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Influence of temperature, water activity and pH on growth of some xerophilic fungi

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Abstract

The combined effects of water activity (a_w), pH and temperature on the germination and growth of seven xerophilic fungi important in the spoilage of baked goods and confectionery were examined. *Eurotium rubrum*, *E. repens*, *Wallemia sebi*, *Aspergillus penicillioides*, *Penicillium roqueforti*, *Chrysosporium xerophilum* and *Xeromyces bisporus* were grown at 25, 30 and 37 °C on media with pH values of 4.5, 5.5, 6.5 and 7.5 and a range of water activities (a_w) from 0.92 to 0.70. The a_w of the media was controlled with a mixture of equal parts of glucose and fructose. Temperature affected the minimum a_w for germination for most species. For example, *P. roqueforti* germinated at 0.82 a_w at 25 °C, 0.86 a_w at 30 °C and was unable to germinate at 37 °C. *E. repens* germinated at 0.70 a_w at 30 °C, but at 25 and 37 °C, its minimum a_w for germination was 0.74. *C. xerophilum* and *X. bisporus* germinated at 0.70 a_w at all three temperatures. The optimum growth occurred at 25 °C for *P. roqueforti* and *W. sebi*, at 30 °C for *Eurotium* species, *A. penicillioides* and *X. bisporus* and at 37 °C for *C. xerophilum*. These fungi all grew faster under acidic than neutral pH conditions. The data presented here provide a matrix that will be used in the development of a mathematical model for the prediction of the shelf life of baked goods and confectionery.

Keywords: Water relations; Xerophilic fungi; Growth; Germination; Water activity; pH; Temperature

1. Introduction

Xerophilic fungi frequently cause spoilage of intermediate moisture and low water activity (a_w) baked goods (Pitt and Hocking,1997), causing considerable economic losses. A better understanding of the responses of these fungi to combinations of pH, temperature and water activity would be of great benefit to manufacturers of baked goods and confectionery, allowing greater confidence in development of new products.

Eurotium species, *Wallemia sebi* and xerophilic *Aspergillus* species such as *Aspergillus restrictus* and *A. penicillioides* are widely distributed fungi that are common contaminants of stored grains, nuts, spices and cereal products (Pelhate, 1968; Pitt and Hocking, 1997). Extreme xerophiles such as *Xeromyces bisporus* and xerophilic *Chrysosporium* species are more common in high sugar environments including confectionery and dried fruits (Pitt and Hocking, 1997). Some *Penicillium* species also cause spoilage in higher a_w baked goods; for example, *Penicillium*

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roqueforti is associated with spoilage of packaged rye bread (Lund et al., 1996). Fungi such as these may become established in the bakery environment often resulting in contamination of baked goods during cooling (Boey et al., 2001). Occasionally, heat-resistant ascospores of *Xeromyces* or *Eurotium* (Pitt and Christian, 1970) may survive mild baking processes and cause spoilage if the a_w and other conditions are conducive. The nutritional composition and physical attributes of the product, especially pH, will affect the potential for the growth of xerophiles.

The a_w limits for growth of many xerophilic fungi have been established. Common *Eurotium* species (*Eurotium rubrum*, *E. repens*, *E. chevalieri* and *E. amstelodami*) are generally able to grow down to 0.70–0.72 a_w under favourable conditions (Snow, 1949; Armolik and Dickson, 1956; Ayerst, 1969; Magan and Lacey, 1984). *A. penicillioides* has a reported minimum a_w of 0.68 in stored grains (Pitt and Hocking, 1997) and *W. sebi* has been shown to grow down to 0.69 a_w (Pitt and Hocking, 1977). The extreme xerophile *X. bisporus* can grow down to 0.61 a_w while xerophilic *Chrysosporium* species may grow down to 0.69 a_w (Pitt and Christian, 1968).

Some previous studies have examined the effects of temperature and a_w (Ayerst, 1969; Magan and Lacey, 1988; Wheeler et al., 1988a,b; Abellana et al., 1999a,b), pH and a_w (Pitt and Hocking, 1977), or the combined effects of a_w, temperature and modified atmosphere packaging (El Halouat and Debevere, 1997) on the growth of xerophilic moulds. However, the combined effects of pH, temperature and a_w on fungal germination and growth have not been systematically investigated. These three parameters largely control germination and growth of xerophilic fungi and determine whether or not certain foods will spoil.

In this study, seven xerophilic fungi of significance in the spoilage of reduced a_w baked goods were grown over a range of temperature, pH and a_w values. The data presented here provide a matrix of growth and germination responses to water activity, pH and temperature that may be used in constructing a mathematical model for the prediction of the shelf life of baked goods. Such a model will be of benefit to manufacturers of these and related products in development of new formulations and processes (manuscript in preparation).

2. Material and methods

2.1. Fungi

The seven fungi used in this study, *A. penicillioides*, *Chrysosporium xerophilum*, *E. repens*, *E. rubrum*, *P. roqueforti*, *W. sebi* and *X. bisporus* (Table 1) were chosen because of their importance in food spoilage. All are capable of growth at very low a_w except *P. roqueforti* which was included because of its ability to grow in the presence of preservatives and in modified atmospheres. All strains used were from the FRR Culture Collection at Food Science Australia, North Ryde, NSW, Australia.

2.2. Media

The basal medium comprised 0.3% malt extract (light brewers malt), 0.3% yeast extract (Oxoid, Australia) and 2.0% agar (food grade; Leiner Davis, Sydney, Australia). Various amounts of phosphoric acid in the forms of KH_2P0_4, K_2HP0_4 and H_3P0_4 were used to adjust pH. All chemicals were analytical reagent grade. High concentrations were used, ranging from 1.3% (pH 4.5) to 1.6% (pH 7.5) to provide substantial buffering capacity. The final pH values of the media were 4.5, 5.5, 6.5 and 7.5. Media with a_w values of 0.92, 0.89, 0.86, 0.82, 0.78, 0.74 and 0.70 were formulated by adding equal weights of glucose and fructose (analytical reagent grade) in the required concentrations as previously described (Pitt and

Table 1
Fungal isolates used in this study

Species	Strain	Source
Aspergillus penicillioides	FRR 3722	Human source, Brazil, 1965
Chrysosporium xerophilum	FRR 3921	Chocolate, NSW, Australia, 1991
Eurotium repens	FRR 382	Dried prunes, Australia, 1969
Eurotium rubrum	FRR 326	Stored high moisture prunes, NSW, Australia, 1965
Penicillium roqueforti	FRR 4903	Lake sediment, Antarctica, 1965
Wallemia sebi	FRR 3055	Dried fish, Indonesia, 1985
Xeromyces bisporus	FRR 2347	Mouldy fruit cake, Australia, 1980

Hocking, 1977). All media were sterilised by steam treatment at 100 °C for 15 min. Water activity values were checked using an Aqualab CX3 instrument with an accuracy of ±0.003 a_w units (Decagon Devices, Pullman, WA, USA). The pH was measured with a 320 pH Beckman meter (Beckman Instruments, Fullerton, CA, USA).

2.3. Germination and growth

Methods used for inoculation and examination were those of Pitt and Hocking (1977). Media (1.5 ml) of the various formulations were poured into the lids of Petrislides (Millipore, Bedford, MA, USA) and immediately covered with the Petrislide body. After cooling, the Petrislides were briefly opened, the medium inoculated at the centre with a single needle-point of mature spores from one of the test species and closed again. The Petrislides were stored in polyethylene food containers with appropriate saturated salt solutions to minimise changes in a_w and incubated at 25, 30 or 37 °C for up to 100 days (Pitt and Hocking, 1977).

To observe germination, Petrislides were examined daily (or less frequently as required, e.g. at low a_w values) using a compound transmitted light microscope at 100× magnification. Germination times were recorded, the criterion for germination being the observation of a significant number of germ tubes of equal length to the spores examined. Colony diameters were measured at intervals (daily to weekly), initially by eyepiece micrometer, then using the microscope stage verniers until growth rate declined or 100 days had elapsed. From these data, radial growth rates, expressed in mm day^{-1} were calculated. For the period over which growth was approximately linear, a mean radial growth rate was calculated for each fungus and each set of conditions. Early exponential growth and late suboptimal growth were not included (Pitt and Hocking, 1977).

3. Results

3.1. Germination

Germination times (days) for each species are shown in Table 2. Germination for all species except *X. bisporus* was fastest at the highest a_w level studied (0.92). Germination times generally increased with decreasing a_w; however, *X. bisporus* germinated faster at lower a_w. It germinated most rapidly at 0.89–0.86 a_w at 25 and 30 °C but at 0.86–0.82 a_w at 37 °C. The majority of the fungi germinated faster at 30 °C; however, the differences between germination times at 25 and 30 °C were minor. At 37 °C, *P. roqueforti* failed to germinate at any combination of a_w and pH, and germination of some species was inhibited by a combination of lower a_w values and low pH.

For *C. xerophilum* and *X. bisporus*, germination was detected at all three temperatures (25, 30 and 37 °C) at 0.70 a_w, the lowest a_w tested here. The minimum a_w at which germination was observed for *E. rubrum* and *A. penicillioides* was 0.70, for *E. repens* and *W. sebi*, 0.74, and for *P. roqueforti*, 0.82, at 25 °C. At 30 and 37 °C, with the exception of *C. xerophilum* and *X. bisporus*, the minimum a_w for germination was higher than that observed at 25 °C (Table 2). Under acidic conditions at pH 4.5 and 5.5, germination rates were generally faster than or equivalent to germination rates observed at higher pH and the same a_w value. Little difference was observed between germination times at pH 6.5 and 7.5.

3.2. Growth rates

Fig. 1 shows growth rates of *E. rubrum* and *A. penicillioides* at 25, 30 and 37 °C with four pH values; Fig. 2 shows similar data for *C. xerophilum* and *X. bisporus* and Fig. 3 shows data for *P. roqueforti* at 25 and 30 °C. Data for *E. repens* and *W. sebi* that have not been presented as growth rates of *E. repens* were very similar to those of *E. rubrum* and *W. sebi* that grew very slowly under all conditions.

As with germination, growth of the species studied, with the exception of *X. bisporus*, was fastest at the highest a_w studied at all temperatures. The optimum a_w for growth of *X. bisporus* varied with temperature. At 25 °C, the growth optimum was between 0.89 and 0.86 a_w (Fig. 2d), at 30 °C, between 0.92 and 0.89 a_w (Fig. 2e) and at 37 °C between 0.86 and 0.82 a_w (Fig. 2f). The maximum growth rates occurred at 30 °C for *E. rubrum* (Fig. 1b), *A. penicillioides* (Fig. 1e) and *X. bisporus* (Fig. 2e), at 37 °C for *C. xerophilum* (Fig. 2c) and 25 °C for *P. roqueforti* (Fig. 3a). The optimum growth occurred at 25 °C for *P. roqueforti*

Table 2
Germination times (days) for food spoilage fungi at various pH and temperature[a]

	pH	Temperature (°C)											
		25				30				37			
		4.5	5.5	6.5	7.5	4.5	5.5	6.5	7.5	4.5	5.5	6.5	7.5
E. rubrum	0.92	0.8	1.0	1.1	1.0	0.8	0.8	0.8	0.7	1.0	0.7	1.0	8.8
	0.89	1.3	1.8	1.9	1.8	1.0	1.1	1.1	1.0	1.3	0.9	0.8	8.0
	0.86	2.0	1.8	1.9	1.8	2.1	1.8	1.8	1.0	1.2	1.0	1.0	8.0
	0.82	4.8	3.8	6.8	3.8	2.8	2.8	2.8	3.8	2.0	2.8	2.7	8.7
	0.78	6.0	10	10	11	6.8	5.8	6.9	4.0	17	8.8	6.8	14
	0.74	15		29	14	14			14				20
	0.7			29									
E. repens	0.92	1.0	1.0	0.8	0.8	0.8	0.8	0.8	0.7	8.7	0.7	1.8	8.7
	0.89	1.3	1.8	0.9	1.8	0.8	0.9	0.8	0.7	8.0	1.0	1.8	8.7
	0.86	1.8	1.8	1.5	1.8	1.0	0.9	0.9	1.8	8.0	2.0	1.8	9.0
	0.82	4.8	3.1	1.9	2.1	1.2	2.0	2.0	2.0	8.7	2.0	6.8	11
	0.78	6.8	7.0	4.1	4.9	2.1	3.8	3.8	4.0	8.8	7.0	23	11
	0.74	7.8	23	7.0	8.9	7.8	8.8	6.9	18		3.8	22	
	0.7						20	9.8					
W. sebi	0.92	1.0	1.0	0.8	0.8	1.0	0.9	1.1	0.7	1.0	1.0	1.8	8.0
	0.89	1.8	1.1	1.1	1.8	1.8	1.1	1.1	1.0	1.0	3.7	1.8	8.0
	0.86	1.8	1.8	1.9	2.1	1.8	1.1	1.8	1.0	2.2	3.8	2.8	8.7
	0.82	3.8	2.8	2.9	2.8	2.1	2.8	1.8	6.9			4.0	9.7
	0.78	7.0	7.0	7.0	6.9	2.1	6.9	6.9					21
	0.74	23	32	16	17								
A. penicillioides	0.92	1.3	1.3	1.5	1.8	1.0	1.1	1.1	1.0	8.0	2.0	0.9	1.0
	0.89	1.8	1.8	1.9	1.8	1.0	1.1	1.1	1.0	8.0	1.1	0.9	1.0
	0.86	1.8	1.8	1.9	2.1	1.8	1.8	1.8	1.8	8.7	1.0	1.8	1.0
	0.82	3.8	2.8	2.9	3.1	2.1	2.8	2.0	2.8	8.7	1.9	1.8	1.9
	0.78	7.0	7.0	7.0	6.9	3.8	6.9	3.8	5.0	8.8	1.8	2.7	10
	0.74	16	23	18.1	14	6.3	18	8.8			6.9	9.8	18
	0.7	91	56										
P. roqueforti	0.92	1.3	1.8	1.8	1.8	1.6	1.2	1.1	1.8				
	0.89	2.8	2.8	2.9	2.8	2.1	2.3	1.8	5.8				
	0.86	5.8	6.9	5.9	7.0	14		6.9					
	0.82	22		29									
C. xerophilum	0.92	1.3	1.8	2.1	1.8	2.0	1.8	0.8	1.0	7.7	0.7	1.0	7.7
	0.89	1.8	1.8	2.9	1.8	1.8	1.8	2.0	1.0	8.0	0.9	0.8	8.0
	0.86	2.7	2.8	2.9	2.1	1.8	1.8	2.8	1.8	8.0	1.0	1.0	8.0
	0.82	2.7	3.8	2.9	3.8	1.8	2.8	2.8	3.8	8.0	1.8	1.8	8.7
	0.78	6.7	7.0	7.0	8.9	3.1	3.8	3.8	7.8	14	4.8	2.7	9.7
	0.74	14	10	18	14	7.8	8.8	21	7.0	14	7.0	4.3	20
	0.7	32	37	25	22	17	11	21					35
X. bisporus	0.92	3.7	1.3	3.9	3.2	2.0	2.0	1.1	2.0	8.7	2	4.0	8.7
	0.89	1.3	2.0	2.2	2.1	0.8	1.8	0.8	1.0	8.0	2	2.0	8.0
	0.86	1.6	2.8	2.3	2.1	0.8	1.1	1.8	8.8	1.2	1	1.0	8.0
	0.82	3.1	4.0	2.9	3.1	2.1	2.8	1.8	8.8	2.0	1	1.8	2.9
	0.78	4.8	4.8	6.8	3.8	2.8	3.8	2.8	22	8.8	2	2.7	14
	0.74	8.8	8.9	10	14	4.9	6.9	7.9	19	9.0	2	20.9	20
	0.7	16	26	25	22	17	9.8		17	14	7		

[a] Underlined figures indicate germination with no subsequent growth.

and *W. sebi*, at 30 °C for *Eurotium* species, *A. penicillioides* and *X. bisporus* and at 37 °C for *C. xerophilum*. At 25 °C, slightly slower growth was observed; however, for most species, growth occurred over a wider a_w range. These fungi all grew faster under acidic than neutral pH conditions.

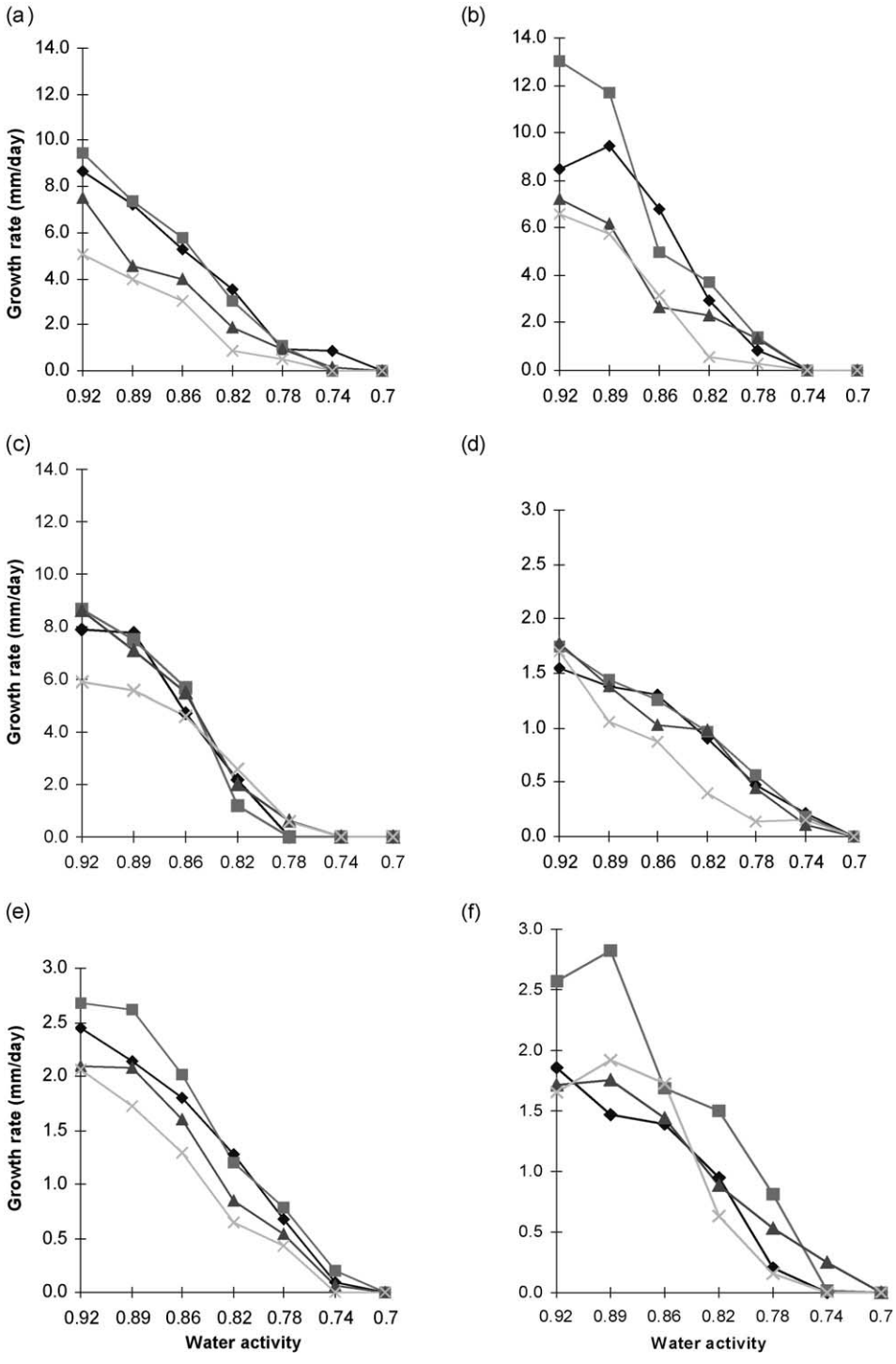

Fig. 1. Effect of water activity on radial growth rates of (a)–(c) *E. rubrum* and (d)–(f) *A. penicillioides* at pH 4.5 (♦), 5.5 (■), 6.5 (▲) and 7.5 (×) at temperatures 25 °C (a and d), 30 °C (b and e) and 37 °C (c and f).

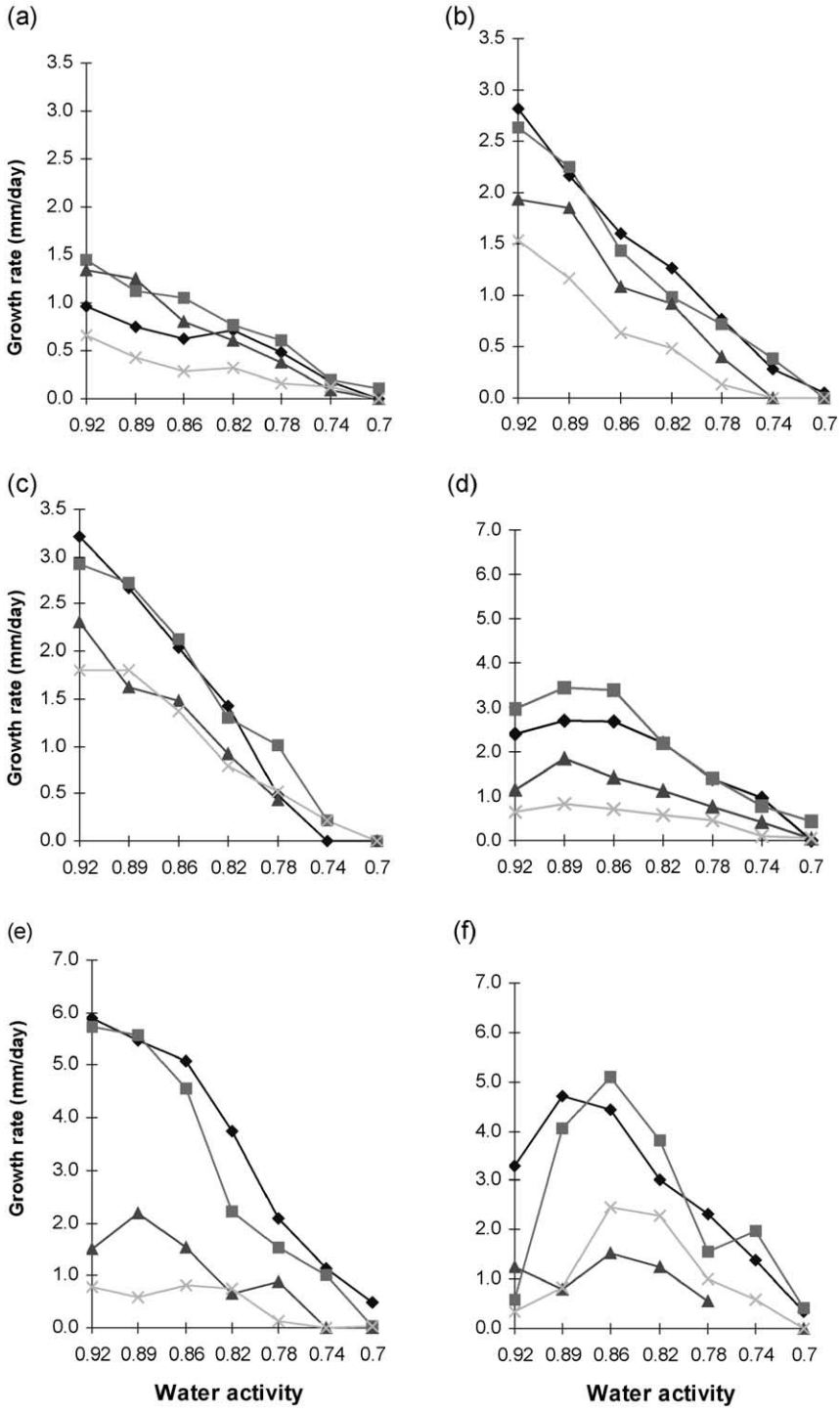

Fig. 2. Effect of water activity on radial growth rates of (a)–(c) *C. xerophilum* and (d)–(f) *X. bisporus* at pH 4.5 (♦), 5.5 (■), 6.5 (▲) and 7.5 (×) at temperatures 25 °C (a and d), 30 °C (b and e) and 37 °C (c and f).

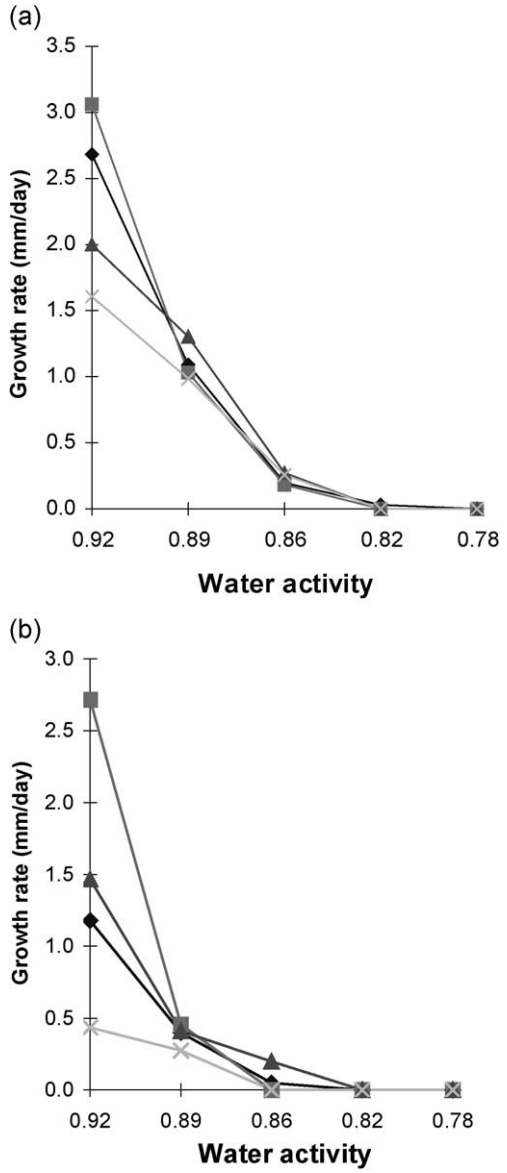

Fig. 3. Effect of water activity on radial growth rates of *P. roqueforti* at pH 4.5 (♦), 5.5 (■), 6.5 (▲) and 7.5 (×) at 25 °C (a) and 30 °C (b).

Growth of fungal species did not always occur at the minimum a_w observed for germination. At 25 °C, the minimum a_w for growth was 0.74 for *E. rubrum*, *E. repens*, *W. sebi* and *A. penicillioides* (Fig. 1a and d), 0.70 for *C. xerophilum* and *X. bisporus* (Fig. 2a and d) and 0.82 for *P. roqueforti* (Fig. 3a). At 30 °C, the minimum a_w for growth was 0.78 for *E. rubrum* (Fig. 1b) and *W. sebi*, 0.74 for *E. repens*, *A. penicillioides* (Fig. 1e) and *C. xerophilum* (Fig. 2b), 0.70 for *X. bisporus* (Fig. 2e) and 0.86 a_w for *P. roqueforti* (Fig. 3b). At 37 °C, the minimum a_w for growth was 0.78 for *E. rubrum* (Fig. 1c) and *W. sebi*, 0.74 for *E. repens*, *A. penicillioides* (Fig. 1f) and *C. xerophilum* (Fig. 2c) and 0.70 a_w for *X. bisporus* (Fig. 2f). *P. roqueforti* did not grow at 37 °C.

The optimum pH for growth for all fungal species studied regardless of temperature was between pH 4.5 and 5.5. The effects of acid conditions were more noticeable at higher a_w values (0.92, 0.89), especially for *E. rubrum*, *C. xerophilum* and *P. roqueforti*. At lower a_w values, the effect of pH was diminished and similar growth rates were seen for all four pH values. No interaction was observed between the effects of temperature and pH on growth rates.

4. Discussion

Germination times for all fungi studied except *X. bisporus* are consistent with results of other workers (Snow, 1949; Armolik and Dickson, 1956; Ayerst, 1969; Scott, 1975; Pitt and Hocking, 1977; Roessler and Ballenger, 1996). For all fungi except *X. bisporus* at all three temperatures (25, 30 and 37 °C), a decrease in a_w resulted in an increase in germination times, but optimal germination of *X. bisporus* occurred at a reduced a_w. Temperature had a considerable effect on the a_w at which *X. bisporus* germinated (Table 2).

For many fungi, germination occurred at a lower a_w than growth. Some authors (Pitt and Christian, 1968; Wheeler et al.,1988a,b) have reported that germination was usually followed by growth; however, others (Hocking et al.,1994) have reported similar effects to those observed here. The lowest a_w examined in our study was 0.70, which previous work has shown is not the minimum a_w for germination or growth of *X. bisporus* or *C. xerophilum* (Pitt and Christian, 1968).

The minimum a_w observed for germination for *E. rubrum* and *A. penicillioides* was 0.70 and for *E. repens* and *W. sebi* was 0.74 at 25 °C. Other studies have shown that *E. repens* has germinated at a_w values as low as 0.70 (Snow, 1949) and 0.72 (Armolik and Dickson, 1956; Wheeler et al.,1988b) at 25 °C.

W. sebi has also been reported as growing at 0.71 a_w (Wheeler et al.,1988a) at 25 °C. *C. xerophilum* and *X. bisporus* have previously been shown to be well adapted to low a_w environments with germination occurring at 0.708 and 0.605 a_w, respectively (Pitt and Christian, 1968). The minimum a_w observed for germination for *P. roqueforti* at 25 °C was 0.82 which is consistent with the minimum a_w for growth of other xerophilic *Penicillium* species (Pitt and Hocking, 1977, Hocking and Pitt, 1979).

The interaction observed between pH and germination of xerophilic fungi was complex. For the species studied, the observed optimum pH for germination was generally between pH 4.5 and 5.5, with slightly longer germination times at pH 6.5–7.5. However, for *W. sebi* at 37 °C, better germination occurred at higher pH values. The minimum a_w for germination was 0.78 at pH 7.5, 0.82 at pH 6.5 and 0.86 at pH 5.5 and 4.5. A similar effect was observed, but to a lesser degree, for *C. xerophilum* at 37 °C.

The more favourable pH values for growth were 4.5 and 5.5 rather than 6.5 and 7.5 for all fungi at all three temperatures, indicating that these food spoilage fungi are adapted better to growth under acid than neutral conditions. This effect was most obvious in the responses of *X. bisporus* at 30 °C. As the a_w decreased and growth rates slowed, the effects of pH were reduced. Previous studies by Pitt and Hocking (1977) demonstrated similar responses. In a glucose–fructose-based medium at 25 °C, *E. chevalieri* and *C. fastidium* grew faster and over a wider a_w range at pH 4.0 than at pH 6.5. Their study showed that although *X. bisporus* grew faster at pH 6.5 than at pH 4.0 over the a_w range 0.95–0.80, below 0.80 a_w, growth was faster at the lower pH value. Moreover, *X. bisporus* grew down to a lower a_w value at the more acid pH (0.68 a_w at pH 4.0 compared with 0.72 a_w at pH 6.5). Wheeler et al. (1991) reported that at high a_w, pH over the range 4–8 had little effect on the growth of selected *Aspergillus*, *Penicillium* and *Fusarium* species at 25, 30 and 37 °C, but growth rates generally declined rapidly below pH 3.

This study has shown that the optimal conditions for growth for the fungi examined were between pH values 4.5 and 5.5 and, with the exception of *X. bisporus*, between a_w 0.92 and 0.89. For *X. bisporus*, the optimal conditions for growth at 25 and 37 °C were 0.89–0.86 a_w at pH 4.5–5.5 and at 30 °C were 0.92–0.89 a_w at pH 4.5–5.5. Optimal growth conditions for *P. roqueforti* would occur at higher a_w values than the range tested here, but the effects of pH and temperature observed in this study would also probably apply to higher a_w.

The study has also defined the a_w limits for growth of the fungal species examined and the effects of pH and temperature on these limits. Such data are of benefit to food manufacturers in the design and formulation of new baked goods, confectionery and similar products.

Acknowledgements

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