Annals of Microbiology, **57 (2)** 209-215 (2007)

The effect of potassium sorbate, NaCl and pH on the growth of food spoilage fungi*

Aycan YIGIT, Mihriban KORUKLUOGLU**

Department of Food Engineering, Faculty of Agriculture, Uludag University, Bursa, Turkey

Received 14 November 2006 / Accepted 30 March 2007

Abstract - In this study, the hurdle technology approach was used to prevent fungal growth of common spoilage fungi in naturally fermented black olives (*Alternaria alternata*, *Aspergillus niger*, *Fusarium semitectum* and *Penicillium roqueforti*). The factors studied included a combination of different concentrations of potassium sorbate (100 up to 1000 mg/L), a range of pH values (4.5, 5, 5.5, 6, and 6.5) and levels of NaCl (0, 3.5, 5, 7.5, and 10%). *Alternaria alternata* was the most sensitive fungus whereas *P. roqueforti* was the most resistant fungi against all hurdle factors. The combination of all hurdles completely inhibited *A. alternata* and *F. semitectum* by lowest inhibitory factors, such as 100 mg/L potassium sorbate with 3.5% NaCl at pH 5. On the other hand, at pH 5, A*. niger* and *P. roqueforti* were totally prevented by a combination of 300 mg/L potassium sorbate with 10% NaCl and 400 mg/L potassium sorbate with 7.5% NaCl, respectively. Potassium sorbate and 5-10% NaCl interaction had significant stimulation effect on *P. roqueforti* and *A. niger* ($p < 0.05$). This study indicates that potassium sorbate is a suitable preserving agent to inhibit growth of fungi in fermented products of pH near 4.5 regardless levels of NaCl. For products of slightly higher pH, the addition of potassium sorbate is suggested in combination with NaCl.

Key words: fungi, inhibition, potassium sorbate, pH, NaCl.

INTRODUCTION

Fungi are a very diverse group of microorganisms and have a significant impact on the safety of food. They are responsible for off-flavour formation and production of allergenic compounds and mycotoxins, which lead to qualitative losses (Farag *et al.*, 1989; Nickelsen and Jakobsen, 1997; Moss, 2000; Nielsen and Rios, 2000). Although industrial standards have been upgraded, food spoilage by fungi is still of great concern. Each year, 30-40% of the world-wide food production is contaminated by mycotoxins with adverse effects for public health and economy (Tzatzarakis *et al*., 2000; Dantigny *et al*., 2005).

Penicillium roqueforti, Alternaria alternata, *Aspergillus niger* and *Fusarium semitectum* are common and well-characterised species which are known to produce the mycotoxins roquefortine C, PR-toxin, alternariol, ochratoxin A, type A trichothecenes and zearalenone (Marasas *et al*., 1984; Hsieh *et al*., 1986; Delgado and Gomez-Cordoves, 1998; Rundberget *et al*., 2004; Bau *et al*., 2005). These fungi are involved in the spoilage of fermented black olives (untreated black olives in brine). Spoilage may result from the development of a mycelial mat on the surface of the brine, from softening of the fruits and changes in the pH of the final product. Generally pH, NaCl and potassium sorbate are regarded as the principal controlling factors during fermentation and subsequent storage of table olives (Sahin and Korukluoglu, 2000).

The major preservation technique currently employed to prevent or delay spoilage in food products is the application of a combination of parameters, which may act synergistically to inhibit or retard fungal growth. The most common parameters applied are slight reductions in a_w (by addition of NaCl or sugar), lowered pH, addition of antimicrobial agents etc. (Chirife and Favetto, 1992; Leistner, 1992; Cuppers *et al*., 1997). Among the most extensively used combinations of treatments there are those in which an antimicrobial acid is applied and its effectiveness is enhanced by lowering the pH (Gould, 1996). Potassium sorbate is a considerable antimicrobial weak acid, owing to its desirable physiological properties, neutral flavour and its effectiveness against fungi (Sofos and Busta, 1981; Thakur *et al*., 1994; Suhr and Nielsen, 2004). A combination of these factors could effectively control the growth of the fungi during fermentation and storage. Different levels of NaCl, which are commonly used by the Turkish table olive industries ranged from 3.5 to 10%, and pH values are expected to range between 6.5-6.0 (initial) and 4.5 (final). In addition, according to Turkish Food Codex, legal limit of potassium sorbate is 1000 mg/L for fermented foods. The range of experimental conditions was chosen to be within the limits of the brine environment.

* This study is A. Yigit's part of the M.S. thesis results.
** Corresponding author. Phone: +90-224-4428970;
Fax: +90-224-4428077; E-mail: mihriban@uludag.edu.tr

The objectives of this study were to investigate (i) the combined effect of pH, NaCl and potassium sorbate on the growth of fungi, causing the main spoilage of fermented table olive, and (ii) to enhance preservative effect and satisfy consumer demands, such as reduced use of preservatives combined with pH and NaCl concentrations.

MATERIALS AND METHODS

Fungi and inoculum preparation. *Alternaria alternata*, *Aspergillus niger*, *Fusarium semitectum* and *Penicillium roqueforti* were isolated from olive and during fermentation process in brine. These isolates are held in the culture collection of the Food Engineering Department of Uludag University, Bursa, Turkey.

The fungi were cultivated on Malt Extract Agar (MEA) slants for seven days at 30 °C and the spores were harvested with 10 mL of 0.1% Tween 80 (Merck, Darmstadt, Germany) solution sterilised by membrane (0.45 µm) filtration. The spore suspensions were adjusted with the same solution to give a final concentration of 10^6 spore/mL and used the same day (Lopez-Malo *et al*., 2002).

Culture conditions. MEA was adjusted by adding NaCl (Merck) in concentrations of 3.5, 5, 7.5 and 10% (w/v). After sterilisation at 121 °C for 15 min, the pH of the medium was adjusted using either 0.1 N lactic acid or NaOH to obtain pH values of 4.5, 5, 5.5, 6, and 6.5; the amount of base or acid needed in each case was previously determined. The necessary amount of potassium sorbate (100, 200, 300, up to 1000 mg/L) was added to sterilised and pH adjusted agar solutions and mechanically mixed under sterile conditions (El-Gazzar *et al*., 1986; Rusul and Marth, 1987; Lopez-Malo *et al*., 2005). Agar solutions were poured into sterile Petri dishes and point-inoculated with 2 mL of spore suspension in the centre. Plates were incubated at 30 °C for 7 days in sealed polyethylene bags in order to maintain appropriate humidity values (Marin *et al*., 2002; Lopez-Malo *et al*., 2005). Culture medium without preservative was used as a control. The Petri plates in which no growth was observed were incubated for 14 days.

Growth and inhibition percentage. Growth was determined by measuring with a scaler the colony diameters. After seven days of incubation, the percentage of inhibition rate calculated as follows (Combina *et al*., 1999; Ozcan and Boyraz, 2000):

$$I = [(C–T) / C] \times 100$$

where I is inhibition (%), C is the colony diameter of the mycelium on the control Petri plate (mm), and T is the colony diameter of the mycelium on the test Petri plate (mm). Experiments were conducted in quadruple.

Statistical analyses. Statistical analysis of data was carried out using the SPSS software package (SPSS 11.5 SPSS Inc, Chicago, IL) and Minitab 14.0 software (Minitab Inc., State Collage, PA, USA).

RESULTS AND DISCUSSION

In this study, the combined effect of three inhibitory factors, pH, potassium sorbate and NaCl, were tested against *A. alternata, A. niger, F. semitectum,* and *P. roqueforti.* As shown in Table 1, all hurdle factors and their interactions showed inhibitory effect on the growth of *A. alternata.* The statistical evaluation demonstrated that variations in the results were significant ($p < 0.05$). The growth decreased with increased levels of potassium sorbate and NaCl and reduced pH values. Combina *et al.* (1999) reported that *A. alternata* ITEM 539 was completely inhibited with 100 mg/kg potassium sorbate at Ground Rice Cornsteep Agar (GRCSA). In this study, *A. alternata* was totally prevented with 400 mg/L potassium sorbate at MEA conditions (at pH 5.5; 0% NaCl). The difference in concentration is most likely due to the medium and strain differences.

Fusarium semitectum and *A. alternata* exhibited similar results against all test treatments. However, *F. semitectum* indicated lower inhibition rates than *A. alternata* at pH 6.5-5.5 (Table 2). Moreover, the growth of *F. semitectum* was stimulated in the presence of 600-1000 mg/L potassium sorbate (at pH 6.5; 0% NaCl). That could be explained by the observation that some fungi are able to metabolise weak organic acid to obtain energy (Thakur *et al*., 1994). No significant differences were found when 100 mg/L potassium sorbate at pH 6.5-5.5 and 0-5% NaCl conditions were applied ($p > 0.05$). Tzatzarakis *et al*. (2000) stated that *F. oxysporium* was inhibited up to 50% with 1292 mg/L potassium sorbate (at pH 6.5 and without NaCl).

Among the test fungi, *P. roqueforti* was the most resistant species against combinations of NaCl, potassium sorbate and pH (Table 3). The growth of *P. roqueforti* was substantially stimulated at pH 5.5 and 6. Also, 100 up to 600 mg/L potassium sorbate showed stimulation activity at pH 6.5 in test NaCl levels, except 10% NaCl. On the other hand, NaCl treatments (3.5-10%) inhibited stimulation at pH 5. It was reported that *Penicillium roqueforti* metabolised 91-100% of 600 ppm potassium sorbate in mycological agar at the end of six days, and usage of potassium sorbate was considered as an additional energy source for *P. roqueforti* (Finol *et al*., 1982; Liewen and Marth, 1985). In our study, the growth of *P. roqueforti* was stimulated by all potassium sorbate concentrations at MEA (at pH 5.5; 0% NaCl). Stimulated growth could be explained due to metabolise of potassium sorbate by this fungi.

As shown in Table 4, levels of NaCl (5-10%) stimulated the growth of *A. niger* at pH 6.5 and 5.5. However, at pH 5.0-4.5 the stimulation effect was deactivated. The growth of *A. niger* was totally prevented in presence of 5.0, 7.5 and 10% NaCl combined with 800, 600 and 300 mg/L potassium sorbate, respectively. Furthermore, the growth was completely inhibited by 600 mg/L potassium sorbate at pH 4.5 and without NaCl conditions. It was found that the differences were insignificant at pH 6.5-6 with 0-10% NaCl and 100-300 mg/L potassium sorbate treatments ($p > 0.05$).

In this study, *A. alternata* was the most sensitive fungi whereas P. *roqueforti* was the most resistant fungi against all hurdle factors. Our results showed that *A. niger* and *P. roqueforti* was completely prevented with potassium sorbate treatments applied at pH 4.5 and 5.0. At pH 4.5, concentrations of 600 mg/L and 700 mg/L potassium sorbate (0% NaCl conditions) were sufficient for complete inhibition of *A. niger* and *P. roqueforti,* respectively.

Antifungal effect of NaCl (control treatment without potassium sorbate) was increased with remaining concen-

TABLE 1 – The inhibition rates (%) of *Alternaria alternata* at different potassium sorbate, NaCl and pH levels after 7 days of incubation.

pH	NaCl (%)	Potassium sorbate (mg/L)									
		100	200	300	400	500	600	700	800	900	1000
6.5	0	3.5 ± 0.9*	4.4 ± 1.0	5.2 ± 2.1	15.8 ± 1.3	24.6 ± 3.0	28.1 ± 2.6	29.8 ± 3.2	36.2 ± 3.9	70.7 ± 5.5	87.3 ± 6.8
	3.5	10.5 ± 1.2	21.3 ± 3.2	26.9 ± 3.5	29.5 ± 2.7	30.8 ± 2.2	34.9 ± 3.3	47.4 ± 3.8	50.7 ± 4.3	62.2 ± 4.7	90.7 ± 8.2
	5.0	14.9 ± 1.5	16.4 ± 2.3	29.4 ± 2.7	30.9 ± 3.6	34.1 ± 3.5	37.3 ± 3.9	50.3 ± 4.4	51.8 ± 4.7	69.7 ± 5.3	–**
	7.5	28.7 ± 3.0	30.3 ± 1.9	33.0 ± 3.4	37.9 ± 4.8	51.4 ± 4.5	55.2 ± 4.1	62.1 ± 5.1	71.7 ± 5.9	–	–
	10.0	31.0 ± 2.5	35.0 ± 2.7	40.0 ± 4.2	44.0 ± 5.1	55.8 ± 4.7	58.3 ± 5.0	68.3 ± 5.7	82.8 ± 6.4	–	–
6.0	0	10.7 ± 0.8	24.4 ± 2.2	34.7 ± 3.5	69.3 ± 4.6	72.7 ± 4.9	74.7 ± 5.3	78.4 ± 5.5	83.3 ± 5.9	88.0 ± 6.4	–
	3.5	19.0 ± 1.7	28.6 ± 2.9	38.1 ± 3.4	57.5 ± 2.7	68.5 ± 3.6	70.2 ± 5.1	71.5 ± 4.8	74.9 ± 5.7	76.7 ± 5.0	–
	5.0	39.7 ± 4.1	47.9 ± 4.8	52.1 ± 5.3	66.7 ± 3.5	71.0 ± 4.4	73.0 ± 4.9	77.4 ± 5.6	82.5 ± 6.2	85.4 ± 6.0	–
	7.5	56.1 ± 4.5	66.7 ± 5.0	69.3 ± 4.6	70.2 ± 5.5	74.8 ± 6.3	80.7 ± 6.0	87.7 ± 5.5	–	–	–
	10.0	59.3 ± 3.9	68.4 ± 4.2	72.9 ± 5.4	73.6 ± 6.8	77.3 ± 5.9	–	–	–	–	–
5.5	0	44.2 ± 3.8	58.9 ± 4.2	67.5 ± 4.6	–	–	–	–	–	–	–
	3.5	53.4 ± 3.5	62.5 ± 4.8	–	–	–	–	–	–	–	–
	5.0	55.3 ± 4.1	65.4 ± 5.7	–	–	–	–	–	–	–	–
	7.5	58.2 ± 4.9	70.1 ± 6.2	–	–	–	–	–	–	–	–
	10.0	69.2 ± 5.7	–	–	–	–	–	–	–	–	–
5.0	0	78.9 ± 5.9	–	–	–	–	–	–	–	–	–

* Mean value (n = 4) ± Standard Deviation ($p < 0.05$); ** – No growth.

TABLE 2 – The inhibition rates (%) of *Fusarium semitectum* at different potassium sorbate, NaCl and pH levels after 7 days of incubation.

pH	NaCl (%)	Potassium sorbate (mg/L)									
		100	200	300	400	500	600	700	800	900	1000
6.5	0	2.8 ± 0.6*	4.2 ± 0.9	4.9 ± 1.6	5.3 ± 1.5	5.8 ± 2.4	11.2 ± 0.8	2.8[a]± 1.3	3.5[a]± 1.2	4.2[a]± 2.2	1.4[a]± 0.7
	3.5	1.1 ± 1.3	1.5 ± 2.0	2.2 ± 3.4	2.7 ± 3.6	3.2 ± 2.7	6.9 ± 3.1	9.4 ± 2.5	13.9 ± 2.8	23.9 ± 3.5	29.4 ± 3.8
	5.0	1.7 ± 0.9	2.4 ± 1.5	3.6 ± 2.8	4.7 ± 3.3	5.5 ± 2.6	7.8 ± 3.9	8.3 ± 3.3	12.8 ± 4.2	17.2 ± 4.4	26.7 ± 4.3
	7.5	5.3 ± 2.4	6.8 ± 3.2	13.2 ± 3.5	15.8 ± 4.6	17.4 ± 4.1	24.0 ± 5.0	30.1 ± 4.8	33.6 ± 5.2	36.2 ± 5.5	57.7 ± 4.9
	10.0	7.8 ± 3.0	22.0 ± 3.7	25.1 ± 3.5	28.3 ± 4.1	29.9 ± 4.4	33.8 ± 3.9	39.4 ± 5.3	40.3 ± 4.8	67.7 ± 6.1	–**
6.0	0	3.0 ± 1.0	5.5 ± 1.5	6.1 ± 0.9	6.7 ± 1.9	7.1 ± 2.4	7.7 ± 2.8	8.0 ± 3.3	12.1 ± 3.2	28.2 ± 4.5	–
	3.5	3.6 ± 1.7	6.2 ± 2.0	6.5 ± 1.9	7.8 ± 0.9	8.2 ± 3.1	8.5 ± 2.6	11.7 ± 3.9	17.2 ± 3.6	42.8 ± 5.1	–
	5.0	4.1 ± 2.2	7.6 ± 3.1	12.8 ± 3.0	13.9 ± 4.5	15.0 ± 4.0	22.8 ± 5.2	25.0 ± 4.9	27.2 ± 3.5	48.3 ± 4.8	–
	7.5	6.5 ± 2.8	10.7 ± 3.4	19.1 ± 3.5	20.6 ± 3.6	32.0 ± 4.9	39.5 ± 4.5	40.9 ± 5.0	47.7 ± 4.6	74.3 ± 6.2	–
	10.0	8.7 ± 2.6	26.3 ± 3.8	29.1 ± 3.3	35.7 ± 4.1	39.8 ± 4.5	57.4 ± 4.4	68.3 ± 5.3	75.0 ± 5.7	–	–
5.5	0	4.4 ± 0.8	18.8 ± 1.7	25.5 ± 3.8	92.0 ± 4.5	–	–	–	–	–	–
	3.5	5.3 ± 1.6	21.7 ± 2.5	31.7 ± 3.7	95.0 ± 4.4	–	–	–	–	–	–
	5.0	8.4 ± 2.0	23.9 ± 3.1	50.6 ± 4.2	–	–	–	–	–	–	–
	7.5	18.2 ± 3.4	28.3 ± 3.9	68.3 ± 5.5	–	–	–	–	–	–	–
	10.0	30.9 ± 4.3	59.1 ± 4.6	–	–	–	–	–	–	–	–
5.0	0	16.7 ± 3.3	–	–	–	–	–	–	–	–	–

* Mean value (n = 4) ± Standard Deviation ($p < 0.05$); ** – No growth.
[a] Stimulated growth rate (%).

TABLE 3 - The inhibition rates (%) of *Penicillium roqueforti* at different potassium sorbate, NaCl and pH levels after 7 days of incubation.

pH	NaCl (%)	Potassium sorbate (mg/L)									
		100	200	300	400	500	600	700	800	900	1000
6.5	0	18.9[a] ± 0.7	15.7[a] ± 0.9	11.8[a] ± 1.3	7.8[a] ± 1.9	3.9[a] ± 2.2	1.0[a] ± 1.1	1.4 ± 2.2	2.0 ± 1.9	3.5 ± 2.8	4.6* ± 3.3
	3.5	24.1[a] ± 1.1	21.3[a] ± 1.4	17.0[a] ± 0.8	12.8[a] ± 1.7	4.2[a] ± 1.5	1.3[a] ± 1.9	4.2 ± 2.5	5.3 ± 2.9	7.1 ± 3.5	12.8 ± 4.5
	5.0	26.2[a] ± 1.8	25.6[a] ± 1.7	20.5[a] ± 1.5	15.4[a] ± 1.2	10.3[a] ± 1.0	10.0[a] ± 0.8	5.0 ± 2.0	6.6 ± 3.4	8.2 ± 4.5	10.3 ± 4.3
	7.5	32.9[a] ± 1.3	30.1[a] ± 1.5	27.4[a] ± 1.1	16.8[a] ± 1.9	14.3[a] ± 0.9	12.5[a] ± 1.4	22.2 ± 3.1	29.6 ± 4.0	30.8 ± 3.3	51.9 ± 4.0
	10.0	11.8 ± 1.5	13.7 ± 1.2	19.7 ± 1.9	23.5 ± 2.3	27.6 ± 3.3	35.3 ± 3.7	35.7 ± 3.3	47.1 ± 4.3	48.5 ± 3.8	58.8 ± 5.1
6.0	0	3.2[a] ± 1.1	10.9[a] ± 0.6	11.4[a] ± 0.9	11.9[a] ± 1.3	13.2[a] ± 1.1	16.3[a] ± 1.5	19.7[a] ± 2.0	22.9[a] ± 1.7	28.5[a] ± 2.2	35.2[a] ± 3.4
	3.5	29.1[a] ± 0.8	21.8[a] ± 1.2	25.5[a] ± 1.4	23.6[a] ± 1.0	18.2[a] ± 0.8	10.9[a] ± 1.1	8.4[a] ± 0.9	7.2[a] ± 1.1	3.6[a] ± 1.0	2.2[a] ± 0.9
	5.0	7.1[a] ± 1.6	9.0[a] ± 1.9	16.5[a] ± 2.1	18.7[a] ± 1.9	18.8[a] ± 1.7	22.5[a] ± 2.2	14.8 ± 1.5	17.7 ± 1.7	18.9 ± 2.0	21.1 ± 2.7
	7.5	7.4[a] ± 0.9	13.5[a] ± 1.3	17.3[a] ± 2.0	19.5[a] ± 1.8	23.0[a] ± 2.2	31.9[a] ± 2.9	29.5 ± 2.6	30.4 ± 3.3	33.9 ± 3.1	54.0 ± 4.5
	10.0	17.9[a] ± 2.0	20.7[a] ± 2.1	21.5[a] ± 1.9	28.6[a] ± 2.7	39.3[a] ± 3.1	7.1[a] ± 1.6	31.4 ± 2.0	35.7 ± 3.6	39.3 ± 3.8	62.6 ± 4.9
5.5	0	2.5[a] ± 1.0	7.7[a] ± 1.2	8.8[a] ± 0.9	10.5[a] ± 1.5	12.1[a] ± 1.9	14.6[a] ± 2.3	17.8[a] ± 2.2	22.4[a] ± 3.1	26.1[a] ± 3.7	31.5[a] ± 3.5
	3.5	3.0 ± 1.3	4.4 ± 1.5	5.6 ± 1.4	6.8 ± 2.0	7.7 ± 2.1	10.6 ± 1.9	12.3 ± 2.5	12.5 ± 2.3	15.4 ± 2.7	30.0 ± 3.1
	5.0	3.6[a] ± 1.2	9.9[a] ± 1.0	11.5[a] ± 0.9	12.2[a] ± 1.6	13.9[a] ± 1.9	16.8[a] ± 2.1	19.3[a] ± 1.7	24.1[a] ± 2.5	28.7[a] ± 2.9	39.0[a] ± 3.0
	7.5	19.6[a] ± 1.6	30.9[a] ± 2.8	45.7[a] ± 4.9	52.1[a] ± 4.7	55.8[a] ± 5.1	58.0[a] ± 5.5	65.3[a] ± 6.1	70.1[a] ± 5.8	77.4[a] ± 6.0	86.7[a] ± 6.3
	10.0	23.1[a] ± 2.0	42.5[a] ± 1.9	53.9[a] ± 2.2	60.0[a] ± 1.8	67.4[a] ± 1.3	71.1[a] ± 0.9	77.2[a] ± 2.0	79.6[a] ± 2.3	80.2[a] ± 2.7	88.5[a] ± 2.9
5.0	0	1.4[a] ± 1.3	4.0[a] ± 1.5	7.6[a] ± 1.2	8.3[a] ± 1.8	9.6[a] ± 1.9	11.3[a] ± 1.6	13.9[a] ± 2.0	17.3[a] ± 2.2	24.7[a] ± 2.0	27.2[a] ± 2.5
	3.5	41.1 ± 2.2	43.8 ± 2.5	53.6 ± 3.8	54.5 ± 3.5	60.7 ± 4.6	73.8 ± 5.0	86.6 ± 6.1	–**	–	–
	5.0	44.3 ± 2.9	50.5 ± 3.7	54.2 ± 4.9	57.7 ± 5.5	66.0 ± 5.3	68.0 ± 5.6	–	–	–	–
	7.5	62.7 ± 4.1	68.7 ± 4.5	82.1 ± 5.4	–	–	–	–	–	–	–
	10.0	–	–	–	–	–	–	–	–	–	–
4.5	0	1.5 ± 1.7	2.8 ± 1.9	4.4 ± 2.4	33.3 ± 3.6	56.7 ± 4.9	66.7 ± 5.3	–	–	–	–
	3.5	50.7 ± 4.5	58.4 ± 5.1	61.3 ± 5.5	69.6 ± 6.2	80.0 ± 6.8	–	–	–	–	–
	5.0	60.7 ± 5.5	62.8 ± 6.1	64.6 ± 6.0	75.2 ± 6.5	–	–	–	–	–	–
	7.5	69.1 ± 5.2	73.3 ± 6.1	80.5 ± 7.2	–	–	–	–	–	–	–
	10.0	–	–	–	–	–	–	–	–	–	–

* Mean value (n = 4) ± Standard Deviation ($p < 0.05$); ** – No growth.
[a] Stimulated growth rate (%).

TABLE 4 - The inhibition rates (%) of *Aspergillus niger* at different potassium sorbate, NaCl and pH levels after 7 days of incubation.

pH	NaCl (%)	Potassium sorbate (mg/L)									
		100	200	300	400	500	600	700	800	900	1000
6.5	0	1.9 ± 2.4*	3.8 ± 2.7	6.1 ± 3.3	7.2 ± 3.8	7.8 ± 3.5	8.8 ± 3.9	9.1 ± 3.4	9.9 ± 4.0	11.3 ± 3.9	12.0 ± 4.1
	3.5	2.4 ± 2.8	5.8 ± 3.0	6.7 ± 2.9	7.9 ± 3.5	8.4 ± 4.0	9.5 ± 4.1	11.1 ± 4.4	13.7 ± 4.5	16.1 ± 3.8	18.4 ± 4.3
	5.0	1.1[a] ± 0.8	2.3[a] ± 1.0	2.8[a] ± 1.1	3.5[a] ± 1.3	4.2[a] ± 1.2	6.5[a] ± 1.5	6.9[a] ± 1.1	7.4[a] ± 1.4	8.3[a] ± 1.2	9.5[a] ± 1.5
	7.5	1.8[a] ± 1.1	3.7[a] ± 1.4	9.4[a] ± 1.5	11.5[a] ± 0.9	13.3[a] ± 1.1	14.0[a] ± 1.3	15.8[a] ± 1.0	16.5[a] ± 1.7	18.1[a] ± 1.5	22.3[a] ± 1.9
	10.0	4.7[a] ± 1.5	5.3[a] ± 1.7	11.2[a] ± 1.6	13.9[a] ± 1.9	15.2[a] ± 2.1	16.6[a] ± 1.8	18.5[a] ± 1.5	19.7[a] ± 1.8	21.8[a] ± 1.7	25.6[a] ± 2.2
6.0	0	2.7 ± 2.6	6.1 ± 2.8	7.4 ± 3.0	9.0 ± 2.9	11.2 ± 3.1	14.8 ± 3.3	16.6 ± 3.5	19.1 ± 3.2	20.6 ± 3.4	23.3 ± 3.8
	3.5	3.1 ± 2.9	6.5 ± 3.3	8.6 ± 3.5	11.9 ± 3.4	15.0 ± 3.5	17.7 ± 2.7	20.3 ± 3.2	21.9 ± 3.7	24.8 ± 3.6	27.0 ± 4.1
	5.0	5.2 ± 3.3	7.7 ± 3.0	9.4 ± 3.2	13.7 ± 3.6	17.2 ± 3.8	18.4 ± 4.1	22.2 ± 4.3	24.5 ± 4.0	30.3 ± 4.5	35.7 ± 4.7
	7.5	5.7 ± 3.7	8.8 ± 3.9	11.9 ± 3.1	8.9[a] ± 1.1	9.6[a] ± 0.9	7.4[a] ± 1.0	7.8[a] ± 1.3	6.3[a] ± 1.0	7.6[a] ± 1.2	15.3[a] ± 1.6
	10.0	6.5 ± 4.0	9.3 ± 4.1	13.1 ± 4.4	2.5[a] ± 2.0	3.6[a] ± 1.9	11.0[a] ± 2.3	16.5[a] ± 2.5	18.2[a] ± 1.9	19.8[a] ± 2.3	20.5[a] ± 2.7
5.5	0	4.4 ± 3.1	8.2 ± 2.8	8.8 ± 3.3	13.7 ± 3.7	15.0 ± 3.6	17.3 ± 3.9	18.9 ± 3.5	21.6 ± 4.0	23.8 ± 3.8	28.8 ± 4.2
	3.5	5.5 ± 3.5	9.3 ± 3.7	11.2 ± 2.8	15.6 ± 3.9	18.5 ± 4.2	20.1 ± 4.4	22.3 ± 4.5	24.9 ± 4.3	26.4 ± 4.7	32.5 ± 5.0
	5.0	5.2[a] ± 1.0	5.2[a] ± 1.1	5.2[a] ± 0.9	5.2[a] ± 1.4	6.7[a] ± 1.6	8.0[a] ± 1.9	8.5[a] ± 2.0	8.8[a] ± 2.4	9.1[a] ± 2.2	9.5[a] ± 2.9
	7.5	11.3[a] ± 1.5	15.8[a] ± 1.7	22.4[a] ± 2.0	23.8[a] ± 2.2	24.5[a] ± 2.5	26.1[a] ± 2.3	27.6[a] ± 2.7	27.9[a] ± 3.0	28.8[a] ± 3.3	29.4[a] ± 3.5
	10.0	13.6[a] ± 2.1	17.5[a] ± 2.5	24.0[a] ± 2.7	27.4[a] ± 3.1	32.3[a] ± 3.0	36.1[a] ± 3.3	40.9[a] ± 3.6	42.3[a] ± 3.8	47.3[a] ± 3.6	45.7[a] ± 4.1
5.0	0	7.1 ± 3.4	10.4 ± 3.7	14.5 ± 4.0	17.8 ± 3.9	22.9 ± 4.4	35.7 ± 4.5	42.0 ± 3.8	55.8 ± 4.7	60.1 ± 5.0	72.3 ± 5.6
	3.5	12.6 ± 3.3	16.9 ± 4.1	20.3 ± 4.4	27.8 ± 3.7	31.5 ± 4.8	44.6 ± 5.1	54.4 ± 5.5	59.3 ± 6.0	67.2 ± 6.3	83.9 ± 6.4
	5.0	6.0 ± 4.0	7.7 ± 4.2	11.3 ± 3.9	15.9 ± 4.6	39.4 ± 5.1	47.7 ± 5.5	55.2 ± 6.7	–**	–	–
	7.5	18.8 ± 3.7	22.6 ± 3.5	36.9 ± 4.2	57.1 ± 5.9	91.1 ± 7.4	–	–	–	–	–
	10.0	29.6 ± 4.3	55.6 ± 5.2	–	–	–	–	–	–	–	–
4.5	0	15.3 ± 4.3	17.5 ± 4.6	24.6 ± 5.0	27.8 ± 4.8	35.1 ± 5.3	–	–	–	–	–
	3.5	18.9 ± 5.0	21.2 ± 5.4	30.0 ± 5.5	43.7 ± 5.9	–	–	–	–	–	–
	5.0	7.6 ± 3.5	9.4 ± 3.8	32.3 ± 4.7	68.3 ± 6.4	–	–	–	–	–	–
	7.5	8.5 ± 4.0	11.8 ± 4.5	37.5 ± 5.0	71.0 ± 7.1	–	–	–	–	–	–
	10.0	12.1 ± 3.9	15.6 ± 4.7	43.8 ± 5.8	–	–	–	–	–	–	–

* Mean value (n = 4) ± Standard Deviation ($p < 0.05$); ** – No growth.
[a] Stimulated growth rate (%).

trations against test fungi at experimental pH. The growth of *A. niger* was reduced in presence of 10% NaCl at pH 4.5 (without potassium sorbate). Kulik and Hanlin (1968) indicated that *A. flavus* was prevented by 15% NaCl in Malt Extract Agar. Statistical evaluation showed the antifungal property of pH against *P. roqueforti and A. niger* was insignificant ($p > 0.05$), whereas it was significant for *A. alternata* and *F. semitectum* ($p < 0.05$). According to the statistical analysis, potassium sorbate had a significant inhibitory effect on the growth of *A. alternata* and *F. semitectum* ($p < 0.05$).

Potassium sorbate and NaCl interaction had no stimulation effect on *A. alternata* and *F. semitectum* whereas *P. roqueforti* and *A. niger* were stimulated by 5-10% NaCl ($p < 0.05$). Potassium sorbate activity increased with decreasing pH against all test fungi, as expected. The results in the present study confirmed the dependence of preservative upon the pH as reported earlier by several studies (Sofos and Busta, 1981; Earle and Putt, 1984; Praphailong and Fleet, 1997; Fustier *et al*., 1998; Stratford and Anslow, 1998). Weak acid preservative, potassium sorbate is more effective towards inhibiting microorganisms in their undissociated forms, which increase in concentration as the pH decreases (Pethybridge *et al*., 1983; Eklund, 1983, 1985; Gould, 1996; Davidson, 1997).

The combination of all hurdles completely inhibited *A. alternata* and *F. semitectum* by lowest inhibitory factors, such as 100 mg/L potassium sorbate with 3.5% NaCl at pH 5.0. On the other hand, at the same pH, A*. niger* and *P. roqueforti* were totally prevented by 300 mg/L potassium sorbate with 10% NaCl and 400 mg/L potassium sorbate with 7.5% NaCl, respectively.

This study indicates that potassium sorbate is a suitable preserving agent to inhibit the growth of fungi in fermented products of pH near 4.5 regardless of NaCl levels. For products of slightly higher pH, the addition of potassium sorbate is suggested in combination with NaCl. Further studies are needed in hurdle factors suitable for application during the fermentation process, especially for fermented foods with reduced NaCl levels.

Acknowledgements

This study was supported by University of Uludag Scientific Research Committee, Bursa, Turkey, Project No. 2000/51.

REFERENCES

Bau M., Bragulat M.R., Abarca M.L., Minguez S., Cabanes F.J. (2005). Ochratoxigenic species from Spanish wine grapes Int. J. Food Microbiol., 98 (2): 125-130.

Chirife J., Favetto J. (1992). Some physico-chemical basis of food preservation by combined methods. Appl Technol., 25:389-396.

Combina M., Dalcero A.M., Varsavsky E., Chulze, S. (1999). Effects of food preservatives on *Alternaria alternata* growth and tenuazonic acid production. Food Addit. Contam., 16: 433-437.

Cuppers H.G.A.M., Oomes S., Brul S. (1997). A model for the combined effect of temperature and salt concentration on growth rate of food spoilage molds. Appl. Environ. Microbiol., 63: 3764-3769.

Dantigny P., Guilmart A., Bensoussan M. (2005). Basis of predictive mycology. Int. J. Food Microbiol., 100: 187-196.

Davidson P.M. (1997). Chemical preservatives and natural antimicrobial compounds. In; Doyle M. P., Beuchat L.R., Montville T.J., Eds, Food Microbiology: Fundamentals and Frontiers, Washington DC, ASM Press, pp. 520-556.

Delgado T., Gomez-Cordoves C. (1998). Natural occurrence of alternariol and alternariol methyl ether in Spanish apple juice concentrates. J. Chromatogr. A, 815 (1): 93-97.

Earle M.D., Putt G.J. (1984). Microbial spoilage and use of sorbate in bakery products. Food Technol., 19: 25-36.

Eklund T. (1983). The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. J. Appl. Bacteriol., 54: 383-389.

Eklund T. (1985). Inhibition of microbial growth at different pH levels by benzoic and propionic acids and esters of p-hydroxybenzoic acid. Int. J. Food Microbiol., 2: 159-167.

El-Gazzar F., Rusul G., Marth E.H. (1986). Growth and aflatoxin production by *Aspergillus parasiticus* in the presence of sodium chloride. J. Food Protect., 49 (6): 461-466.

Farag R.S., Daw Z.Y., Abo-Raya S.H. (1989). Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. J. Food Sci., 54: 74-76.

Finol M.L., Marth E.H., Lindsay R.C. (1982). Depletion of sorbate from different media during growth of *Penicillium* species. J. Food Protect., 45: 398-404.

Fustier P., Lafond A., Champagne C.P., Lamarche F. (1998). Effect of inoculation techniques and relative humidity on the growth of molds on the surfaces on yellow layer cakes. Appl. Environ. Microbiol., 64: 192-196.

Gould G.W. (1996). Methods for preservation and extension of shelf life. Int. J. Food Microbiol., 33: 51-64.

Hsieh K.P., Yu S., Wei Y.H., Chen C.F., Wei R.D. (1986). Inhibitory effect *in vitro* of PR toxin, a mycotoxin from *Penicillium roqueforti*, on the mitochondrial bicarbonate-ATPase of the rat brain, heart and kidney. Toxicon, 24 (2):153-160.

Kulik M.M., Hanlin R.T. (1968). Osmophilik strains of *Aspergillus* species. Mycologia, 60: 961-964.

Leistner L. (1992). Food preservation by combined methods. Appl. Techol., 25: 151-158.

Liewen M.B., Marth E.H. (1985). Growth of sorbate-resistant and -sensitive strains of P*enicillium roqueforti* in the presence of sorbate. J. Food Protect., 48 (6):525-529.

Lopez-Malo A., Alzamora S.M., Palou E. (2002). *Aspergillus flavus* dose-response curves to selected natural and synthetic antimicrobials. Int. J. Food Microbiol., 73: 213-218.

Lopez-Malo A., Alzamora S.M., Palou E. (2005). *Aspergillus flavus* growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds. Int. J. Food Microbiol., 99: 119-128.

Marasas W.F.O., Nelson P.E., Toussoun T.A. (1984). Toxigenic *Fusarium* species. Identify and Mycotoxicology. The Pennsylvania State University Press, University Park, London, UK.

Marin S., Guynot M.E., Neira P., Bernado M., Sanchis V., Ramos A.J. (2002). Risk assessment of the use of sub-optimal levels of weak-acid preservatives in the control of mould growth on bakery products. Int. J. Food Microbiol., 79: 203-211.

Moss M.O. (2000). Toxigenic fungi and mycotoxins. In: Lund B.M., Baird-Parker T.C., Gould G.W., Eds, Microbiological Safety and Quality of Food. Aspen Publishers, Inc., Gaithersburg, Maryland, pp. 1490-1517.

Nickelsen L., Jakobsen, M. (1997). Quantitative risk analysis of aflatoxin toxicity for the consumers of ‘‘kenkey’- a fermented maize product. Food Contr., 3: 149-159.

Nielsen P.V., Rios R. (2000). Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. Int. J. Food Microbiol., 60: 219-229.

Ozcan M., Boyraz N. (2000). Antifungal properties of some herb decoctions. Eur. Food Res. Technol., 212: 86-88.

Pethybridge A.D., Ison R.W., Harrigan W.F. (1983). Dissociation constant of sorbic acid in water and water-glycerol mixtures at 25 ^{0}C from conductance measurements. J. Food Technol., 18, 789-784.

Praphailong W., Fleet G.H. (1997). The effect of pH, sodium chloride, sucrose, sorbate and benzoate on the growth of food spoilage yeasts. Food Microbiol., 14: 459-468.

Rundberget T., Skaar I., Flaoyen A. (2004). The presence of *Penicillium* and *Penicillium* mycotoxins in food wastes. Int. J. Food Microbiol., 90 (2): 181-188.

Rusul G., Marth, E.H. (1987). Growth and aflatoxins production by *Aspergillus parasiticus* NRRL 2999 in the presence of potassium benzoate or potassium sorbate and at different ınitial pH values. J. Food Protect., 50 (10): 820-825.

Sahin I., Korukluoglu M. (2000). Kuf-Gida-insan. Uludag Universitesi Guclendirme Vakfı Vipas A.S. ISBN975-564-095-9, Bursa.

Sofos J.N., Busta F.F. (1981). Antimicrobial activity of sorbate. J. Food Protect., 44: 614-622.

Stratford M., Anslow P.A. (1998). Evidence that sorbic acid does not inhibit yeast as a classic weak acid preservative. Lett. Appl. Microbiol., 27: 203-206.

Suhr K.I., Nielsen P.V. (2004). Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activities and pH values. Int. J. Food Microbiol., 95: 67-78.

Thakur B.R., Singh R.K., Arya S.S. (1994). Chemistry of sorbate - a basic perspective. Food Rev. Int., 10: 71-91.

Tzatzarakis M., Tsatsakis A.M., Liakou A., Vakalounakis D.J. (2000). Effect of common food preservatives on mycelial growth and spore germination of *Fusarium oxysporum*. J. Environ. Sci. Health, B35 (4): 527-537.