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The Efficacy of Potassium Sorbate and Organic Acids in the Control of Food Spoilage Yeasts

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Abstract: In this study, the inhibition effect of some organic acids as natural antimicrobial agents and also, the efficiency of potassium sorbate which is known and used as a food preservative were researched to compare with each other. *Saccharomyces cerevisiae* ATCC 9763 and *Kluyveromyces lactis* ATCC 8585 strains were activated in Malt Ekstract Broth. The inhibitory effects of some concentrations of potassium sorbate (0, 300, 500, 750, 1000 mg L^{-1}) and organic acids which are acetic, lactic and citric acids (0, 0.1, 0.3, 0.5, 0.7, 1.0%) at pH 6.0 was researched. It was determined that the inhibitory effect of K-sorbate and organic acid concentrations are quite different. According to the results of this research, acetic acid concentrations are the most effective and citric acid is the second effective for inhibition of *S. cerevisiae*, on the other hand, for *K. lactis* as K-sorbate is the most effective one, acetic acid is the second effective inhibitor. But, lactic acid has the weakest effect for both of yeasts.

Key words: Yeast, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, inhibition, preservatives, K-sorbate, acetic acid, lactic acid, citric acid, food

INTRODUCTION

Food spoilage, a metabolic process that results foods to be unacceptable for consumption in sense of changes in sensorial properties, is often mediated by microbial activity and is a serious problem for the food industry since it leads to high economical losses. The microorganisms that could proliferate in any foodstuff highly depend on the microorganism itself that come into contact with the ingredients and the product, moisture content, chemical composition (nutrients), pH, temperature and the storage conditions (Loureiro and Malfeito-Ferreira, 1993; Loureiro, 2000).

Chemical preservatives are commonly used to preserve nutritional and sensorial quality, to prolong shelf-life and to control the growth of microorganisms that can constitute a health hazard for humans. Antimicrobial agents are added to the food product as supplements to prevent microbial spoilage caused by bacteria, moulds, yeasts, pathogen or non-pathogen microorganisms. Examples of antimicrobial agents that are generaaly used in food industry are benzoic acid, propionic acid, sorbic acid and their salts, acetic acid and acetates, nisin, nitrite and its compounds, sulphur dioxide and various sulphides (Cakmakci and Celik, 1994; Kucukoner, 2006; Yanochko *et al*., 2010).

Yeasts, a subset of a large group of organisms called fungi are widely distributed in nature and play an important role in food spoilage. The spoilage caused by yeasts is manifested by a variety of changes in sensory attributes, such as off-colors, off-odors, off-flavor, rancidity, acidity, softening and slime in wines, cheese, beverages, juices, fruits, salads, oils, sugar and meat (Ray, 1996; Souza *et al*., 2007; Sarnoski *et al*., 2012). The growth of spoilage yeasts in foods is interrelated with low pH, high sugar, organic acids and other easily metabolized carbon sources, certain temperature, oxygen and salt content. Those yeasts include Brettanomyces, Candida, Pichia, Rhodotorula, Torulopsis, Saccharomyces, Zygosaccharomyces, Hansenula and Trichosporon (Fleet 1992; Tudor and Board, 1993; Wojtatowicz *et al*., 2002; Forsythe, 2004; Querol and Fleet, 2006; Serpaggi *et al*., 2012).

Food spoilage can take place by the usage of lactate and acetate during the growth of yeasts or by the production of CO_2 and ethanol during fermentation without any measurable microbial growth (Moon, 1983; Thomas and Davenport, 1985; Savard *et al*., 2002).

In recent years, demand for quality and convenience besides the increased awareness regarding safety and health have significantly shifted consumption patterns and agricultural trade towards natural foods or food components. Processed food formulations may include antimicrobial compounds, either chemical or natural, in order to limit or inhibit the growth of spoilage

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microorganisms. Since, chemical antimicrobial agents pose high health hazard, consumer perceptions have led to exploration of applications of natural antimicrobial components in food systems. Therefore, using organic acids which have significant efficiency to control spoilage microorganisms is gaining popularity to provide increased food safety and prolonged shelf life (Warth, 1977; Smulders *et al*., 1986; Gould, 1990; Savard *et al*., 2002; Ullah *et al*., 2012).

The present study discusses the concentration dependent-inhibition effect of potassium sorbate and some organic acids (i.e., lactic acetic and citric acid) on two yeast species at fixed pH value (6.0). Since, it is well-defined by several researchers that K-sorbate and organic acids exhibit the highest inhibitory effect at low pH levels, the experimental design was planned to investigate the change of inhibition at high pH levels. The yeasts species were *Saccharomyces cerevisiae* and *Kluyveromyces lactis* and were chosen due to their well-known potential to grow and spoil the food (Betts *et al*., 1999; Loureiro and Querol, 1999; Souza *et al*., 2007; Arroyo-Lopez *et al*., 2008).

MATERIALS AND METHODS

Yeasts and inoculum preparation: *Saccharomyces cerevisiae* ATCC 9763 and *Kluyveromyces lactis* ATCC 8585 were obtained from DSMZ (German Collection of Microorganisms and Cell Cultures), Germany.

Cultur conditions: Malt Extract Agar and Broth (Merck) were used to grow yeast cultures (Kasemets *et al*., 2009; Sarlin and Philip, 2011).

After Malt Extract Medium was sterilized at 121°C for 15 min, the specified concentrations (0, 300, 500, 750, 1000 mg L^{-1}) of sterile K-sorbate (10%) was added to the medium and pH value was adjusted to pH 6.0 with sterile 1N NaOH under aseptic conditions. Same procedure was applied organic acids, to the sterilized medium, the specified concentrations (0, 0.1, 0.3, 0.5, 0.7 and 1.0%) of sterile organic acids (20%) were added and pH was adjusted (Yigit and Korukluoglu, 2007).

Inoculation of yeasts: Yeasts were stored on Malt Extract Agar slants by activating twice a month during the study. Cultures were activated after incubation at 30°C for 18-24 h in Malt Extract Broth (Souza *et al*., 2007). About 100 μL was taken from the yeast suspension and was inoculated to the each tube including K-sorbate, organic acids and control medium (without K-sorbate or organic acids) at pH 6.0 (Yigit and Korukluoglu, 2007).

Determination of viable microorganisms: About 0.1 mL yeast suspension was uniformly inoculated on the sterile Petri dishes containing Malt Extract Agar at six different times (0, 1, 4, 8, 14 and 24 h) and incubated at 30°C for 24 h. After 24 h incubation, visible yeast colonies were counted and the results were expressed as log CFU mL^{-1}. The inhibition was encountered by comparing the colony counts.

Statistical analysis: Statistical analysis of results was carried out by SPSS Software package (SPSS 15.0 SPSS Inc, Chicago, IL).

RESULTS

The growth of *K. lactis* was linear in Malt Extract Broth at pH 6.0 , whereas *S. cerevisiae* displayed a non-linear growth (Fig. 1).

The inhibitory effect of organic acids (acetic, citric and lactic acid) and potassium sorbate on *K. lactis* are shown in Table 1. The statistical analyses demonstrated that the addition of K-sorbate and organic acids and time of treatment was significant ($p<0.05$) on growth of *K. lactis*. Generally, the increase in sorbate concentration produced a decrease in yeast growth rate.

The growth of *Kluyveromyces lactis* was found to be dependent on the concentration and time of application of organic acids and K-sorbate.

As seen control results, stimulation effect of organic acids, at 0.1% concentration in general was determined; in other words, the main effect of organic acids were observed after 8 h.

The inhibition rates (%) of *Kluyveromyces lactis* ATCC 8585 at different organic acids and potassium sorbate concentrations for incubation period are demonstrated in Table 2.

The highest inhibitory effect on growth of *K. lactis* at 4th h was observed with 0.3% acetic acid as 38.8% and

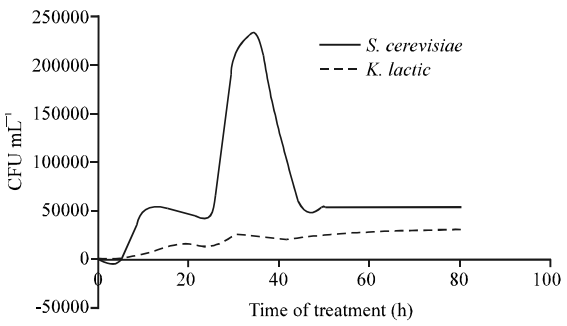

Fig. 1: The growth curves of *S. cerevisiae* and *K. lactis*

Table 1: *Kluyveromyces lactis* ATCC 8585 counts at different organic acid and potassium sorbate concentrations (log CFU mL^{-1}±SD)

	Time of treatment (h)			
Treatments	4	8	14	24
Control	5.36±0.00^{m}	6.34±0.02^{h}	6.71±0.00^{h}	7.37±0.00^{a}
Acetic acid (%)				
0.1	5.71±0.00^{f}	6.66±0.00^{b}	6.40±0.01^{l}	5.92±0.00^{m}
0.3	5.13±0.00^{p}	6.63±0.00^{q}	6.98±0.00^{b}	7.03±0.02^{g}
0.5	5.36±0.00^{m}	5.17±0.00^{e}	7.00±0.00^{a}	6.81±0.00^{j}
0.7	5.83±0.00^{d}	6.04±0.02^{l}	6.89±0.00^{e}	6.90±0.00^{i}
1.0	5.73±0.00^{e}	6.42±0.00^{e}	6.60±0.00^{k}	5.80±0.00^{o}
Lactic acid (%)				
0.1	5.45±0.00^{l}	6.35±0.00^{gh}	6.61±0.01^{j}	6.07±0.00^{l}
0.3	5.67±0.00^{g}	6.25±0.01^{i}	6.89±0.00^{e}	7.13±0.01^{d}
0.5	5.54±0.02^{i}	6.44±0.00^{d}	6.94±0.00^{d}	7.10±0.00^{e}
0.7	5.85±0.00^{c}	6.47±0.00^{c}	6.90±0.00^{e}	7.19±0.01^{c}
1.0	6.11±0.00^{a}	5.97±0.01^{n}	6.96±0.01^{c}	5.68±0.01^{q}
Citric acid (%)				
0.1	5.36±0.01^{m}	5.35±0.00^{f}	6.80±0.00^{f}	5.88±0.00^{n}
0.3	5.48±0.00^{j}	6.09±0.02^{k}	6.76±0.00^{g}	7.07±0.02^{f}
0.5	5.62±0.00^{h}	6.20±0.02^{j}	6.69±0.01^{i}	6.95±0.00^{h}
0.7	5.48±0.00^{j}	6.39±0.01^{f}	6.96±0.00^{c}	7.27±0.00^{b}
1.0	6.01±0.00^{b}	7.06±0.00^{a}	6.70±0.00^{h}	5.75±0.00^{p}
Potassium sorbate (mg L^{-1})				
300	5.46±0.00^{kl}	6.37±0.00^{g}	6.29±0.01^{m}	4.67±0.00^{r}
500	5.28±0.00^{n}	5.78±0.00^{o}	6.11±0.00^{n}	5.89±0.00^{n}
750	5.24±0.00^{o}	5.67±0.00^{p}	6.05±0.02^{o}	6.22±0.00^{k}
1000	5.47±0.00^{jk}	6.01±0.00^{m}	6.04±0.00^{o}	4.37±0.00^{s}

Mean value (n = 3) ±Standard deviation (p<0.05); $^{a-s}$The difference between the averages shown in the same column with different letters are important

Table 2: The inhibition rates (%) of *Kluyveromyces lactis* ATCC 8585 at different organic acids and potassium sorbate concentrations for incubation period

	Time of treatment (h)			
Treatments	4	8	14	24
Acetic acid (%)				
0.1	120.40^{a}	108.30	53.45	96.61
0.3	38.81	80.80	80.37^{a}	55.73
0.5	2.99	93.20	88.78^{a}	72.92
0.7	194.81^{a}	49.31	47.66^{a}	66.82
1.0	132.61^{a}	20.60^{a}	24.67	97.41
Lactic acid (%)				
0.1	22.42^{a}	3.80	20.74	95.01
0.3	100.80^{a}	19.40	48.59^{a}	43.42
0.5	50.81^{a}	25.80^{a}	63.55^{a}	46.31
0.7	203.40^{a}	32.11^{a}	54.20^{a}	33.60
1.0	455.10^{a}	57.70	71.02^{a}	98.00
Citric acid (%)				
0.1	0.62	89.80	19.43^{a}	96.80
0.3	30.30^{a}	43.40	9.72^{a}	51.61
0.5	78.60^{a}	27.60	8.22	62.32
0.7	29.51^{a}	12.60^{a}	77.57^{a}	20.54
1.0	346.90^{a}	423.50^{a}	3.55	97.62
Potassium sorbate (mg L^{-1})				
300	24.41^{a}	5.90^{a}	63.36	99.82
500	17.12	72.40	75.32	96.71
750	25.21	78.60	78.69	93.20
1000	27.20^{a}	53.20	78.69	99.91

^{a}Stimulated growth rate (%)

at 8th h with 0.5% acetic acid as 93.10%. At the end of 14 h of treatment, the inhibition declines to 53.45% whilst at 24 h acetic-acid-induced growth inhibition was 97.51% with 1% acid addition (Table 2). Lactic and citric acid additions did not cause growth inhibition at 4th h of treatment; on the contrary acid-inducement stimulated the growth of yeasts. The growth was rather stimulated till 24th h where high inhibitions were observed with 0.1 and 1% lactic/citric-acid-inducement. Application with 1% lactic acid significantly inhibited the growth over 24 h. Nonetheless, potassium sorbate displayed more pronounced inhibition over 24 h, as 1000 mg L^{-1} K-sorbate addition displayed the highest growth inhibition rate on *K. lactis* as 99.91%. It is apparent that the growth of *K. lactis* was inhibited in all media including any concentration of acid and K-sorbate at 24 h.

Table 3: *Saccharomyces cerevisiae* ATCC 9763 counts at different organic acid and potassium sorbate concentrations (log CFU mL^{-1}±SD)

	Time of treatment (h)			
Treatments	4	8	14	24
Control	6.06±0.00^{h}	7.04±0.00^{bc}	7.32±0.00^{e}	8.22±0.00^{a}
Acetic acid (%)				
0.1	6.16±0.01^{f}	6.84±0.00^{h}	7.26±0.00^{f}	7.41±0.01^{l}
0.3	6.18±0.00^{e}	6.87±0.00^{g}	7.23±0.01^{g}	7.32±0.00^{n}
0.5	6.16±0.01^{f}	6.84±0.00^{h}	7.18±0.00^{i}	7.34±0.01^{m}
0.7	6.12±0.01^{g}	6.80±0.00^{i}	6.95±0.00^{n}	7.30±0.00^{o}
1.0	5.93±0.00^{j}	6.62±0.00^{l}	6.97±0.00^{m}	7.11±0.01^{p}
Lactic acid (%)				
0.1	5.62±0.00^{o}	6.93±0.00^{d}	7.26±0.01^{f}	7.85±0.00^{e}
0.3	5.82±0.00^{k}	7.04±0.01^{b}	8.00±0.00^{a}	7.95±0.00^{c}
0.5	5.90±0.00^{j}	7.03±0.01^{c}	7.41±0.00^{b}	7.91±0.00^{d}
0.7	5.79±0.00^{l}	7.06±0.01^{a}	7.36±0.00^{c}	7.97±0.00^{b}
1.0	5.72±0.00^{n}	6.85±0.00^{h}	7.11±0.01^{j}	7.95±0.00^{c}
Citric acid (%)				
0.1	6.28±0.00^{c}	6.87±0.00^{g}	7.37±0.01^{c}	7.46±0.01^{k}
0.3	6.22±0.01^{d}	6.78±0.00^{j}	7.21±0.01^{h}	7.52±0.01^{i}
0.5	6.29±0.01^{b}	6.89±0.00^{e}	7.34±0.00^{d}	7.54±0.01^{h}
0.7	6.23±0.00^{d}	6.88±0.00^{f}	7.11±0.00^{j}	7.53±0.00^{i}
1.0	6.31±0.00^{a}	6.89±0.00^{e}	7.09±0.00^{k}	7.35±0.01^{m}
Potassium sorbate (mg L^{-1})				
300	5.92±0.00^{i}	6.72±0.00^{k}	7.00±0.01^{l}	7.59±0.00^{f}
500	5.82±0.00^{k}	6.62±0.00^{l}	7.21±0.01^{h}	7.57±0.01^{g}
750	5.75±0.00^{m}	6.48±0.00^{m}	6.77±0.00^{p}	7.49±0.00^{j}
1000	5.83±0.00^{k}	6.39±0.00^{n}	6.86±0.00^{o}	7.29±0.00^{o}

Mean value (n = 3) ±Standard deviation (p<0.05); $^{a-p}$The difference between the averages shown in the same column with different letters are important

The inhibitory effect of organic acids and potassium sorbate on the growth of *S. cerevisiae* is given in Table 3. As shown in Table 3, among the preservatives used, 0.1% lactic-acid-inducement displayed the highest inhibition on *S. cerevisiae* at the end of 4th h whilst all concentrations (0.1, 0.3, 0.5, 0.7 and 1%) of citric acid stimulated the growth. At the end of 8th h, acid-inducement and K-sorbate addition, except 0.7% lactic acid, inhibited the growth while the inhibitory rates of all preservatives showed a declining inhibitory efficiency by the 14th h. It is definite that to obtain an efficient inhibition with any preservative application of 24 h is required (Table 3). Inclusion of weak-acids and K-sorbate as antimicrobial agent exhibited a variable inhibitory effect on *S. cerevisiae* growth within 0.70-92.40% (Table 4).

Table 4: The inhibition rates (%) of *Saccharomyces cerevisiae* ATCC 9763 at different organic acids and potassium sorbate concentration doses at different hours

	Time of treatment (h)			
Treatments	4	8	14	24
Acetic acid (%)				
0.1	20.00[a]	37.84	13.55	84.91
0.3	26.66[a]	45.23	20.09	87.83
0.5	21.66[a]	36.77	28.50	87.19
0.7	13.33[a]	42.88	58.64	88.24
1.0	29.58	62.34	56.31	92.40
Lactic acid (%)				
0.1	64.96	23.08	14.96	58.02
0.3	44.28	0.70	365.81[a]	47.33
0.5	33.33	4.20	20.94[a]	51.91
0.7	48.17	4.20[a]	8.12[a]	45.80
1.0	55.96	36.36	39.74	48.07
Citric acid (%)				
0.1	58.33[a]	36.77	9.81[a]	83.15
0.3	40.00[a]	45.23	24.30	80.35
0.5	64.16[a]	29.19	3.74[a]	79.59
0.7	43.33[a]	30.99	37.39	80.35
1.0	72.50[a]	29.09	42.06	86.84
Potassium sorbate (mg L^{-1})				
300	30.17	52.45	52.99	77.10
500	45.01	62.24	24.35	78.32
750	52.55	72.38	72.65	81.83
1000	43.55	77.97	65.81	88.55

[a]Stimulated growth rate (%)

DISCUSSION

There appears a wide diversity in the growth characteristics of *S. cerevisiae* and *K. lactis* with acetic, citric, lactic acid and K-sorbate inducement at 6.0 pH. According to the data obtained from the present study, K-sorbate, particularly at 1000 mg L^{-1} concentration had the highest growth inhibition on both *S. cerevisiae* and *K. lactis*. Similar findings were reported by Romano and Suzzi (1985) with *S. cerevisiae*. In their research, they found a positive correlation between K-sorbate concentration and its inhibitory effect on the growth over time.

K-sorbate is thought to have various potential inhibition mechanisms affecting essential intracellular and membrane enzymes of microorganisms (Sofos, 2000), since any direct effect due to acidic hydrolysis by K-sorbate is not expected within approved concentrations as such concentration are relatively low. Han and Floros (1998) noted that K-sorbate was absorbed by *S. cerevisiae* after nutrient uptake did not interfere/prevent nutrient absorption and inhibited the growth of only active cells that uptake nutrients. They suggested that K-sorbate inhibited the critical biochemical reactions within yeast cell which are required for survival after nutrient and preservative uptake and the inhibition mechanism might be similar to bacteriostatic antibiotics. The present study expressed compatible inhibition efficacy of K-sorbate on *S. cerevisiae* with the researchers.

No relevant research is available concerning the effects of K-sorbate and weak-acid-inducement on growth of *K. lactis*. The reason of the stimulated growth of *K. lactis* until the end of 8th h of inoculation is associated with the extreme resistance of *K. lactis* to preservatives and consumption of them as a nutrient. It is assumed that the high inhibitory effect at 24th h could be due to consumption of all nutrients during the incubation, thus resulting in lacking of requisite nutrients for maintaining cell vitality.

Antimicrobial effect of weak-acids increases as pH decreases. This implies that the activity of acids is directly related to the amount of undissociated molecules (protonated acids) which increase as pH decreases in accordance to the increase in protons (Levine and Fellers, 1940; Ingram *et al*., 1956; Eklund, 1989; Ray and Sandine, 1992; Savard *et al*., 2002). However, a high concentration of organic acids in growth media is stressful. Organic acid stress does not result in a toxic effect due to high hydrogen ion concentration but is also dependent on the chemical nature of the organic acid to which the organism is exposed (Bayrock and Ingledew, 2004). Organic acids exert stronger inhibitory effects at low pH where they are substantially undissociated.

When acetic acid is dissolved in solution, it dissociates to release free protons and decreases the solution's pH. The increased amount of protons on the outer surfaces of microorganisms can disrupt membrane function via denaturing enzymes or altering cell permeability and change membrane stabilization (Booth, 1985; Cassio *et al*., 1987; Ray and Sandine, 1992; Casal *et al*., 1998; Savard *et al*., 2002), as well as can traverse the lipid bilayer of yeasts and cause acidification of the cytoplasm (Pampulha and Loureiro-Dias, 1989; Young and Foegeding, 1993; Casal *et al*., 1998; Guldfeldt and Arneborg, 1998; Quintas *et al*., 2005). When pH value is high, the amount of undissociated molecules will be relatively small and at this stage it is suggested that acetic acid can cause extracellular damage as the pH value is around neutral. In contrast, at lower pH values where the amount both of protons and undissociated acetic acid are high, significantly more internal and external cell damage is observed (Ray and Sandine, 1992; Fialova *et al*., 2008). In this case, in the study the stimulated effect of acetic acid on *K. lactis* until the end of 8th h was due to low concentration and inconsiderable undissociated molecules, so it is thought that the yeast could utilize acetic acid as nutrient.

The inhibition data on *S. cerevisiae* with acid and K-sorbate addition (Table 4) were similar to Pattison and von Holy (2001), who reported that acetic acid (≥0.1%) is a potential growth inhibitor of *S. cerevisiae*, eventhough being a by-product of alcohol fermentation by the yeast.

The antimicrobial effect of an acid is dependent upon its pKa value and the pH value of the external medium (Sorrells *et al*., 1989; Savard *et al*., 2002). Acetic acid (pKa 4.75) is a weaker acid than lactic acid (pKa 3.86) however, displays higher inhibition. This is mainly due to its function as having 2 or 4 times more molecules in undissociated form than lactic acid. The weak lipophylic undissociated acid can easily diffuse into the negatively charged cell and is dissociated in the cytoplasm where it reacts with essential cell substances. The extent of dissociation depends on intracellular pH and consequently, the cell activity is limited and inhibition occurs (Warth, 1977; Moon, 1983; Booth, 1985; Booth and Kroll, 1989; Eklund, 1980, 1989; Savard *et al*., 2002). Based on only its pKa (pKa 3.7), lactic acid is expected to be more efficient in growth control since it displays a higher pH decrease effect however, research on use of lactic acid in antimicrobial studies revealed that it is less effective than sorbic, acetic, propionic and benzoic acids (Chung and Goepfert, 1970; Minor and Marth, 1970; Sofos, 2000). The limited growth inhibition effect could be a result of lactic acid being in more ionized form than the other acids at a given pH, since the inhibitor form is the protonized form (not ionized) of the molecule.

The inhibitory activity of citric acid was variable on *K. lactis* and *S. cerevisae*. All concentrations of citric acid caused stimulation in growth at 4 h after inoculation. Hereafter, *K. lactis* was found to be more resistant to citric acid inhibition than *S. cerevisiae*. Most yeast isolated from dairy products are reported to grow at even low temperatures, in fermented lactose or sucrose and assimilate lactic and citric acids (Fleet, 1990; Laubscher and Viljoen, 1999), therefore citric acid is considered to behave as a growth-stimulant growth for *K. lactis* (Table 1 and 2).

Kawahata *et al*. (2006) explained that citric acid is inhibitory to *S. cerevisiae* at a much higher concentrations than other acids. Citric acid does not induce osmotic stress, however induces a general stress response and glycerol synthesis in the cell (Lawrence *et al*., 2004). There is very small amount of fermentable sugars, acetic and lactic acid left if *S. cerevisiae* is found in the medium because of its ability to metabolize these nutrients into acetyl-Coenzyme A (CoA) which enters the TCA and glyoxylate cycles to exercise the energy needs and synthesis of metabolites (Casal *et al*., 1996; Flores *et al*., 2000).

Lawrence *et al*. (2004) suggested that citric acid is not a so-called weak-acid preservative, rather acts as a chelator and inhibits the growth by chelating divalent metal ions from the medium. Nielsen and Arneborg (2007) investigated the effect of citric acid on growth and metabolism of anaerobic *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* cultures. They observed that growth inhibition of both yeast species increased with increasing pH values under citric-acid-inducement which indicated the dissimilarity of antimicrobial mechanism of citric acid from that of classical weak-acid preservatives. Citric acid is found to cause a high glycerol production and changes in transcryptome and proteome of aerobic yeasts (Omori *et al*., 1995; Lawrence *et al*., 2004).

CONCLUSION

If any organic acid is decided to be used as preservative whole properties of the microorganism and the food product should be taken into consideration. Further research is needed to verify antimicrobial efficacy of organic acids and potassium sorbate in food matrices as well as to evaluate their effectiveness to protect foods against spoialge by microorganisms throughout shelf-life. The results may also be useful for development of predictive models for control of spoilage yeasts.

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