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The fungicidal efficacy of various commercial disinfectants used in the food industry

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Abstract - The antifungal effects of eight commercial disinfectants namely alcohol, peracetic acid, iodophors, aldehydes, quaternary amine compounds (QAC, a, b and c), and a chlorine-based agent were assessed at different concentrations. The time taken for these disinfectants to kill different microorganisms was used to assess their efficacy. The microorganisms tested were six yeasts*, Saccharomyces cerevisiae, Saccharomyces uvarum, Kloeckera apiculata, Candida oleophila, Metschnikowia fructicola, Schizosaccharomyces pombe,* and two moulds, *Aspergillus niger* (5 strains) and *Penicillium roqueforti* (5 strains). The disinfectants QAC (a) and QAC (c) were the most effective against all the microorganisms tested. The chlorine-based disinfectant worked most efficiently against the moulds at all concentrations used (0.5, 1.0, 1.5 and 2.0%). Peracetic acid and alcohol based disinfectants were most effective against the yeasts than mould. Tested yeasts were more resistant to the aldehyde and iodophors base disinfectants than the others.

Key words: yeast; mould; food industry; disinfectant efficacy.

INTRODUCTION

Eighty percent of the microbiological spoilage that affects fresh and processed fruits and vegetables is caused by fungi (Fields, 1979; Kreger-Wan Rij, 1982; Acar, 1998). Cleaning and disinfection are of enormous importance for the prevention of spoilage caused by these fungi, particularly in fruit and vegetable processing factories and cold stores (Defigueiredo and Splittstoesser, 1976; Guthrie, 1980). And new trends in food production and consumption have increased the need for efficient sanitary practices in the food processing industry (Langsrud *et al.,* 2003).

The focus placed on the need for safer foods and a longer shelf life has led to the more frequent use of chemical disinfection. The aim of disinfection is to eliminate microorganisms present on the food-contact surface, thereby avoiding contamination of raw materials and products with spoilage fungi. In most cases, the failure of the disinfection process to eliminate yeast and mould to an acceptable level is due to the use of incorrect process parameters (disinfectant concentration, exposure time) or a failure of the cleaning process to disinfect microorganisms on the surface. Occasionally the user may have unrealistic expectations of the spectrum of activity of the disinfectant applied (Langsrud *et al.,* 2003).

In some areas of food processing, a comprehensive daily cleaning procedure is used in combination with disinfection once or twice daily. Disinfection should minimise the number of microorganisms, both on the premises and the equipment to an insignificantly low level, with reduction rates of approximately 5 to 8 Log_{10} (Reuter, 1998).

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Birzele *et al.* (1997) assessed how psychotropic yeasts are resistant to the disinfectants peracetic acid, quaternary ammonium compounds and formaldehyde. They investigated the effect of disinfection since microorganisms in sugar factories are known to cause product losses during the diffusion process. The microorganisms in the flora during diffusion were reported to be bacteria with aerobic spores; the yeasts primarily belonged to the genus *Saccharomyces.* The most effective level of inhibition was obtained by using 25 ppm of quaternary ammonium compounds in combination with 50% formalin. The osmophylic yeasts were inactivated regardless of the treatment period used with formaldehyde and peracetic acid (Fiedler, 1994).

Baca and Wieczarek (1998) investigated disinfection of the tanks, pipes and bottles in a beer factory using peracetic acid. They found that this disinfectant used in 500 ppm killed 100% of spoilage microorganisms in pipes whereas killed 98.9% of the total number of spoilage microorganisms in the tanks. This level of disinfection was also achieved in the bottles if they were cleaned prior to disinfection.

Laubscher and Viljoen (1999) determined antimicrobial activity of 9 different commercial detergents and disinfectants against *Rhodotorula mucilaginosa, Candida versatilis, Candida rugosa, Trichosporon beigelii, Debariomyces hansenii,* and *Torulaspora delbrueckii* isolated from a cheese factory. This study showed that a peroxide-based disinfectant was effective only at the end of a treatment period of 45-60 min.

Aspergillus and *Penicillium* species such as *Aspergillus niger* and *Penicillium roqueforti* are responsible for the spoilage of many food product, and feeds (Sahin and Korukluoglu, 2000; Overy *et al.,* 2003). Furthermore, *A. niger* can produce malformins, naphtho-r-pyrones, nigerazine B, nigragillin and ochratoxin A, while roquefortine C, PR toxin and mycophenolic acid are produced by *P. roqueforti* (Sahin and Korukluoglu, 2000; Varga *et al.,* 2000; Taniwaki *et al.,*

TABLE 1 – A list of the fungi examined the source of isolation and the supplier

Microorganisms	Source of isolation	Suppliers institute (Agricultural Fac., Uludag University)
Saccharomyces cerevisiae	ATTC 9763	
Saccharomyces uvarum	Wine	Food Engineering Dept.
Kloeckera apiculata	Decayed apple	Plant Protection Dept.
Candida oleophila	Decayed sweet cherries	Plant Protection Dept.
Metschnikowia fructicola	Decayed sweet cherries	Plant Protection Dept.
Schizosaccharomyces pombe	Wine	Food Engineering Dept.
Aspergillus niger 1	Olive	Food Engineering Dept.
Penicillium roqueforti 1	Olive	Food Engineering Dept.
A. niger 2 and *P. roqueforti* 2	Decayed tomatoes	Food Engineering Dept.
A. niger 3 and *P. roqueforti* 3	Kashar cheese	Food Engineering Dept.
A. niger 4 and *P. roqueforti* 4	Bread	Food Engineering Dept.
A. niger 5 and *P. roqueforti* 5	Peanut	Food Engineering Dept.

2001; Erdogan *et al.,* 2003; Blumenthal, 2004; Rundberget *et al.,* 2004). Thus, the presence and growth of these fungi in food products and feed threaten human and animal health.

The aim of this study was to assess the efficacy of commercial disinfectants, used to sanitise external surfaces in the food industry, against some fungi. The financial burden generated by the use of ineffective and excess amounts of disinfectants may be reduced by choosing an appropriate disinfectant according to the dominant species of microorganism. Environmental pollution from the use of excessive quantities of chemicals may similarly be avoided.

MATERIALS AND METHODS

Test microorganisms. *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Kloeckera apiculata*, *Candida oleophila*, *Metschnikowia fructicola*, *Schizosaccharomyces pombe*, *Aspergillus niger* (5 strains) and *Penicillium roqueforti* (5 strains) were the microorganisms tested. The source of each of these microorganisms is shown in Table 1.

Disinfectants. Commercial disinfectants were chosen based on recommendations for their effectiveness against fungus, their frequency of usage in the food industry for cleaning the surfaces and their representation of different disinfectant groups (Langsreud *et al.,* 2003). All the disinfectants used were mixed with sterile distilled water to the desired concentration. The highest and the lowest concentration used were those recommended on the manufacturer's label. The contents of the disinfectants and the concentrations used are provided in Table 2.

Fungal strains and the production of conidia. The fungi used in this study were cultured on Sabouraud Dextrose Agar (Difco, Detroit, I11) plates at 30 °C for 7 days. Tween 20 (1%, 10 mL) was added for spore collection. Conidia were harvested by centrifugation at 1000 rpm for 15 min and washed with 10 mL of sterile distilled water. This step was repeated three times and the spore suspension was stored in sterile distilled water at 4 °C until use. The concentration of spores in the suspension was determined by a viable spore count on Sabouraud Dextrose Agar plates using the spread plate, surface count technique (Yin and Tsao, 1999).

Preparation of yeast. Yeasts taken from the stock cultures were inoculated onto a nutrient medium consisting of 10 mL Malt Extract and the tubes were incubated at 30 °C for 24 h. This culture was diluted as required, and than inoculated onto Malt Extract Agar plates. The Petri dishes were incubated

TABLE 2 – A list of the disinfectants and the concentrations used

Disinfectants	Concentration (%)*	Main active components
Alcohol-based	Direct	Isopropyl alcohol
Peracetic acid-based	0.1 - 0.3	Peracetic acid, peroxide
Iodophors	0.5 - 1.0	Iodine
Aldehydes	0.2 - 0.5	Formaldehyde
QAC-based (a)**	2.0	Alkyldimethylbenzylammonium chloride
QAC-based (b)	0.5 - 1.0	Didecyldimethylammonium chloride
QAC-based (c)	0.5 - 1.0 - 1.5 - 2.0	Benzalkanium chloride
Chlorine-based	0.5 - 1.0 - 1.5 - 2.0	Sodium hypochloride

* All the concentrations given for commercial products are as recommended by the manufacturer. ** QAC, quertarnary amine compounds; a, b, c, defines different product provided by different suppliers.

at 30 °C for 48 h; colony counts were than performed at the end of this period.

Test procedures. To determine effects of disinfectants on fungi, we used a modified version of the methods of Orr and Beuchat (2000) and Senel and Basoglu (2002). The recommended concentrations of the disinfectants (Table 2) were prepared immediately after inoculation of the microorganism cultures onto agar plates. Each disinfectant solution (5 mL) was combined with 1 mL of the 24 h fungi culture. These were incubated at room temperature for periods ranging from 1 to 60 min (being the period selected as the max time for disinfection in the food industry). After thorough mixing, 1 mL of each of the yeast and mould plus disinfectant solutions was used to inoculate 5 mL Malt Extract (for yeast) or Sabouraud Broth (for mould). These were incubated at 30 °C for 48 h and were evaluated for growth at the end of this period. Visible turbidity was used to indicate positive (+) existent growth and negative (-) or non-existent growth. Furthermore, survival was monitored by applying surface plate method onto Sabouraud Dextrose and Malt Extract Agar plates from the tubes thought to be negative. Those solutions where survival was still observed following 60 min of disinfection were monitored for 14 days. All tests were performed 4 times.

Statistical analysis. Statistical inferences hierarchical cluster analysis and one-way variance analysis were used. The analyses were carried out using the statistical package the SPSS 10.0.

RESULTS AND DISCUSSION

The results obtained from this study are summarised in Table 3. The antifungal effect of each disinfectant is as follows.

Alcohol

Alcohol was shown to be very effective against the yeasts *S. cerevisiae*, *S. uvarum, K. apiculata* and *S. pombe* and no survival was observed even after inoculation onto the nutrient medium immediately following disinfectant treatment. *Metschnikowia fructicola* showed the highest level of resistance to alcohol-based disinfectant followed by *C. oleophila*. Other investigators have also reported similar results. Kıvanc *et al*. (1989) investigated the effects of alcohol and phenol compounds on *S. cerevisiae* and *K. apiculata* and showed that *K. apiculata* was the more sensitive to both of these disinfectants. Fraise (1999) reported that alcohol based disinfectants had rapidly fungicidal activity.

Among the test microorganisms tested, moulds exhibited a greater resistance to alcohol compared with yeasts. Alcohol showed no inhibitory effect on *P. roqueforti* 3 and 5 isolated from kashar cheese and peanuts, whereas it was effective against *P. roqueforti* 1 and killed it within 2 min. The inhibition periods of *A. niger* strains were similar although *A. niger* 2 was the least resistant (10 min), and *A. niger* 3 was the most resistant (25 min).

Peracetic acid

The peracetic acid-based disinfectant was very effective against yeast even at 0.1% concentration. No growth was observed in *K. apiculata* and *C. oleophila*, while *S. uvarum* was the most resistant species (7 min). When used at 0.3% concentration no survival was observed in all the yeasts, except *S. uvarum*, which was killed within 2 min of exposure to the disinfectant. Fraise (1999) explained that peracetic acid was a strong oxidising agent and had fungicidal activity against a range of fungi. Winniczuk and Parish (1997) determined that 0.02% peracetic acid killed *S. cerevisiae* isolated from spoilage orange juice. Orth (1998) found that 0.5% peracetic acid demonstrated ≥ 5.7 log reduction rates after 5 min. Similarities and differences might be explained with different strains used in our study.

This disinfectant (0.3%) was able to kill *A. niger* 2 and *A. niger* 4 after exposures of 60 and 55 min respectively, while it had no effect on the other strains. The minimum inhibitory period for *P. roqueforti* 3 was found to be 32 min at a disinfectant concentration of 0.1% and at a concentration of 0.3% was killed within 5 min. The most resistant strain of *P. roqueforti* was found to be *P. roqueforti* 4, which was killed within 56 and 16 min with a disinfectant concentration of 0.1% and 0.3% respectively.

Iodophors

The iodine-based disinfectants showed no inhibitory effects on *S. uvarum* and *S. cerevisiae* at the concentrations used (0.5-1.0%). *Schizosaccharomyces pombe* was the most resistant microorganism to the type of disinfectant (killed within 52 min at a concentration of 0.5% and within 31 min at 1.0%) and *K. apiculata* was the most sensitive (killed within 12 min at a disinfectant concentration of 0.5% and within 5 min at a concentration of 1.0%). *Aspergillus niger* strains (killed within 12-49 min) were more resistant than *P. roqueforti* (killed within 8-18 min) to this disinfectant at concentration of 1.0 %.

Aldehydes

In yeasts the greatest resistance to the aldehyde-based disinfectant, when used at a concentration of 0.5% was exhibited by *M. fructicola* whereas *S. pombe* was the most sensitive. Moulds displayed a greater sensitivity to aldehyde-based disinfectants and at a concentration of 0.5% they were inhibited within a period of 1-12 min.

In our study, while disinfectant containing 0.5% aldehyde showed fungicidal effect against *A. niger* maximum in 5 min and *P. roqueforti* in 12 min, Bundgaard-Nielsen and Nielsen (1996) observed that the use of a disinfectant containing 2% formaldehyde applied for 10 min had no fungicidal effects on *A. niger* and P. *roqueforti.*

Quaternary ammonium compounds (QAC)

Among the QAC-based disinfectants tested the most effective was QAC(c). QAC(c) was effective against *S. uvarum* (3 min), *C. oleophila* (1 min), *A. niger* 3 (3 min) and *A. niger* 5 (2 min) at a concentration of 1.0%, all the other microorganisms were killed at this concentration. QAC(a), when used at a concentration of 2% was very effective (within 2 to 11 min) against all the microorganisms tested.

The greatest resistance to QAC-based disinfectants was demonstrated by *S. uvarum* and *A. niger* 3 among the yeasts and moulds, respectively. However, *P. roqueforti* demonstrated sensitivity to this type of disinfectant and effective inhibition was achieved within a short time, depending on the concentration used. Ozyurt (2000) announced that *A. niger* was killed with QAC disinfectant at a concentration of 1% in < 2 min. This result is similar to our study.

TABLE 3 – The minimum inhibitory time in different concentrations of the commercial disinfectants tested against various fungi (n = 4) inoculated at the 10^6 level

Fungi*	Minimum inhibitory time (min)**																
	Alcohol (direct)	Peracetic acid (%)		Iodophor (%)		Aldehydes (%)		QAC (a) (%)***	QAC (b) (%)		QAC (c) (%)			Chlorine (%)			
		0.1	0.3	0.5	1	0.2	0.5	2	0.5	1	0.5	1	1.5	0.5	1	1.5	2
S. cerevisiae	-	3±0.2	-	Nd	Nd	Nd	10±0.3	5±0.6	Nd	Nd	5±0.0	-	-	Nd	Nd	Nd	Nd
S. uvarum	-	7±0.3	2±0.1	Nd	Nd	Nd	15±0.6	11±0.1	Nd	Nd	16±0.3	3±0.1	-	Nd	Nd	43±0.8	12±0.6
K. apiculata	-	-	-	12±0.6	5±0.2	Nd	8±0.2	-	15±0.4	12±0.3	-	-	-	12±0.2	-	-	-
C. oleophila	23±0.2	-	-	18±0.5	6±0.2	47±0.7	23±0.2	-	12±0.4	4±0.2	8±0.1	1±0.1	-	33±0.9	25±1.1	4±0.3	-
M. fructicola	37±0.7	2±0.1	-	25±0.5	11±0.2	58±0.6	25±0.3	-	8±0.2	4±0.2	8±0.1	-	-	Nd	56±0.7	18±0.7	3±0.1
S. pombe	-	4±0.1	-	52±0.4	31±0.3	23±0.6	-	-	15±0.2	6±0.1	5±0.1	-	-	Nd	Nd	Nd	36±0.1
A. niger$_1$	20±0.3	Nd	Nd	Nd	35±0.4	10±0.0	5±0.1	2±0.0	Nd	Nd	5±0.3	-	-	1±0.2	-	-	-
A. niger$_2$	10±0.1	Nd	60±2.0	57±1.1	32±0.2	8±0.0	2±0.1	-	60±1.2	51±0.8	-	-	-	-	-	-	-
A. niger$_3$	25±0.6	Nd	Nd	Nd	49±1.0	15±0.5	5±0.3	5±0.2	Nd	Nd	12±0.3	3±0.2	-	3±0.1	1±0.1	-	-
A. niger$_4$	12±0.6	Nd	55±1.3	53±0.7	28±0.6	5±0.1	2±0.0	3±0.1	Nd	45±0.8	3±0.1	-	-	-	-	-	-
A. niger$_5$	18±0.2	Nd	Nd	34±0.7	12±1.3	7±0.1	3±0.1	5±0.3	Nd	Nd	6±0.6	2±0.1	-	2±0.0	-	-	-
P. roqueforti$_1$	2±0.0	45±1.1	10±0.4	29±0.2	11±0.5	15±0.3	3±0.2	-	45±1.5	12±0.2	-	-	-	-	-	-	-
P. roqueforti$_2$	5±0.1	45±0.6	13±0.4	18±0.1	12±0.5	22±0.5	8±0.5	2±0.1	51±1.3	21±0.4	2±0.0	-	-	-	-	-	-
P. roqueforti$_3$	Nd	32±0.8	5±0.6	33±1.6	15±0.7	13±0.3	5±0.2	-	38±0.9	8±0.4	-	-	-	-	-	-	-
P. roqueforti$_4$	8±0.3	56±0.8	16±0.9	42±1.2	18±0.5	25±0.6	12±0.5	3±0.1	50±0.9	19±0.3	3±0.0	-	-	-	-	-	-
P. roqueforti$_5$	Nd	43±1.5	8±0.3	26±0.7	8±0.1	9±0.7	1±0.1	-	22±1.0	13±0.2	-	-	-	-	-	-	-

*$_{1,2,3,4,5}$: Microorganisms isolated from different sources; ** Average±SD; *** QAC, quertarnary amine compounds; a, b, c, defines different product provided by different suppliers; -: no growth; Nd: no death detected within 14 days.

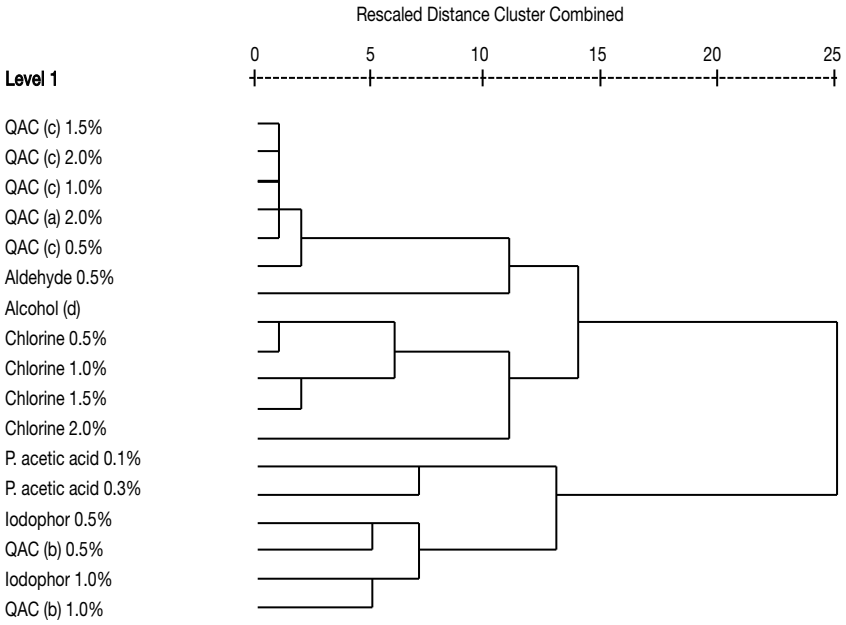

FIG. 1 – Dendrogram from a cluster analysis grouping the different disinfectants based on their effect on various food spoilage fungi.

Chlorine

Chlorine-based disinfectant activity increased as depending on concentration leveland inhibition was determined at different time and concentrations on the tested yeasts, except *S. cerevisiae* that was the most resistant yeast even at a disinfectant concentration of 2%. On the other hand, *K. apiculata* was the most sensitive test yeast with that being observed within 12 min at a disinfection concentration of 0.5%.

This disinfectant was most effective against the moulds. *Penicillium roqueforti* did not show any growth, even at a concentration of 0.5%, while *A. niger* was inhibited within a maximum of 3 min.

Results are comparable to the antifungal activity of hypochlorite reported in literature. In these studies disinfectant containing hypochlorite inhibited *S. cerevisiae* with 0.1% (Winniczuk and Parish, 1997), *A. niger* with 0.2% concentration (Ozyurt, 2000). In addition Reynolds *et al.* 2004 used NaOCl against *Penicillium, Cladosporium, Mucor, Rhizopus, Stachybotrys, Alternaria, Aspergillus, Helminthosporium* and *Trichophyton* and found that these moulds were reduced by > 5 logs within 5 min.

Cluster analysis units were used for dividing homogenous groups in some similarity or differences calculated between variables (Özdamar, 2003). Hierarchical cluster analysis was applied to compare the effects of disinfectants, which were classified into 3 groups (Fig. 1). These groups were (1) peracetic acid (0.1% and 0.3%), iodophor (0.5% and 1.0%) and QAC(b) (0.5% and 1.0%); (2) chlorine (0.5%, 1.0%, 1.5% and 2.0%) and aldehyde (0.2%) and (3) QAC(a) (2.0%), QAC(c) (0.5%, 1.0%, 1.5% and 2.0%), aldehyde (0.5%) and alcohol (direct). The results from the cluster analysis indicate that, at the concentrations used QAC(b) and iodophor-based disinfectants were similarly effective against all of the microorganisms tested, while peracetic acid-based disinfectant was only effective against the moulds. PAA has shown good disinfection efficiency against enteric bacteria in wastewaters, but viruses, bacterial spores and protozoan cysts are more resistant (Liberti and Notarnicola, 1999; Stampi *et al.,* 2001, 2002; Salgot *et al.,* 2002; Wagner *et al.,* 2002; Veschetti *et al*., 2003; Koivunen and Heinonen-Tanski, 2005). In addition, peracetic acid-based disinfectant might be used for the inhibition of food borne fungi as an industrial surface disinfectant. In the second group, all concentrations of chlorine-based disinfectant were similarly effective against the microorganisms tested, and this level of effectiveness was similar to that of the aldehyde-based disinfectant at a concentration of 0.2%. For this reason, it is thought that both of these disinfectants have the same effect on yeast. The microorganisms tested were shown to have a fast death time in the 3rd group, except ethyl alcohol. By contrast, no-anti-fungal effect on the part of the alcohol-based disinfectant was observed against *P. roqueforti* isolated from kashar cheese and peanuts. *Penicillium roqueforti* may show a different resistance when isolated from varying sources and/or species.

The results of this study showed clearly that the choice of disinfectant along with the optimum concentration and the time of action is very important when destroying food spoilage fungi in the manufacturing industry. While planning the sanitising process, it is important that the resistance of fungi varies with respect to the genus, species, strain and source from which it was isolated as well as the active ingredients of the disinfectant used. These factors will be included in a working HACCP system for factories. In addition the disinfectant should be tested for its effectiveness against microorganisms according to the manufacturer's instructions and this should be supported by a cost analysis.

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