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Research Article

Effect of chemical factors and clove oil to decrease the growth of film yeast on fermented bamboo shoots

Jaruwan Maneesri*[1] and Jirajin Maneesri[2]

[1]Department of Food Science and Nutrition, Faculty of Science and Technology, Prince of Songkla University, Pattani, Thailand.

[2]Planning Division**,** Prince of Songkla University, Pattani, Thailand.

*Author to whom correspondence should be addressed, email: mjaruwan@bunga.pn.psu.ac.th

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Abstract

This research examined the effect of chemical factors (pH, sodium chloride, sugars (glucose and sucrose), preservatives (benzoic acid and sorbic acid)) and clove oil to decrease the growth of film yeasts (*Saccharomyces cerevisiae* J1, *Candida krusei* J2 and *Candida krusei* J3) on fermented bamboo shoots. All yeasts grew at pH 3.0-7.0 and did not grow greater than 7.5%(w/v) of sodium chloride concentration. The ability to grow in the presence of sodium chloride decreased at low pH. Both *Candida krusei* strains were more tolerant to sodium chloride concentration than *S. cerevisiae* J1. All strains grew well in the presence of 7.5%(w/v) sodium chloride at pH 4.0-7.0, whereas, *Saccharomyces cerevisiae* was only tolerant to 7.5%(w/v) sodium chloride at pH 6.0. All yeasts grew well at 60-70%(w/v) sugar concentration at pH 3.0-7.0. The effect of benzoic and sorbic acids found that all yeasts were tolerant at 1000 mg/l and pH range 6.0-7.0. No strains grew in the presence of preservatives at pH 2.0-4.0. Clove oil (*Syzygium aromaticum* L.) inhibited completely all yeast film strains at 2%(v/v). However, clove oil treatment reduced the organoleptic properties of fermented bamboo shoots with their strong flavour.

Keywords: sodium chloride, *Candida krusei*, sugars, *Saccharomyces cerevisiae*, preservatives, clove oil, film yeast, *Syzygium aromaticum*, fermented bamboo shoots, Thailand.

Introduction

Other than bacterial pathogens, spoilage yeasts are one of the major problems found in fermented food. For example, Savard *et al*. [1] suggest that spoilage yeasts of fermented vegetables is caused by *Saccharomyces bayanus* and *S. unisporus*, whilst James and Stratford [2] suggest that the most problematic spoilage yeasts in the food and drinks industry are those belonging to the genus *Zygosaccharomyces*. It is known that the fermentation and processing techniques are important in decreasing the viability of yeast spoilage. Studies of yeast growth in such conditions demonstrate that different yeast species respond in widely different ways to environmental conditions.

Particularly, chemical factors such as pH, concentration of sodium chloride, concentration of sucrose and concentration of sorbic and benzoic acids are used to inhibit yeasts as *Debaryomyces hansenii*, *Yarrowia lipolytica*, *Kloeckera apiculata*, *Zygosaccharomyces bailii*, *Z. rouxii*, *Kluyveromyces marxinus*, *Pichia membranaefaciens*, *Pichia anomala* and *Saccharomyces cerevisiae* [3]. In addition, the synergistic effect of sodium chloride, temperature and pH on the growth of spoilage yeasts has been studied [4, 5]. These studies show the need to find safe and effective replacements for chemical treatment, such as natural food preservatives. Clove oil has been used throughout the world for such applications. Eugenol (4-allyl-2-methoxyphenol), the active substance, makes up 90-95% of clove oil [6], and as a food additive is classified as a substance that is generally regarded as being safe. Many reports have shown the potential application of clove oil to food. Prasad and Seenayya [7] used solar salt brines with clove oil at 0.02 and 0.5% (v/v) concentrations in salt cured fish, which could completely eliminate *Halomonas* spp. and red halophilic bacteria after 30 s and 1 min exposure, respectively. Singh *et al.* [8], reported the essential oil in clove was highly effective against *Listeria monocytogenes* in hotdogs. Also, Mytle *et al*. [9], determined that 1% or 2% clove oil in frankfurters inhibited *L. monocytogenes*. There are few reports in the literature about the inhibitory effects of clove on the growth of food spoilage yeasts.

In a previous study, our laboratory had found *Saccharomyces cerevisiae* J1, *Candida krusei* J2 and *C. krusei* J3 as film yeasts on the surface of the fermentation solution from fermented bamboo shoot products and altered the odor, colour, taste and texture of the products [10]. Little research has been undertaken the growth or non-growth responses of film yeast to environmental and natural food preservatives.

Therefore, the purpose of this investigation was to determine the effects of pH, sodium chloride, sugars, preservatives and clove oil on the viable growth of film yeast.

Materials and Methods

Yeast species

Three strains of yeast [10], were isolated from fermented bamboo shoot products produced by villagers in Amphur Kokpho, Pattani Province. *Saccharomyces cerevisiae* J1, *Candida krusei* J2 and *Candida krusei* J3 were maintained on slants of YPD agar medium (1% yeast extract, 2% peptone, 2% glucose and 2% agar) with pH 5.0 at 10^{o}C until required for use.

Preparation of inoculum

A loopful of inoculum was taken from a pure culture of film yeast grown on slants and inoculated into 100 ml of YPD broth. It was then incubated at 30^{o}C on a shaker incubator at 110 rpm for 18 h. The growth so obtained was adjusted to 10^4-10^6 cfu/ml and used as inoculum.

Effect of chemical factors on the growth of film yeast

The presence or absence of yeast growth in response to chemical factors were determined in 96-welled microlitre trays. Medium (0.3 ml) was aseptically dispensed into the wells of the tray and each well was inoculated with 0.03 ml of yeast suspension. The trays were incubated at 30^{o}C for 48 hours and growth was recorded visually as the presence or absence of turbidity in the wells. Experiments were repeated in triplicate.

To examine the effect of pH (2.0, 3.0, 4.0, 5.0, 6.0 and 7.0) on yeast growth, sodium chloride (2.5, 5.0, 7.5, 12.5,15.0 and 20.0%(w/v)) at different pH values and sugars (sucrose and glucose) were incorporated into the YPD medium to give final concentrations of 20, 30, 40, 50, 60 and 70%(w/v) and preservatives (benzoic acid or sorbic acid) were added to the YPD medium of different pH values to give final concentrations of 250, 500, 750, 1000 mg/l. To maintain a constant concentration all components were filtered through a 0.45 μm membrane.

Effect of clove oil on the growth of film yeast in fermented bamboo shoot

Young edible shoots of bamboo were peeled and chopped into thin pieces and soaked in 5% (w/v) sodium chloride for 2 days. They were then divided into 170 g portions, 400 ml of 5% sodium chloride was added, the solution kept in the bottle, covered tightly and stored at ambient temperature to allow fermentation for 15 days.

The experiments (three replications) were carried out for each addition of different concentrations of clove oil (0, 0.5, 1.0, 1.5 and 2.0 %(v/v)) and 1%(v/v) of inoculum. They were incubated at room temperature. The solution of fermented bamboo shoot was taken for pH and microbiological analysis at 0, 24 and 48 h. The pH values were determined with a digital pH meter (Schott, Germany). For determination of microbiological count, serial 10-fold dilutions were prepared by diluting 1 ml of sample solution in 9 ml of 0.1%(w/v) peptone. Yeasts and moulds was enumerated in potato dextrose agar (PDA, Himedia) and incubated at 30^{o}C for 3-5 days. Microbial counts were expressed as log cfu/ml.

Preliminary sensory evaluation of fermented bamboo shoot treated by the application of clove oil was carried out. It was evaluated for colour, odor and overall acceptance by 50 untrained panelists. A five-point hedonic scale was used to assess the treated fermented bamboo shoot. The panelists were asked to evaluate the samples on a scale from 5 to 1 indicating decreasing taste.

All experiments were run in triplicate. Data were subjected to analysis of variance (ANOVA) and differences among the means were determined for significance using Duncan's multiple range test. Statistical analysis was performed using the Statistical Package for Social Science (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL).

Results and Discussion

Effect of chemical factors

pH

In general, fermented bamboo shoot is an acidic food. The growth of film yeasts was determined at the pH range of 2.0-7.0 (Table 1). All species exhibited strong growth in the pH range of 3.0-7.0. It has been reported that most yeasts initiate growth within this range [11, 12, 13]. *C. krusei* J2 and J3 grew weakly at pH 2.0, while *S. cerevisiae* J1 did not grow at all. *C. krusei* strains were tolerant of pH 2.0 and closely resembled *C. magnoliae*, the low pH resistant yeast that grew at pH 1.75 [14]. However, the obtained results showed reduction of pH to 2.0 was not sufficient to inhibit growth of film yeasts.

Table 1. Effect of variable pH (2-7) on the growth response of yeast species in YPD broth.

pH	Yeast species		
	J1	J2	J3
2	-	+	+
3	+++	+++	+++
4	+++	+++	+++
5	+++	+++	+++
6	+++	+++	+++
7	+++	++	+++

Yeast species; J1, *S. cerevisiae* J1, J2, *C. krusei* J2, J3, *C. krusei* J3
Growth reaction : -, no growth; +, weak growth; ++, good growth; +++, strong growth as determined by relative amount of turbidity (visual observation) in wells of microlitre trays.

The ability of yeasts to tolerate acid pH values is related to the activity of plasma membrane ATPase which regulates intracellular pH by exporting protons [15]. Presumably, yeasts which tolerate low pH values were more efficient or had a more stable plasma membrane ATPase system, but this would need to be demonstrated experimentally by comparing with different environments.

Sodium chloride

The growth of film yeasts at different concentrations of sodium chloride were varied with range of pH (Table 2). None of the strains examined in this study grew at 12.5-20%(w/v) sodium chloride. *S. cerevisiae* J1exhibited tolerance to salt occurring at high pH values of 5.0-6.0 and the ability to grow in presence of sodium chloride was decreased at low pH. The greater tolerance of *S. cerevisiae* to sodium chloride has been reported by Praphailong and Fleet [3]. It is not clear why the lower pH limits for growth of some yeasts are extended by the presence of salt. It is normally a preservative and used for product stability. *C. krusei* J2 and J3 were more tolerant to salt than *S. cerevisiae* J1. They showed greatest tolerance to 7.5% and 5.0%(w/v) sodium chloride at pH 4.0-7.0 and 2.0-3.0, respectively. pH and sodium chloride did not affect *C. krusei*. However, *S. cerevisiae* J1 and both *C. krusei* did not grow at a concentration greater than 7.5%(w/v) sodium chloride.

Table 2. Effect of sodium chloride concentrations at different pH values on the growth response of yeasts in YPD broth.

pH	Maximum sodium chloride (%w/v)		
	J1	J2	J3
2	NG	5.0	5.0
3	2.5	5.0	5.0
4	2.5	7.5	7.5
5	5.0	7.5	7.5
6	7.5	7.5	7.5
7	2.5	7.5	7.5

Yeast species; J1, *S. cerevisiae* J1, J2, *C. krusei* J2, J3, *C. krusei* J3
NG, no growth in the absence of NaCl

Sugars

The tolerance to sugars (glucose and sucrose) of yeast films were studied. All strains tested were able grow in YPD medium containing 70%(w/v) sugars (glucose and sucrose) (Table 3). The ability for growth in presence of 60-70%(w/v) glucose and sucrose was shown at pH 3.0-7.0.

Varying pH was shown to have a positive effect on growth. Yeast species tolerated to high sugar concentration have been studied by Martorell *et al*. [14]. Therefore, they could be considered a possible extreme adaptation of yeast.

Table 3. **Effect of glucose and sucrose concentrations at different pH values on the growth response of yeasts in YPD broth.**

pH	Maximum glucose (%w/v)			Maximum sucrose (%w/v)		
	J1	J2	J3	J1	J2	J3
2	NG	30	30	NG	70	70
3	60	60	60	70	70	70
4	70	70	60	70	70	70
5	70	70	70	70	70	70
6	70	60	70	70	70	70
7	60	60	60	60	70	70

Yeast species; J1, *S. cerevisiae* J1, J2, *C. krusei* J2, J3, *C. krusei* J3
NG, no growth in the absence of glucose or sucrose

Preservatives

The tolerance of yeasts for preservatives of weak carboxylic acid (benzoic and sorbic acid) were tested (Table 4). It was observed that *S. cerevisiae* J1 and *C. krusei* J2 were tolerant of benzoic acid at concentration 750 mg/ml (pH 5.0) and 1000 mg/ml (pH 6.0-7.0), whereas *C. krusei* J3 showed strong tolerance to benzoic acid at concentration 1000 mg/ml (pH 5.0-7.0). For the effect of sorbic acid it was also found that all species were tolerant at concentration 1000 mg/ml (pH 6.0-7.0). *S. cerevisiae* J1 and both *C. krusei* were tolerant at concentration 250 mg/ml (pH 5.0) and 500 mg/ml (pH 5.0), respectively. All yeast species were inhibited by a lower concentration of preservatives (<250 mg/ml) at low pH (2.0-4.0). At pH 5.0 and the presence of benzoic acid (750-1000 mg/ml), yeasts were more tolerant than in the presence of sorbic acid (250-500 mg/ml). All strains were not resistant to benzoic acid and sorbic acid at low pH. These results are in agreement with Ferreira *et al*. [16]. The weak acid inhibition of fermentation showed a good correlation with the lipid solubility of weak acids suggesting that the acids interact with the hydrophobic regions of cell membranes. The combined effects of pH and preservatives were also reported by Arroyo Lopez *et al.* [17].

Table 4. **Effect of benzoic and sorbic acid concentrations at different pH values on the growth response of yeasts in YPD broth.**

pH	Maximum benzoic acid (mg/l)			Maximum sorbic acid (mg/l)		
	J1	J2	J3	J1	J2	J3
2	NG	-	-	NG	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	750	750	1000	250	500	500
6	1000	1000	1000	1000	1000	1000
7	1000	1000	1000	1000	1000	1000

Yeast species; J1, *S. cerevisiae* J1, J2, *C. krusei* J2, J3, *C. krusei* J3
NG, no growth in the absence of benzoic acid or sorbic acid; - no growth in the presence of benzoic acid or sorbic acid.

Effect of clove oil

The effects of natural food preservatives such as clove oil on the growth of film yeasts are shown in Figure 1. Treatment with 2% clove oil, the yeast and moulds were completely eliminated with no growth at 24 h. Clove oil clearly inhibits the growth of film yeasts.

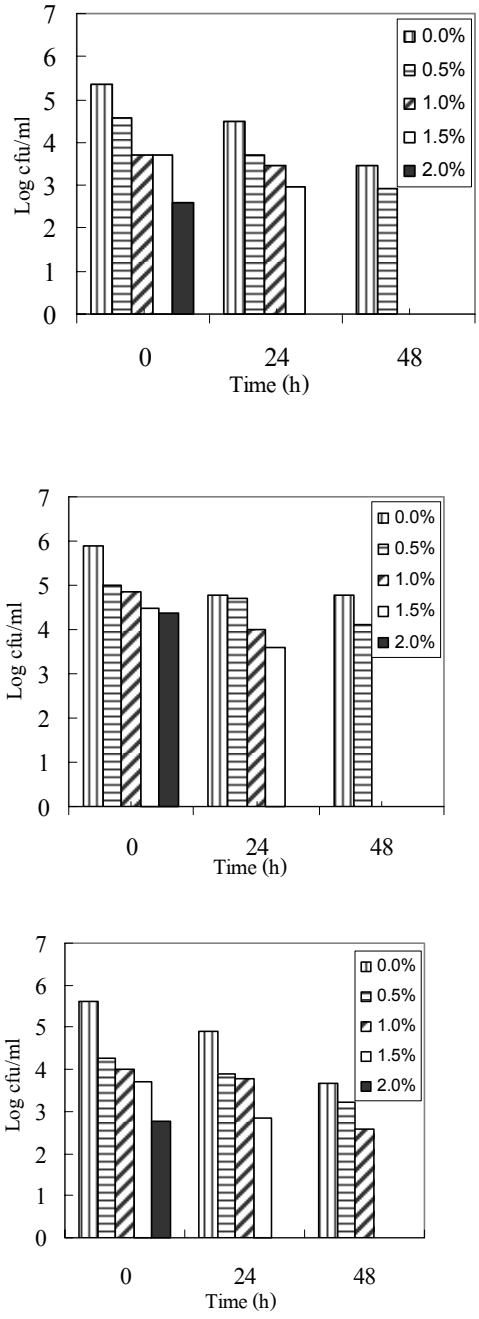

Figure 1. Effect of clove oil concentrations at different time on the yeast and moulds in the fermented bamboo shoot and addition *S. cerevisiae* J1 (a) *C. krusei* J2 (b) and *C. krusei* J3 (c).

Origanum vulgare L. essential oil has been considered as a strong inhibitor to *C. albicans* and *C. krusei* [18]. The reactions were the prevention of any complex formation, especially the cell membrane which is widely regarded as one of the primary target sites for plant essential oils [19, 20]. Cytoplasm membrane disturbance, rupture of proton motive force and cytoplasm content coagulation are some mechanisms involved in the antimicrobial properties of essential oils [21, 22]. To reach an adequate shelf life, fermented bamboo shoot products require less film yeast.

Statistical analysis of the sensory panel evaluations for colour, odor and overall acceptability revealed significant differences ($P<0.05$). The sensory studies showed the 2% clove oil treatment had a lower score than the untreated. The flavor was not acceptable to the consumers (Fig. 2).

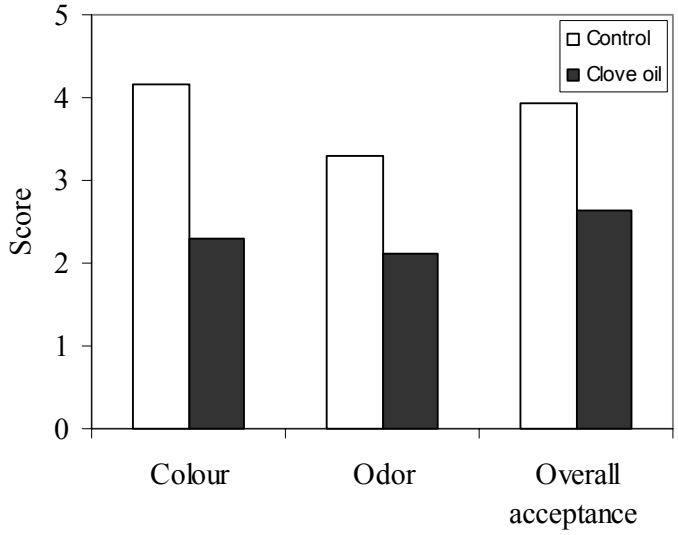

Figure 2. Taste panel results of fermented bamboo shoot prepared by adding clove oil.
□ control (0%); ■ 2%(v/v) clove oil

This result was of much importance since the sensory quality of food products often limits the use of plant essential oil as an antimicrobial agent. Similar results have been reported in hotdogs [9]. Thus a strong flavor would be dispersed when the package is opened by the consumer. For example, Matan *et al*. [23] reported higher concentrations of clove oil should be explored to provide adequate protection for intermediate moisture food (IMF) products. In addition, the advantages of using a volatile gas phase of essential oil for food products are that it may have lesser influence on the final taste and aroma of the product and its release may better be able to be regulated.

Conclusion

The study has revealed that film yeasts (*S. cerevisiae* J1, *C. krusei* J2 and *C. krusei* J3) from fermented bamboo shoot respond to some environments on their growth. These strains displayed particular physiological behaviours, including growth at low pH, resistance to sodium chloride and sugar, extreme osmotolerance, ability to adapt to high sugar concentration, no resistance to preservatives at low pH. The prevention of the growth of these strains could be considered by applying a low concentration of benzoic or sorbic acid in combination with low pH values that can markedly inhibit the growth of film yeasts. 2% clove oil was able to suppress the growth of

all film yeast strains. However, it has a strong flavour, thereby reducing the resultant organoleptic properties. Therefore, it could be useful to preserve and enhance food safety in fermented bamboo shoot. The application of clove oil would need to consider other techniques to minimize the reduction of flavor.

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