 000		290	60	$216 \cdot 10^5$	72 000		490	80
W8	$216 \cdot 10^5$				7 210	$216 \cdot 10^5$				9 170
W9	$216 \cdot 10^5$	620 000		830	160	$216 \cdot 10^5$	780 000		810	90

Table 6. *Trichosporon beigelii*

	cfu/mL									
	10 °C					25 °C				
Detergent	0 min	10 min	20 min	45 min	60 min	0 min	10 min	20 min	45 min	60 min
W1	$96 \cdot 10^5$				18 620	$96 \cdot 10^5$				17 620
W2	$96 \cdot 10^5$				20 120	$96 \cdot 10^5$				19 610
W3	$96 \cdot 10^5$			14 520	8 930	$96 \cdot 10^5$			12 820	9 260
W4	$96 \cdot 10^5$	248 000	4 200	460	90	$96 \cdot 10^5$	310 000	5 090	510	110
W5	$96 \cdot 10^5$	3 200	640	0	0	$96 \cdot 10^5$	280 000	560	0	90
W6	$96 \cdot 10^5$	480 000		426	230	$96 \cdot 10^5$	400 000	530	380	210
W7	$96 \cdot 10^5$	340 000		500	140	$96 \cdot 10^5$	310 000	690	480	80
W8	$96 \cdot 10^5$				8 920	$96 \cdot 10^5$			15 820	7 980
W9	$96 \cdot 10^5$				7 920	$96 \cdot 10^5$			12 820	6 950

Table 7. *Debaryomyces hansenii*

	cfu/mL									
	10 °C					25 °C				
Detergent	0 min	10 min	20 min	45 min	60 min	0 min	10 min	20 min	45 min	60 min
W1	$372 \cdot 10^5$				20 160	$372 \cdot 10^5$				18 600
W2	$372 \cdot 10^5$			51 000	16 800	$372 \cdot 10^5$			49 600	15 760
W3	$372 \cdot 10^5$			16 540	7 280	$372 \cdot 10^5$				9 620
W4	$372 \cdot 10^5$		12 000	1 390	690	$372 \cdot 10^5$		11 800	1 490	580
W5	$372 \cdot 10^5$	782 000		1 080	520	$372 \cdot 10^5$	862 000	8 700	980	490
W6	$372 \cdot 10^5$		84 000	1 000	580	$372 \cdot 10^5$		9 880	9 880	460
W7	$372 \cdot 10^5$	980 000		620	320	$372 \cdot 10^5$	1 021 000	8 000	580	170
W8	$372 \cdot 10^5$				9 890	$372 \cdot 10^5$				10 800
W9	$372 \cdot 10^5$				8 200	$372 \cdot 10^5$				9 670

Table 8. *Dekkera custersiana*

	cfu/mL									
	10 °C					25 °C				
Detergent	0 min	10 min	20 min	45 min	60 min	0 min	10 min	20 min	45 min	60 min
W1	$416 \cdot 10^5$				12 000	$416 \cdot 10^5$				11 800
W2	$416 \cdot 10^5$	728 000	8 980	502 000	168 000	$416 \cdot 10^5$	682 000	928 000	560 000	1 420
W3	$416 \cdot 10^5$			10 540	5 860	$416 \cdot 10^5$			10 520	4 820
W4	$416 \cdot 10^5$	520 000	8 600	940	190	$416 \cdot 10^5$	480 000	9 200	890	80
W5	$416 \cdot 10^5$	460 000	980	0	0	$416 \cdot 10^5$	392 000	860	0	0
W6	$416 \cdot 10^5$	738 000	48 000	980	480	$416 \cdot 10^5$	920 000	52 000	1 002	520
W7	$416 \cdot 10^5$	680 000	1 680 000	1 010	280	$416 \cdot 10^5$	810 000	1 720 000		470
W8	$416 \cdot 10^5$				8 930	$416 \cdot 10^5$				10 500
W9	$416 \cdot 10^5$			870 000	3 820	$416 \cdot 10^5$			920 000	2 980

Table 9. *Torulaspora delbrueckii*

	cfu/mL									
	10 °C					25 °C				
Detergent	0 min	10 min	20 min	45 min	60 min	0 min	10 min	20 min	45 min	60 min
W1	$528 \cdot 10^5$					$528 \cdot 10^5$				19 600
W2	$528 \cdot 10^5$	146 000	2 080	1 120	600	$528 \cdot 10^5$	138 000	1 940	980	520
W3	$528 \cdot 10^5$	890	250	50	20	$528 \cdot 10^5$	1 000	290	30	10
W4	$528 \cdot 10^5$	4 800	980	640	340	$528 \cdot 10^5$	5 000	1 010	720	290
W5	$528 \cdot 10^5$	620 000	920	640	320	$528 \cdot 10^5$	71 000	880	580	300
W6	$528 \cdot 10^5$	18 000	960	640	320	$528 \cdot 10^5$	192 000	1 680	580	270
W7	$528 \cdot 10^5$	460 000	1 040	20	10	$528 \cdot 10^5$	380 000	1 020	10	0
W8	$528 \cdot 10^5$				10 800	$528 \cdot 10^5$				14 600
W9	$528 \cdot 10^5$			6 700	8 640	$528 \cdot 10^5$			5 600	8 250

W1 = Chlorinated general cleaner, W2 = Concentrated acid detergent, W3 = Germicidal hand soap, W4 = Heavy duty caustic detergent powder (cold wash), W5 = Iodophor sanitizer, W6 = Pasteuriser detergent, W7 = Peroxide based acid sanitizer, W8 = Heavy duty chlorinated alkaline detergent, W9 = Heavy duty caustic detergent powder (hot wash)

yoghurt (*20*), cheese (*6*) and raw milk (*4*). In all of these studies, the species emerged as a post-pasteurisation contaminant, isolated from a wide range of sources including the brine, whey, curd, air, surface equipment, workers' hands and aprons. Consequently, it has to be controlled by cleaning compounds or sanitizers to prevent spoilage of the final product.

Rhodotorula mucilaginosa was killed within 45 min when exposed to the peroxide based sanitizer, whereas *Trichosporon beigelii* and *Dekkera custersiana* were killed when exposed to the iodophor sanitizer. None of the remaining yeasts was killed by any of the cleaning compounds or sanitizers. The resistance of the yeast species, attributed to their thicker cell walls (*17*), may lead to spoilage as they are all typical dairy associated yeasts (*5*) capable of peptonising casein and attack butterfat readily (*4,20*).

The »peroxide based acid sanitizer« proved to be the most effective inhibitor against all the yeasts, resulting in final counts ranging from zero to $3.2 \cdot 10^2$ cfu/mL after 60 min of contact. Poor killing effects of hydrogen peroxide (*19*) and peracetic acid (*17*) were attributed to low concentrations or too short contact time (10 min). Despite the general use of chlorinated cleaning compounds and concentrated detergents in the food industry, the compounds had little effect on the survival of the yeasts, resulting in the high viable yeast counts after 60 min of contact time. All the yeasts showed viable counts exceeding 10^4 cfu/mL. Bundgaard-Nielsen and Nielsen (*19*) also reported poor killing effects of the alkaline disinfectants like potassium hydroxide and sodium hydroxide. High yeast counts were also observed with the use of heavy duty chlorinated alkaline and caustic detergents (Tables 3–9). The usage of iodophor as a sanitizer resulted in the total inhibition of *Trichosporon beigelli* and *Dekkera custersiana*, and generally exhibited good killing effects against most of the yeast species. The usage of this sanitizer in the South African dairy industry, however, was recently prohibited.

Conclusions

These results clearly demonstrate that, individual yeast species exhibits different responses to cleaning compounds and sanitizers and have the potential to survive on surfaces and during sanitation of processing equipment. Therefore, to obtain proper manufacturing hygiene it is important to determine the resistance of the dominant yeast types against all the compounds. Eventually, it may be necessary to apply more than one sanitizer to assure efficient cleaning.

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Otpornost kvasaca u mljekarskoj industriji prema komercijalnim sredstvima za čišćenje i dezinfekciju

Sažetak

Ispitana je otpornost sedam kvasaca izoliranih iz tvornice sira prema uobičajenim sanitarnim postupcima u industriji. Pojedinačno je ispitivan inhibitorski učinak devet komercijalnih sredstava za čišćenje i dezinfekciju na izolate kvasaca pri raznim temperaturama.

Candida rugosa pokazala je najveću otpornost prema svim upotrijebljenim sredstvima. Sredstva za dezinfekciju na bazi peroksida bila su najdjelotvorniji inhibitori za najveći broj izolata, uzrokujući nakon dodira od 45 do 60 minuta najmanji rast. Nijedan od upotrijebljenih spojeva nije bio sposoban ubiti sve kvasce. Promjena temperature nije bitno utjecala na inhibitorsko djelovanje primijenjenih sredstava na izolate kvasca.