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Food spoilage yeasts: effects of pH, NaCl and temperature on growth

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Abstract

The effects of temperature (8 and 22°C), pH (2.5–6.0) and NaCl concentration (0.4–8% w/v) were evaluated on the time to growth for 13 strains of yeasts representing five genera: *Debaryomyces*, *Pichia*, *Zygosaccharomyces*, *Candida* and *Saccharomyces*. Laboratory media were made at various combinations of the three factors, inoculated with the individual yeasts, incubated for up to a maximum of 56 days and observed for signs of turbidity. At 22°C, 12 of the 13 yeast species tested were able to grow at 8% NaCl, although the rate of growth varied considerably, taking between 1 day (*Candida guilliermondii*) and 32 days (*Candida holmii*) to reach turbidity. All 13 species were able to grow at pH 2.5, although the maximum NaCl concentration permitting growth at this pH ranged from 0.8% (*Debaryomyces* sp.) to 8% (eight species). When the temperature was reduced to 8°C, the maximum salt level permitting growth was generally lower and only five species were able to grow at 8% NaCl. In addition, the minimum pH permitting growth was increased and only 10 of the 13 species grew at pH 2.5. It was evident that there was a synergistic effect between NaCl and pH at the lower temperature. For example, for *Candida parapsilosis* the maximum NaCl concentration permitting growth was 6.4, 4.8, 3.2 and 1.6% when the pH was 4.5, 4, 3.5 and 3 respectively. These data have an application to producers of low pH chilled products and could be used to assess the spoilage potential of new and existing product formulations.

Keywords: Yeasts; Growth; Spoilage

1. INTRODUCTION

Yeasts are responsible for the microbiological spoilage of a wide range of chilled and ambient stable products including milk and dairy, fermented salads and mayonnaises, bakery goods, jams and preserves, fruit products, raw meat and fish.

In some cases, there is a diverse yeast flora present, for example Viljoen and Greyling (1995) isolated 187 species of yeasts from a Cheddar and Gouda factory belonging to nine different genera (*Saccharomyces*, *Yarrowia*, *Zygosaccharomyces*, *Torulaspora*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Kluyveromyces* and *Trichosporon*). Similar genera of yeasts have been isolated from Camembert and blue-veined cheeses (Roostita and Fleet, 1996).

In other cases the spoilage flora is less diverse; for example, Fleet (1990) suggests that spoilage of dairy products is caused by a relatively small group of organisms, including *D. hansenii*, *Candida holmii*, *Pichia membranaefaciens* and *Rhodotorula glutinis*, whilst Guerzoni et al. (1993) found that the yeast populations of commercial chilled products were unexpectedly uniform, comprising principally *Y. lipolytica*, *D. hansenii* and *P. membranaefaciens*.

It is known that the particular processing technique used for each product will select for the growth of particular types of yeasts which are adapted to grow under the environmental stress created. For example, foods which contain high levels of salt and sugar will select for yeasts capable of growth under low water activity, such as *Debaryomyces hansenii* in the case of high salt and *Zygosaccharomyces rouxii* in the case of high sugar (Board, 1994).

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Fermented salads, salad dressings and mayonnaises will select for yeasts which have a degree of resistance to acetic acid which is often present in these products, for example *S. dairiensis* and *S. exiguus* (Brocklehurst et al., 1983) and *S. cerevisiae* (Hunter et al., 1994).

In a recent review on Fungi and Food Spoilage, Pitt and Hocking (1997) stated that in their experience only 10 species of yeasts are responsible for spoilage of foods which have been processed and packaged according to GMP: these include *Z. bailii*, *S. cerevisiae*, *D. hansenii* and *Candida krusei*.

There are a number of other excellent reviews which fully describe the association of spoilage yeasts in a range of food commodities (Deak, 1991; Fleet, 1990, 1992).

It has been shown that a wide range of yeast species are able to grow in chilled dairy, fermented products and low pH foods such as salad dressings and mayonnaise; however, relatively few data exist on the interactive effectiveness of salt, pH and temperature on the time to growth of a range of species most commonly associated with food spoilage. Data are available to describe the effects of single factors, e.g. temperature, on the growth of yeasts, although there is relatively little information on the behaviour of yeasts at low temperatures, especially for yeasts other than *S. cerevisiae* (Fleet, 1992).

There appears therefore to be gaps in the knowledge on the influence of environmental factors on the physiological properties of spoilage yeasts.

The aim of this study was to evaluate the time taken for visible growth to occur for common spoilage yeasts in order to determine the effect of changes in growth temperature, pH and salt concentration.

2. MATERIALS AND METHODS

2.1. Microorganisms

Thirteen strains of yeast, which had been isolated from dairy sources, salad dressings or fruit environments were chosen from the Campden and Chorleywood Food Research Association culture collection. These represented five of the major genera of yeasts associated with spoilage of these food types.

Candida holmii, CRA627 (Yoghurt factory); *Candida krusei*, CRA629 (Yoghurt base); *Pichia anomala*, CRA626 (Sugar solution in yoghurt factory); *Hansenula polymorpha*, CRA636 (Yoghurt); *Zygosaccharomyces* spp., CRA104 (Salad cream); *Zygosaccharomyces* spp., CRA105 (Sandwich spread); *Candida parapsilosis*, CRA106 (Mayonnaise); *Candida parapsilosis*, CRA209 (Cottage cheese); *Candida guilliermondii*, CRA290 (Cottage cheese); *Saccharomyces cerevisiae*, CRA585 (18% sherry); *Debaryomyces* spp., CRA3238 (Dried Grape Powder); *Pichia anomola*, CRA6402; *Saccharomyces cerevisiae*, CRA6413; *Zygosaccharomyces bailii*, CRA230.

A freeze-dried sample of each strain of yeast was rehydrated in 10 ml Malt Extract Broth (Oxoid CM57) supplemented with 2% glucose (MEBG) and incubated at 25°C for 5 days. Following incubation, the broths were streaked onto the surface of slopes and plates of Malt Extract Agar (LabM LAB 37, MEA), incubated at 25°C for 3 days and checked for purity.

The MEA slopes were then maintained at 4 ± 2°C until required for use.

2.2. Growth studies

Various combinations of salt (0.4–8% w/v NaCl) and pH (2.5–7.0) were produced in an MEBG broth base.

The correct amount of salt was weighed out for each concentration and added to a volumetric flask. Deionized water was then added to make a total volume of 1 litre and this was added to the MEBG powder.

The pH was adjusted using HCl or NaOH and the media autoclaved at 115°C for 10 min. The pH was rechecked after autoclaving.

Each MEBG produced (200 μl) was added to a single well of a 96-well microtitre plate.

In preparation for inoculation of the wells, a sample of each yeast strain was taken from the MEA slope, inoculated into MEBG and incubated at 25°C for 5 days. MEB yeast culture (50 μl) was added to each well to give a concentration of approximately 10^4 cells per ml. Allowances were made for this dilution effect on the final concentration of salt.

The microtitre plates were incubated at 22 ± 1°C and 8 ± 1°C and examined visually for signs of turbidity for up to 22–57 days (see Tables 1 to 5).

3. RESULTS AND DISCUSSION

Tables 1–5 show the time taken for visible turbidity to occur under the various conditions of salt, pH and temperature. For the sake of brevity, the data for each genus studied have been combined and the range is given for the shortest and longest time to turbidity.

Table 1 contains data for *Zygosaccharomyces* (three strains), whilst the data for *Candida* (five strains), *Debaromyces* (one strain), *Pichia* (two strains) and *Saccharomyces* (two strains) are shown in Tables 2–5 respectively.

3.1. Effect of NaCl

Of the 13 species of yeasts tested, 12 were able to grow at 8% NaCl when the pH and temperature were optimum for growth.

Table 1
Time to visible growth (days) of *Zygosaccharomyces* species* in MEBG at 22 and 8°C

pH	Salt (%w/v)										
	0.4	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8
(a) 22°C											
7	1–7[a]	2–8	3–16	4–16	8–NG	4–NG	7–NG	4–NG	NG	10–NG	NG
6	1–2	1–2	1–4	1–8	3–NG	3–NG	4–NG	7–NG	7–NG	NG	NG
5.5	1–2	1–2	1–2	1–3	1–4	3–8	3–18	3–NG	4–NG	4–NG	4–NG
5	1–2	1–2	1–2	1–2	1–4	3–7	3–18	3–24	3–18	4–21	4–NG
4.5	1–2	1–2	1–2	1–2	1–3	2–4	2–8	2–8	3–8	3–NG	4–NG
4	1–2	1–2	1–2	1–2	1–3	2–4	2–7	2–16	3–8	3–24	4–NG
3.5	1–2	1–2	1–2	1–2	2	2–7	2–16	2–16	3–8	3–18	4–NG
3	1–3	1–3	1–3	2–3	2–3	2–3	2–4	3–4	3–7	3–7	4–7
2.5	2–3	2–3	2–3	2–4	2–4	2–4	3–7	3–7	3–7	4–14	4–14
(b) 8°C											
	14–NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	10–18	18–NG	14–NG	NG	NG	NG	NG	NG	NG	NG	NG
5.5	10–17	10–18	13–NG	14–NG	NG	NG	NG	NG	NG	NG	NG
5	10–13	10–17	10–25	13–25	13–NG	NG	NG	NG	NG	NG	NG
4.5	10–13	10–13	10–13	10–17	13–NG	13–NG	14–NG	22–NG	NG	NG	NG
4	10–13	10–13	10–13	10–13	10–NG	13–NG	14–NG	22–NG	NG	NG	NG
3.5	10–13	10–13	10–13	10–13	10–NG	13–NG	17–NG	22–NG	22–NG	NG	NG
3	13–NG	13–NG	13–NG	13–NG	22–NG	22–NG	22–NG	NG	NG	NG	NG
2.5	13–NG	13–NG	13–NG	17–NG	22–NG	22–NG	NG	NG	NG	NG	NG

Z. bailii CRA230, *Z. bailii* CRA104, *Z. bailii* CRA105.
NG = no growth after 22 days.
[a]Growth first detected (days), range of three species shown.

Table 2
Time to visible growth of *Candida* species* in MEBG at 22 and 8°C

pH	Salt (%w/v)										
	0.4	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8
(a) 22°C											
7	1[a]	1	1	1	1–2	1–2	1–3	1–4	1–7	1–11	1–NG
6	1	1	1	1	1–2	1–2	1–2	1–2	1–4	1–14	1–NG
5.5	1	1	1	1	1–2	1–2	1–2	1–2	1–4	1–14	1–32
5	1	1	1	1	1–2	1–2	1–2	1–2	1–3	1–NG	1–NG
4.5	1	1	1	1	1–2	1–2	1–2	1–2	1–3	1–NG	1–NG
4	1	1	1–2	1–2	1–2	1–2	1–2	2–3	2–NG	2–7	3–23
3.5	1	1	1–2	1–2	1–2	2–3	2–4	2–7	2–NG	3–NG	3–NG
3	1	1	1–2	1–2	1–4	1–NG	2–7	2–NG	2–NG	2–NG	3–NG
2.5	1	1	1–2	1–NG	1–NG	1–NG	2–NG	2–NG	2–NG	2–NG	3–NG
(b) 8°C											
7	6–10	6–14	6–NG	6–NG	6–NG	6–NG	10–NG	10–NG	10–NG	10–NG	10–NG
6	6–14	6–14	6–14	6–14	6–14	10–NG	10–NG	10–NG	10–NG	10–NG	14–NG
5.5	6–14	6–14	6–14	6–14	6–14	10–NG	10–NG	10–NG	10–NG	10–NG	14–NG
5	6–14	6–14	6–14	6–14	6–NG	10–NG	10–NG	10–NG	10–NG	14–NG	14–NG
4.5	6–14	6–14	6–17	7–14	10–NG	10–NG	10–NG	10–NG	10–NG	14–NG	22–NG
4	6–14	6–14	6–14	6–17	7–NG	14–NG	14–NG	14–NG	22–NG	22–NG	NG
3.5	6–14	6–14	6–17	6–NG	7–NG	17–NG	22–NG	22–NG	22–NG	22–NG	NG
3	6–17	6–22	6–17	7–NG	7–NG	10–NG	13–NG	22–NG	22–NG	22–NG	22–NG
2.5	7–NG	6–NG	6–NG	7–NG	7–NG	10–NG	13–NG	17–NG	22–NG	NG	NG

C. krusei CRA629, *C. parapsilosis* CRA209, *C. parapsilosis* CRA106, *C. holmii* CRA627, *C. guilliermondii* CRA296.
[a]Growth first detected (days), range of five species shown.
NG = no growth after 32 days (22°C) or 22 days (8°C).

Table 3
Time to visible growth (days) of *Debaryomyces* species (CRA3238) in MEBG at 22 and 8°C

pH	Salt (%w/v)										
	0.4	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8
(a) 22°C											
7	1[a]	1	1	1	1	1	1	1	2	2	2
6	1[a]	1	1	1	1	1	2	2	2	2	2
5.5	1	1	1	1	2	2	2	2	2	2	2
5	1	1	1	1	2	2	2	2	2	2	2
4.5	1	1	1	1	2	2	2	22	2	2	2
4	1	1	1	2	2	2	3	3	3	3	4
3.5	1	2	2	2	2	3	7	4	7	7	8
3	2	2	2	2	2	3	3	3	4	3	7
2.5	4	8	NG	NG	NG	NG	NG	NG	NG	NG	NG
(b) 8°C											
7	4	4	4	4	4	4	4	7	7	8	8
6	7	4	4	4	4	7	7	7	7	8	8
5.5	7	4	4	4	7	7	7	7	7	8	8
5	4	4	4	7	7	7	7	8	8	8	8
4.5	4	4	7	7	7	8	8	8	8	8	8
4	4	4	7	7	7	7	11	18	8	11	11
3.5	4	4	7	11	11	7	37	21	17	17	21
3	7	7	7	7	7	8	7	8	8	7	11
2.5	11	15	NG	NG	15	23	15	23	23	NG	NG

[a] = Growth first detected (days).
NG = no growth after 22 days.

Table 4
Time to visible growth (days) of *Pichia anomola** in MEBG at 22 and 8°C

pH	Salt (%w/v)										
	0.4	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8
(a) 22°C											
7	1[a]	1	1	1	1–2	1–2	1–2	1–3	1–3	2–4	2–7
6	1	1	1	1	1–2	1–2	1–2	1–2	2–3	2–3	2–NG
5.5	1	1	1	1	1–2	1–2	1–2	1–2	2–3	2–3	2–4
5	1	1	1	1	1–2	1–2	1–2	1–2	2	2–3	2–4
4.5	1	1	1	1	1–2	1–2	1–2	1–2	2–3	2–3	2–7
4	1	1	1–2	1–2	1–2	1–2	1–2	2–3	2–3	2–4	2–7
3.5	1	1	1–2	1–2	1–2	1–3	2–4	2–3	2–4	2–7	2–NG
3	1	1	1	1–2	1–2	2–3	2–3	2–7	2–7	2–7	2–18
2.5	1	1–2	2	3	3	3–8	3–3	3–18	3–14	4–5	17–NG
(b) 8°C											
7	6–7	6–7	6–7	7	7	7	7–14	14–17	14–17	14–NG	14–NG
6	6–7	6–7	6–7	7	7	7	7–17	8–17	14–17	14–NG	25–NG
5.5	6–7	6–7	7	7	7	7–13	7–17	8–17	14–17	14–17	14–NG
5	6–7	6–7	7	7	7–17	7–17	7–17	8–NG	8–17	14–17	14–NG
4.5	6–7	6–7	7–13	7–14	7–17	7–NG	8–NG	8–NG	8–NG	21–NG	25–NG
4	6–7	6–7	7–13	7–13	7–22	7–NG	8–NG	14–NG	21–NG	25–NG	NG
3.5	6–7	7	7	7–14	8–22	11–NG	21–NG	14–NG	25–NG	29–NG	NG
3	6–7	8	6–7	6–11	6–11	6–15	6–11	7–18	7–23	16–18	10–NG
2.5	6–15	15	6–18	6–NG	6–NG	6–NG	7–NG	7–NG	10–NG	13–NG	14–NG

**Pichia anomola* CRA6402, *Pichia anomola* CRA626.
[a]Growth first detected (days) (range of two species).
NG = no growth after 56 days (22°C) or 37 days (8°C).

Table 5
Time to visible growth (days) of *Saccharomyces cerevisiae** in MEBG at 22 and 8°C

pH	Salt (%w/v)										
	0.4	0.8	1.6	2.4	3.2	4	4.8	5.6	6.4	7.2	8
(a) 22°C											
7	1[a]–2	2	2	2–3	2–3	2–8	3–16	7–NG	8–NG	11–NG	16–NG
6	1–2	1–2	1–2	2	2–3	2–24	3–NG	4–NG	7–NG	11–NG	16–NG
5.5	1–2	1–2	1–3	2–3	3	3–7	7–11	7–NG	7–NG	11–NG	11–NG
5	1–2	1–2	1–3	2–7	3	7–8	3–NG	7–NG	7–NG	11–NG	11–NG
4.5	1–2	1–2	1–3	2–3	3	3–8	3–NG	7–NG	7–NG	11–NG	NG
4	1–2	1–2	2	2–4	3–NG	3–NG	3–NG	8–NG	8–NG	14–NG	16–NG
3.5	1–2	1–2	2	3–8	3–8	8–NG	32–NG	11–NG	11–NG	NG	NG
3	1–2	1–2	1–2	3	3–NG	4–NG	3–NG	7–NG	7–NG	7–NG	10–NG
2.5	1–4	2–4	4–7	NG	7–NG	10–NG	NG	NG	NG	NG	NG
(b) 8°C											
7	8	14–25	14–25	14–NG	25–NG	17–NG	17–NG	32–NG	32–NG	32–NG	32–NG
6	7–21	11–21	17–11	18–NG	NG	21–NG	17–NG	21–NG	NG	NG	37–NG
5.5	7–18	7–NG	11–25	11–NG	25–32	NG	32–NG	NG	NG	25–NG	NG
5	7–14	7–25	11–NG	11–21	25–NG	NG	32–NG	NG	NG	NG	32–NG
4.5	7–11	7–11	11–21	11–21	14–21	NG	21–NG	14–NG	21–NG	32–NG	25–NG
4	7–8	7–8	8–9	8–14	8–NG	8–NG	8–18	8–NG	8–NG	8–NG	14–NG
3.5	7	7	7–9	7–17	7–NG	11–NG	8–NG	11–NG	11–NG	11–NG	15–NG
3	7	7–8	7–8	7–11	7–NG	11–NG	8–NG	11–NG	11–NG	11–NG	15–NG
2.5	7–8	8–11	8–11	NG	18–NG	NG	NG	NG	NG	NG	NG

S. cerevisiae CRA6413, *S. cerevisiae* CRA585.
[a]Growth first detected (days) (range of two species).
NG = no growth after 56 days.

The time to growth varied considerably, ranging from 1 day for *Candida guilliermondii* to 32 days for *Candida holmii* (Table 2). This shows the variation in growth characteristics by different *Candida* spp., particularly under extreme environmental conditions.

One yeast species, *Saccharomyces cerevisiae* CRA6413, which did not grow at 8% (Table 5), had a maximum NaCl concentration permitting growth of 4.8%.

Previous studies (Praphailong and Fleet, 1997) showed that *S. cerevisiae* did not grow at salt levels higher than 7.5% within a 14-day storage period. In this study, the tolerance of *S. cerevisiae* to salt showed strain to strain variation as one strain of *S. cerevisiae* tested grew at a level of 8% salt within 8 to 11 days whilst the other did not tolerate > 4.8% salt.

3.2. Effect of pH

When the temperature and NaCl were at non-inhibitory levels, all strains tested were able to grow at a pH of 2.5 within 1 to 4 days.

When the temperature was reduced to 8°C the minimum pH which allowed growth increased for three of the 13 strains. For example, the minimum pH allowing growth of both strains of *C. parapsilosis* (Table 2) and *Z. bailii* (CRA230) (Table 1) increased from 2.5 to 3.0 and 3.5 respectively.

An interesting observation from these data is the relationship between the different species of yeasts and optimum pH range for growth. All three species of *Zygosaccharomyces* tested had an optimum pH range for growth of 3.5 to 5.5; particularly at 8°C, the time to visible growth decreased either side of this pH range. The optimum pH range for growth became narrower, e.g. 3.5 to 4.5, as the salt level increased.

Similar data were found by Praphailong and Fleet (1997) where *Z. bailii* (six strains) grew in the pH range 3.0 to 6.5 but not at pH 2.0 and 7.0.

Other species of yeasts tested did not exhibit such a marked pattern of growth in relation to the pH of the medium, although most grew better at pH 6.0 or below than they did at pH 7.0.

3.3. Effect of temperature

All 13 yeast strains grew at 8°C, although the time to visible growth was markedly slower than at 22°C; for example, the time to turbidity at 0.4% NaCl ranged from 4 to 22 days at 8°C compared to 1 to 7 days at 22°C.

3.4. Interactions between pH, salt and temperature

It is apparent from these data that pH and salt have a synergistic effect in relation to growth of spoilage

yeasts. For example, the time to growth generally increased at any single pH value as salt increased or at any single NaCl concentration as the pH decreased. This can be seen from *P. anomola* (Table 4) where the time to growth was 1 day at pH 2.5 when the NaCl concentration was 0.4% and up to 51 days at pH 2.5 when it was 8%.

The synergistic effect of pH and salt was greatly enhanced at 8°C. At this temperature, the maximum salt concentration permitting growth was generally much lower than that seen at 22°C. For example, *Z. bailii* grew in 8% NaCl/pH 5.0 at 22°C within 4 days but was only able to grow in 6.4% salt/pH 3.5 at 8°C within 22 days (Table 1). *C. parapsilosis* CRA106 was able to grow in 8% NaCl/pH 2.5 within 3 days at 22°C, yet when the incubation temperature was 8°C, this strain did not grow at pH 2.5 and was only able to tolerate levels of 2.4% salt at pH 3 and 3.2% salt at pH 3.5 (Table 2).

This effect has recently been reported for *D. hansenii* where growth was observed at 10% NaCl at pH 3–7, but only occurred at a maximum of 2.5% NaCl at pH 2.0 (Praphailong and Fleet, 1997).

Similar effects have been seen in food systems where *Z. bailii* grew at water activity (Aw) 0.90 (16% NaCl) when the pH was 3.6 but only grew at Aw 0.95 (8% NaCl) when the pH was reduced to 3.2 (Meyer et al., 1989).

From the five genera of spoilage yeasts tested, *Debaryomyces* appeared to be most tolerant of the combined effects of pH, salt and temperature.

4. CONCLUSIONS

It has been shown that there is a wide diversity in the growth characteristics of species of yeasts commonly associated with dairy and fermented salad products. Of the 13 strains of yeasts evaluated, all but one (*S. cerevisiae*) were able to grow in broths containing 8% sodium chloride (NaCl) when incubated at 22°C. In addition, all species were able to grow at pH 2.5, although the maximum levels of salt permitting growth ranged from 0.8% (*Debaryomyces* sp.) to 8% (eight strains).

The time to visible growth of some species was very rapid; for example, with *Candida guilliermondii*, turbidity was observed within 1 day at 8% NaCl/pH 4.5 and within 3 days at 8% NaCl/pH 2.5. For other species, growth was markedly slower under the same conditions; for example, *Candida holmii* took 32 days to grow at 8% NaCl/pH 5.5. This prolonged lag phase and slow growth rate is of significance to manufacturers of low pH, high salt products where spoilage may not occur within the first few weeks but may develop a month into the shelf-life.

At a reduced temperature of 8°C, the maximum salt level permitting growth was generally much lower than at 22°C. Only five of the 13 species were able to grow at 8°C in 8% NaCl compared to 12 species at 22°C. The minimum pH permitting growth was also affected by the low temperature. At 8°C only 10 yeast species were able to grow at pH 2.5 compared to all 13 species at 22°C.

It was also evident that there was a synergistic effect between pH and NaCl at the lower temperature. For example, *C. parapsilosis* grew at 5.6% NaCl when the pH was 7 to 4.5 but could only grow at 4.8, 3.2 and 1.6% NaCl when the pH was 4, 3.5 and 3.0 respectively.

Of the five genera tested, *Debaryomyces* appeared to be most resistant to the interactive effects of pH, salt and temperature, and was able to grow under 95% of the conditions tested within 1 to 37 days. This is perhaps not surprising due to the high tolerance shown by this organism to salt (Pitt and Hocking, 1997; Deak, 1991). Measures other than pH, temperature and salt level (up to 8% w/v) would be needed to inhibit growth of this organisms in chilled products with an extended shelf-life.

For the other four genera tested, the synergistic effect of the three factors studied did show inhibition of growth. These data can be used to assess the spoilage potential of new and existing chilled, acidified food products with respect to the most common food spoilage yeasts.

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