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NOTE

EFFECT OF VANILLIN ON GERMINATION TIME AND RADIAL GROWTH OF MOULDS IN APPLE PUREE

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ABSTRACT

The inhibitory effects of various concentrations of natural vanillin on the growth of *Aspergillus niger, A. flavus, Pencillium expansum* and *P. italicum* in potato dextrose agar (PDA) and apple puree-based agar at pH of 3.5 and water activity (a_w) of 0.98 were studied. The most resistant mould was *A. niger*, followed by *A. flavus, P. italicum* and *P. expansum*. For each mould the increase in vanillin concentration affected radial growth rate. The vanillin inhibitory concentrations were in general lower than 1500 mg l^{-1}.

Key words: *A. flavus, A. niger, P. expansum, P. italicum,* Apple puree, Germination time, Mould, Radial growth.

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اثر ضد میکروبی وانیلین بر رشد کپکها در پوره سیب

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چکیده

اثر ضد میکروبی اسانس وانیلین بر رشد چهار کپک *Aspergillus niger, A. flavus* و *P. italicum* و *P. expansum* در پوره سیب و محیط کشت PDA مورد بررسی قرار گرفت. با افزودن اسید سیتریک، pH پوره ها به ۳/۵ کاهش یافت و a_w پوره سیب و PDA به ترتیب ۰/۹۸۷ و ۱/۰۰۳ تعیین شد. غلظت موثر اسانس وانیلین در سیستم PDA معادل ۵۰۰ $mg\ l^{-1}$ برای کپک های *P. italicum* و *P. expansum* ، ۱۰۰۰ $mg\ l^{-1}$ برای کپک *A. flavus* و برای *A. niger* برابر ۱۵۰۰ $mg\ l^{-1}$ تعیین شد. در پوره سیب، غلظت لازم برای جلوگیری از رشد کپک های *A. flavus* و *A. niger* برابر ۱۵۰۰ $mg\ l^{-1}$ و برای کپک های *P. italicum* و *P. expansum* برابر ۱۰۰۰ $mg\ l^{-1}$ به دست آمد.

INTRODUCTION

New trends in food preservation have led to reduction in the levels of preservatives and the use of naturally occurring antimicrobials (2). Therefore, the examination of natural antimicrobials has been stimulated and several reports about antimicrobial activity of spices, herbs and plants have been published (8). The potential for an extensive use of many of these natural antimicrobials appears promising. However, the challenge is to isolate, purify, stabilize and incorporate these natural antimicrobials into foods without adversely affecting the sensory, nutritional and safety characteristics of the product (1). On the other hand, antimicrobial activities may vary widely depending on the type of spice or herb, microorganism, and food product (7), and it is necessary to examine their suitability for use in each particular case. Otherwise, the effectiveness of natural antimicrobials can only be enhanced by

combination with other factors (3).The antimicrobial activities of extracts from several types of plant parts used as flavoring agents in foods and beverages have been recognized for many years (10). Among these, vanillin (4-hydroxy-3-methoxybenzaldehyde), a major constituent of vanilla beans, has been reported as a possible antimicrobial agent (11). In this study the use of vanillin at organoleptically acceptable levels and in combination with reduced pH and a_w to inhibit the growth of different strains of food spoilage moulds in laboratory medium and in apple puree was evaluated.

MATERIALS AND METHODS

Microorganisms and Inocula Preparation

Aspergillus niger PTCC-5010, *A. flavus* PTCC-5006 (from BBRC,Tehran, Iran), *Pencillium expansum* and *P. italicum* (from Alzzahra Univ., Tehran, Iran) were obtained. The strains were cultured on PDA (Merck Co.) slants for about 5-10 days at 25°C and the spores were harvested in 10 ml of sterilized distilled water containing 0.1% Tween 80 (Merck Co.) The spore suspensions were adjusted with the same solution to give a final concentration of 10^6 spores per ml.

Preparation of the Systems

Apples were purchased (4-5 kg) from a local market and stored under refrigeration until use for no longer than 5 days. The fruit was washed, and hand peeled, cut in cubes (~ 1 cm) and blanched with saturated vapor for 5 min. Then it was aseptically homogenized in a blender with 1% w/w of agar-agar (Merck Co.) and the necessary quantity (previously determined) of citric acid added to give a pH of 3.5. The fruit-based agar was heated for 1 min. at 110°C, cooled, aseptically divided into four portions and to each one, 0, 500, 1000 and 1500 mg l^{-1} of crystalline natural vanillin (Merck Co.) were added and mechanically mixed under sterile conditions. The fruit-based agar was poured into sterile petri dishes. For the PDA system, the agar solutions with the quantity of sucrose needed to reach an a_w of 0.98 (from 1.00 to 0.98 with addition of 11.5% sucrose) were sterilized for 15 min at 120°C and cooled and

acidified with citric acid to pH 3.5. Systems with different vanillin concentrations were prepared as above.

Inoculation and Incubation

Four petri dishes of each system and for each mould were centrally inoculated by puncturing the agar with the spore suspension to give a circular inoculum of 0.6 mm in diameter. Three plates of each system were maintained without inoculation as controls. The inoculated plates and controls were covered and sealed with plastic film and stored at 25°C for 2 months.

Measurements of Water Activity and pH

The pH was determined using a 766 Calimatic Knick pH meter Germany). The a_w was measured with Thermoconstantor Novasina.

Colony Radial Growth

The inoculated systems were examined daily and after the colonies were confluent, their increase in size was followed by measuring colony diameter. The increase in diameter on each plate was plotted as a function of incubation time and the radial growth rate was obtained from the slope by linear regression of the linear phase of growth (9). The mean radial growth rate and the standard deviation were calculated and reported. To calculate the germination time or lag, the linear equation was extrapolated to a zero increase in diameter (0.6 mm diameter), and the intercept on the time axis was defined as the lag.

RESULTS AND DISCUSSION

The pH and the a_w of the fruit-based and PDA control were determined at the beginning and end of 2 months of storage at 25°C showed that pH and a_w remained constant under storage conditions. The addition of up to 1500 mg l^{-1} of natural vanillin did not affect pH and the a_w of the studied systems. Fig. 1

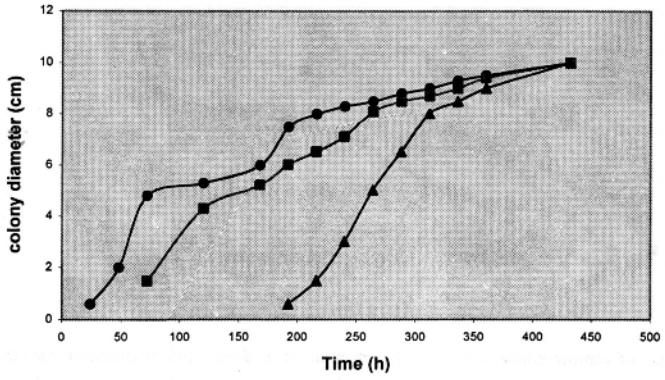

Fig. 1. Effect of natural vanillin concentration on the variation of *A. niger* colony diameter (cm) in apple puree-based agar at pH=3.5 and a_w = 0.98 during incubation at 25°C. (●) 0 mg l^{-1}, (■) 500 mg l^{-1}, (▲) 1000 mg l^{-1}

shows the increase in colony diameter of apple-based agar inoculated with *A. niger* with different natural vanillin concentrations. After a lag period, that increased with vanillin concentration, the increase of colony diameter followed a zero order kinetics (linear increase with incubation time). Similar results were obtained for the other moulds (Fig. 2 to 8).

Table 1 shows the mean radial growth rate in the different systems tested for *A. niger, A. flavus, P. expansum, and P. italicum*. In the systems where the moulds grew, the mean radial growth rate varied between 0.0021(*P. italicum*, apple puree, 500 mg l^{-1}) to 0.058 mmh^{-1} (*A. niger*, PDA, 0 mg l^{-1}) and the standard deviations from 0.001-0.013 mmh^{-1}. The most resistant mould to the conditions assayed was *A. niger*. For the PDA systems, the presence of 1500 mg l^{-1} natural vanillin inhibited *A. niger* growth for more than 2 months at 25°C, while growth of *A. flavus* was inhibited by 1000 mg l^{-1} and *P. expansum* and *P. italicum* were inhibited by 500 mg l^{-1}. Inhibitory concentration of vanillin

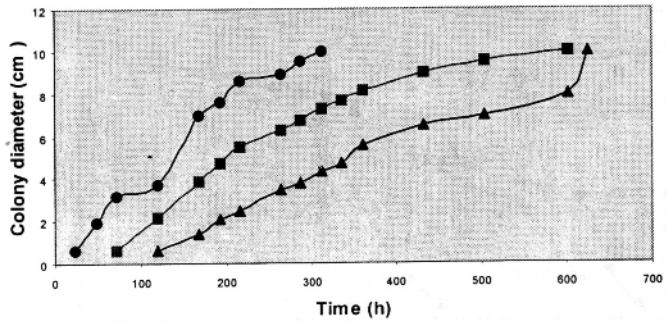

Fig. 2. Effect of vanillin concentration on the variation of *A. flavus* colony diameter (cm) in apple puree-based agar at pH=3.5 and a_w=0.98 during incubation at 25°C. (●) 0 mg l^{-1}, (■) 500 mg l^{-1}, (▲) 1000 mg l^{-1}.

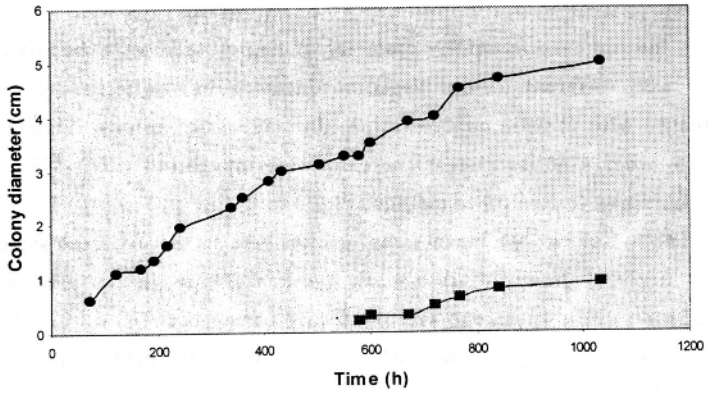

Fig. 3. Effect of vanillin concentration on the variation of *P. expansum* colony diameter (cm) in apple puree-based agar PDA system at pH=3.5 and a_w=0.98 during incubation at 25°C. (●) 0 mg l^{-1}, (■) 500 mg l^{-1}.

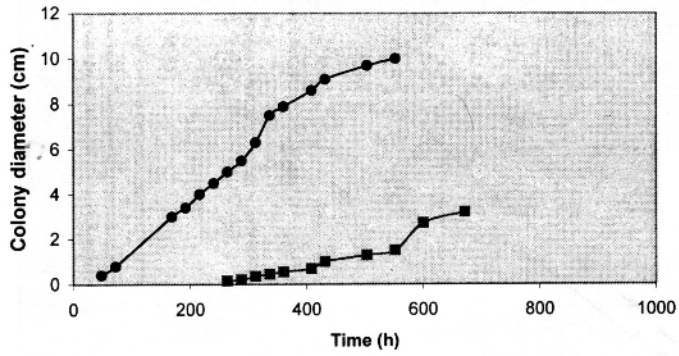

Fig. 4. Effect of vanillin concentration on the variation of *P. italicum* colony diameter (cm) in apple puree-based agar and PDA system at pH=3.5 and a_w=0.98 during incubation at 25°C. (●) 0 mg l^{-1}, (■) 500 mg l^{-1}

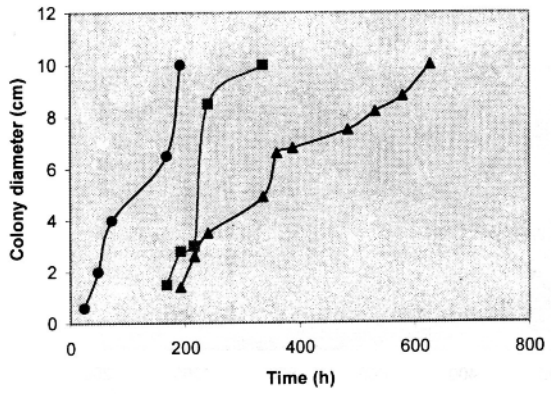

Fig. 5. Effect of vanillin concentration on the variation of *A. niger* colony diameter in (cm) PDA system at pH=3.5 and a_w=0.98 during incubation at 25°C. (●) 0 mg l^{-1},(■) 500 mg l^{-1}, (▲) 1000 g ml^{-1}.

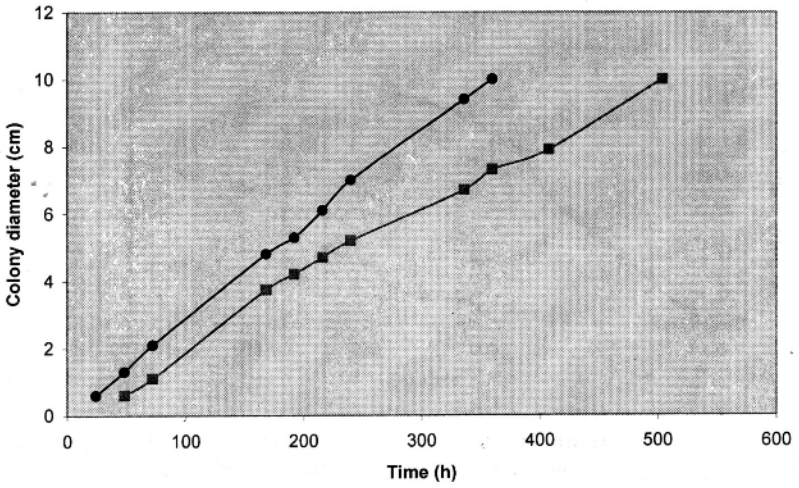

Fig. 6. Effect of vanillin concentration on the variation of *A. flavus* colony diameter (cm) in PDA system at pH= 3.5 and a_w=0.98 during incubation at 25°C. (●) 0 mg l^{-1}, (■) 500 mg l^{-1}.

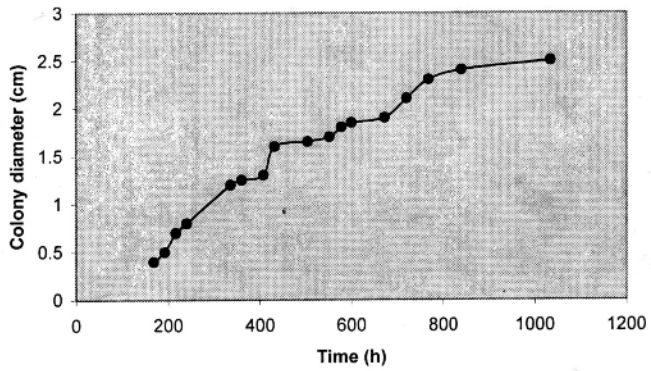

Fig. 7. Effect of vanillin concentration on the variation of *P. expansum* colony diameter (cm) in PDA system at pH=3.5 and a_w=0.98 during incubation at 25°C. (●) 0 mg l^{-1}.

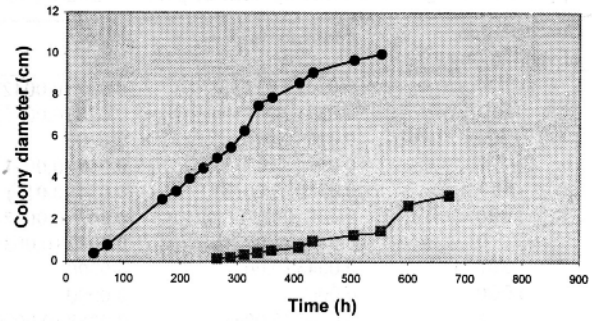

Fig. 8. Effect of vanillin concentration on the variation of *P. italicum* colony diameter (cm) in PDA system at pH=3.5 and a_w=0.98 during incubation at 25°C. (●) 0 mg l^{-1}, (■) 500 mg l^{-1}.

for all moulds in apple-based agars were greater than those found in PDA systems. Table 2 shows the effect of vanillin concentration on the germination time lag phase of the 4 moulds studied. The lag time increased as the vanillin concentration increased. For the conditions where no growth was observed after 2 months of storage, the lag time was more than 1440 hr. These results are different from the results obtained by Lopez-Malo and Alzamora (4) (who found that the minimum inhibitory concentrations of vanillin were in the range of 1000-2000 mg l^{-1}) perhaps due to the different nature of the apple used in our work. We did not use sucrose in apple puree as the a_w of the apple was 0.98, but in the PDA system the a_w was 1.00 so sucrose was added up to 11.5% (w/w). In their work, sucrose was added to both systems.

CONCLUSIONS

The results obtained suggest that natural vanillin could be used as an antimicrobial agent to prevent or delay mould spoilage in apple puree. The mechanism of inhibitory effect of vanillin is reported by Zumer *et al.* (12),

Table 1. Effect of vanillin concentration on radial growth rate† (mmh^{-1}) of 4 moulds in apple-based agar and PDA system at pH=3.5 and a_w=0.98.

	Concentration of vanillin ($mg\ l^{-1}$)	Apple puree	PDA
	0	0.054 (0.014)	0.029 (0.0042)
A. flavus	500	0.033 (0.002)	0.026 (0.0045)
	1000	0.016 (0.006)	0.0000
	0	0.058 (0.007)	0.058 (0.007)
A. niger	500	0.0389 (0.0018)	0.054 (0.013)
	1000	0.037 (0035)	0.043 (0.00184)
	0	0.0104 (0.0024)	0.0042 (0.003)
P. expansum	500	0.0045 (0.001)	0.0000
	1000	0.0000	0.0000
	0	0.0167 (0.004)	0.0068 (0.0026)
P. italicum	500	0.0021 (0.003)	0.0000
	1000	0.0000	0.0000

† Mean of 4 replicates, standard deviation in parentheses.

Table 2. Effect of vanillin concentration on lag phase† (h) of 4 moulds in apple-based agar and PDA system at pH=3.5 and a_w=0.98.

	Vanillin Concentration ($mg\ l^{-1}$)	Apple puree	PDA
	0	14	4
A. flavus	500	56	30
	1000	87	>1440§
	0	15	15
A. niger	500	26	42
	1000	180	163
	0	20	86
P. expansum	500	547	>1440
	1000	>1440	>1440
	0	29	32
P. italicum	500	219	>1440
	1000	>1440	>1440

† Mean of 4 replicates § More than 1440 hr.

that it inhibits O_2 uptake by *S. cervisiae*. The addition of vanillin in combination with a slight reduction of a_w (depending on the nature of the fruit) and pH may be a promising form of the fruit preservation. The

minimum inhibitory concentration of vanillin for the moulds studied is higher than those reported for common food preservatives (in the range of 1000-1500 mg l^{-1}), which are organoleptically compatible with the fruit nature and do not impair the acceptance of the fruit (6). Vanillin is widely used as a flavoring agent on ice cream, soft drinks, baked goods, cakes, cookies, etc. (5), so its organoleptic feature is well accepted by consumers.

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