The Pharma Innovation Journal 2022; 11(12): 2402-2406

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 2402-2406

www.thepharmajournal.com
Received: 21-09-2022
Accepted: 24-10-2022

VD Surve
Associate Professor, Department of Post Harvest and Food Biotechnology, V D College of Agricultural Biotechnology, VNMKV, Parbhani, Latur, Maharashtra, India

US Shaikh
M. Sc. Student, Department of Post Harvest and Food Biotechnology, V D College of Agricultural Biotechnology, VNMKV, Parbhani, Latur, Maharashtra, India

AA Pawar
M. Sc. Student, Department of Post Harvest and Food Biotechnology, V D College of Agricultural Biotechnology, (VNMKV, Parbhani, Latur, Maharashtra, India

Corresponding Author:
VD Surve
Associate Professor, Department of Post Harvest and Food Biotechnology, V D College of Agricultural Biotechnology, VNMKV, Parbhani, Latur, Maharashtra, India

Efficacy of vanillin in preventing yeast spoilage of pomegranate fruit juices

VD Surve, US Shaikh and AA Pawar

Abstract
In the present investigation attempts were made to use vanillin as an antimicrobial agent against *Saccharomyces cerevisiae* and *Candida parapsilosis* in pomegranate juice stored at (room temperature) 25 °C and 10 °C for five weeks. In present study it was found that the concentrations of 20 and 10 mM vanillin were required to achieve complete inhibition of *S. cerevisiae* investigated in the pomegranate juice at 25 °C and 10 °C respectively even after five weeks. The antimicrobial activity of vanillin against *C. parapsilosis* was studied and it was found that the concentrations of 20 and 10 mM vanillin were required to achieve complete inhibition of *C. parapsilosis* investigated in the pomegranate juice stored at 25 °C and 10 °C respectively, after five weeks. The results of this investigation indicate the potential for using vanillin as an alternative to synthetic preservative agents. The vanillin levels required to inhibit growth of yeasts at the lower temperature compare favorably for practical use in fruit juices and soft drinks that are low in both lipid and protein content, which are thought to interfere with the antimicrobial activity of vanillin. However, as high vanillin concentration gives the strong aromatic flavor of vanillin, lower levels would have to be added if vanillin was to be used as a widespread preservative.

Keywords: Pomegranate juice, yeast, vanillin, antimicrobial activity

1. Introduction
Modern consumer opinion suggests a desire for high quality foods that are more natural, minimally processed and preservative free, while remaining safe and with an extended shelf life. This along with tighter legislation regarding current preservatives has challenged the food industry and has led to increased research into and the use of "naturally derived" antimicrobials (Beuchat and Golden, 1989; Gould, 1996) [2, 9]. Certain plants and their extracts, normally added to foods for their flavoring properties are known to possess antimicrobial activity, thereby offering a potential alternative to synthetic preservatives (Jay and Rivers, 1984; Juven *et al*., 1994; Hao *et al*., 1998) [11, 12, 10]. Low-molecular weight phenolic compounds represent the major flavour components of these plants (Gould, 1996; Davidson and Naidu, 2000) [9, 6].

Phenolic compounds are probably the major antimicrobial components of spices and their essential oils, for instance, thymol from thyme and oregano; cinnamic aldehyde from cinnamon; eugenol from cloves, allspice and cinnamon; carvacrol from oregano and anethole from anise (Dziezak, 1989) [8]. Vanillin (4-hydroxy-3-methoxybenzaldehyde), the crystalline component of vanilla pods and structurally similar to eugenol, is also antimycotic. Vanillin or methyl vanillin, or 4-hydroxy-3-methoxybenzaldehyde, or vanillaldehyde is an organic compound with the molecular formula $C_8H_8O_3$. Its functional groups include aldehyde, ether and phenol. It is the primary component of the extract of the vanilla bean. Synthetic vanillin is used as a flavoring agent in foods, beverages, and pharmaceuticals. Methyl vanillin is used by the food industry as well as ethyl vanillin. The ethyl is more expensive but has a stronger note and differs by having an ethoxy group ($-O-CH_2CH_3$) instead of a methoxy group ($-O-CH_3$).

A study of yeast flora of frozen fruit juice concentrates reported the isolation of 12 genera and 21 species of yeast. *Saccharomyces cerevisiae* was most frequently isolated yeast species (24.7 %) followed by *Candida stellata* (22.1 %) and *Zygosaccharoces rouxii* (14.3%. Five other candida strains including *Candida parapsilosis* were also isolated (Deak and Beuchat, 1993) [7]. Jay and Rivers (1984) [11] studied in laboratory media minimum inhibitory concentrations (MIC) of 1000, 1000, 125 and 250 pg/ml for *Staphylococcus aureus* 196E, *Escherichia coli*, *Torulopsis candida and Aspergillus niger*, respectively (nutrient broth, pH 6.0, incubation at 30 °C for 48 h).

In a study to explain the effects of vanillin concentration, pH (3-4) and incubation temperature (10-30 °C) on growth of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus* And *Aspergillus parasiticus* using potato dextrose agar adjusted to water activity (a_w) 0.98, it was reported that the lag period depends on vanillin concentration, pH and incubation temperature. The inhibitory conditions depend on the type of mold. *A. niger*, the most resistant species, was inhibited at 15 °C, pH 3.0, vanillin concentration of 1000 ppm whereas, for *A. ochraceus*, the most sensitive species, vanillin concentration of 500 ppm, pH 3.0 and temp < 25 °C or pH 4.0 low temp < 15 °C (Lopez-Malo *et al*., 1997) [13].

Cerrutti and Alzamora (1996) [4] reported that the addition of approximately 13.2 mM vanillin to laboratory media with water activity (a_w) of 0.99 was sufficient to inhibit growth of *S. cerevisiae* for 40 days. Increasing the concentration of vanillin resulted in the extension of the lag phase of growth and reduction of cell density. The present study aims to determine the antimicrobial activity of vanillin when applied to pomegranate juice inoculated with *S. cerevisiae* or *C. parapsilosis* at two different incubation temperatures of 25 °C and 10 °C.

2. Materials & Methods

2.1. Strain Collections

Saccharomyces cerevisiae (3209) and *Candida parapsilosis* (3323) were obtained from NCL Pune, (Maharashtra.) Yeast cells were maintained at 4 °C on yeast extract peptone dextrose (YEPD) agar. Then yeasts were grown overnight (18h) in YEPD broth (pH 4.0) at 25 °C with continuous agitation.

2.2 Media Preparation

YEPD agar can be prepared by dissolving measured amount of yeast extract, peptone, dextrose, in distilled water and these ingredients were completely dissolved by heating the flask. The pH of media was adjusted to 4.0 and agar-agar was dissolved and Flask was heated on hot plate till complete dissolution of contents. Further it was tightly plugged by cotton plug and sterilized in an autoclave at 121 °C for 15 minutes. Then flask was cooled and media was poured in clean dried, sterilized Petri plate to solidify. The media consists of 10g yeast extract, 20g peptone, 20g dextrose and 15 g agar per liter.

2.3 Vanillin Preparation

A, stock solution of 2.5 mM vanillin was prepared in 99.7 to 100% (vol/vol) ethanol. This solution was stored at -20 °C in dark until required. Since vanillin has very low solubility in water on the order of 0.5 % at 20 °C and 30% in ethanol. So ethanol was used for stock solution of vanillin.

2.4 Beverage Preparation

The pomegranate juice was purchased locally and stored at 4 °C according to the manufacturer's instructions. The pure pomegranate juice contained trace amount of fat and protein per 100 ml and had undergone pasteurization during its production. The pH of the juice was confirmed in the laboratory and found to be 3.0.Juice contained no any preservative and color. The pomegranate juice was aseptically dispensed (25 ml) into sterile 30 ml screw capped bottles. These conditions simulated those of a sealed, bottled beverage. Product was initially checked for absences of contaminants by surface plating onto YEPD agar and inoculation into YEPD broth followed by incubation at 25 °C for 72 hour. No contaminants were detected in product.

2.5 Inhibition of Cell Viability

Overnight yeast cultures were harvested by centrifugation at 5 °C at 4400 rpm for 10 minutes and resuspended in quarter strength Ringer's Solution (pH 7) (Ringer's solution contains NaCl-8 g. Kel-0.42 g. $CaCl_2$-0.24 g. $NaHCO_3$, 0.2 g and final volume made to 1 liter using distilled water).The cultures obtained after centrifugation were diluted with 50 ml of quarter strength Ringer's Solution each. The juice samples containing different level of vanillin were then inoculated in duplicate to give an equal initial viable cell count. Samples (0.1 ml) were taken immediately and the decimal dilutions made in quarter strength Ringer's solution were surface plated (in duplicate) on to YEPD agar. The viable cell counts/colonies were enumerated after incubation at 25 °C for 48 to 72 hours. Beverage samples containing 1.6% (vol/vol) ethanol acted as the controls. The inoculated sample sets were incubated at 25 °C. Further samples which were stored at 25 °C and 10 °C were taken at intervals over a 5 week period.

3. Results and discussion

In present study attempts have been made to use vanillin as a preservative of pomegranate juice. Antimicrobial activity of vanillin in pomegranate juice was examined against *S. cerevisiae* and *C. parapsilosis* at (room temperature) 25 °C and 10 °C as it is more susceptible to yeast attack due to its high sugar content.

3.1 Effect of vanillin concentration on viable counts of *Saccharomyces cerevisiae* in Pomegranate Juice stored at 25 °C.

Vanillin, the extract of vanilla beans plays a key role in inhibiting the growth of *S cerevisiae* in pomegranate juice at 25 °C. From Fig.1 it is observed that the viable count in control sample was found to be of 1250 at the beginning and it increased to 1540 after 3 weeks and then reduced to 940 due to depletion in nutrients. It was found that addition of 5 mM vanillin inhibited the growth of *Saccharomyces cerevisiae* with gradual decrease in viable counts from 1300 to 540 after 5 weeks. Similar trend was found in juice with 10 mM vanillin. The viable count was reduced from 1100 to 10 after three weeks and 900 to 2 after second week with vanillin concentration of 20 mM and 40 mM respectively.

So it was revealed from results that the *S. cerevisiae* cells were no longer detected in juice after 1-2 week, 3 week and 5 week in presence of 40 mM, 20 mM and 10 mM vanillin respectively. This reduction in viable counts was might be due damage of a variety of enzyme systems of yeasts affecting structural component synthesis and energy production by the essential oils.

3.2 Effect of vanillin concentration on viable counts of *Saccharomyces cerevisiae* in Pomegranate Juice stored at 10 °C

The storage temperature was maintained at 10 °C, this showed significant improvement in effect of Vanillin against yeast culture. Fig. 2 shows that the viable count in control sample was found to be 1250 at the beginning and it increased to 1420 after 4 weeks and then reduced to 920.It was found that addition of 5 mM vanillin inhibited growth of *Saccharomyces*

cerevisiae with gradual decrease in viable counts from 1300 to 135 after five weeks. The viable count reduced from 1250 to 12 after three weeks and from 1100 to 12 after second week in juice 3 containing 10mM vanillin and 20 mM vanillin.
So it was observed that, lower temperature extended the lag phase and decreased growth rates of all culture samples compared to those grown at 25 °C. The presence of 5 mM vanillin was sufficient to inhibit growth for the duration of study with parallel reduction in viable cell counts to 135 in 5 weeks. Furthermore additions of 10 mM, 20 mM, and 40 mM vanillin resulted in complete loss of viability within 2-3 week, 2 week, and up to 1 week respectively. These results were consistent with those of a previous study that reported that the activity of vanillin against number of *Aspergillus* strains could be increased by lowering the incubation temperature from 25 °C to 15 °C (Lopez-Malo *et al*., 1997) [13].

3.3 Effect of Vanillin Concentration on Viable counts of *Candida parapsilosis* in Pomegranate Juice at 25 °C

The activity of vanillin concentration on the growth and viability of *Candida parapsilosis* in Pomegranate Juice stored at 25 °C was studied. Data presented in fig. 3 shows that the viable count was highest at beginning in controlled sample (1300) followed by juice containing 5 mM, 10 mM, 20mM and 40mM Vanillin (1280, 1220, 1050, 940 respectively). Same trend was found the after 2nd, 3rd, 4th and 5th week. It was found that addition of 5 mM vanillin, inhibited the growth of *Candida parapsilosis* with gradual decrease in viable counts from 1280 to 180 after 5 weeks. Similar trend was found in juice with 10 mM vanillin. The viable count was reduced from 1050 to 14 after three weeks and 940 to 8 after second week with vanillin concentration of 20 mM and 40 mM respectively.
Thus it was found that addition of either 5 or 10mM vanillin extended the lag phase and decreased the growth rate of *Candida parapsilosis* but was unable to prevent spoilage of juice at 25 °C (Table 4). The addition of 10 mM, 20 mM and 40 mM vanillin again resulted in complete loss of viability within 5 weeks, 3 weeks and up to 2 weeks respectively.

3.4 Effect of Vanillin Concentration on Viable counts of *Candida parapsilosis* in Pomegranate Juice at 10 °C

The antimicrobial activity of vanillin was enhanced by lowering storage temperature to 10 °C and this activity was studied in following table.It may be observed from Fig. 4 that the viable count in control sample was found to be of 1300 at the beginning and it decreased to 740 after five weeks due to depletion in nutrients. It was found that addition of 5 mM vanillin inhibited the growth of *C.parapsilosis* with gradual decrease in viable counts from 1280 to 280 after 5 weeks. Similar trend was found in juice with 10 mM vanillin. The viable counts were reduced from 1050 to 6 after two weeks and 940 to 2 after second week with vanillin concentration of 20 mM and 40 mM respectively. Thus it was found that viable cells were not detected after 2-3 weeks, 1-2 weeks and up to 1 week when yeast was grown in presence of 10 mM, 20 mM and 40 mM vanillin concentration in juice stored at 10 °C.

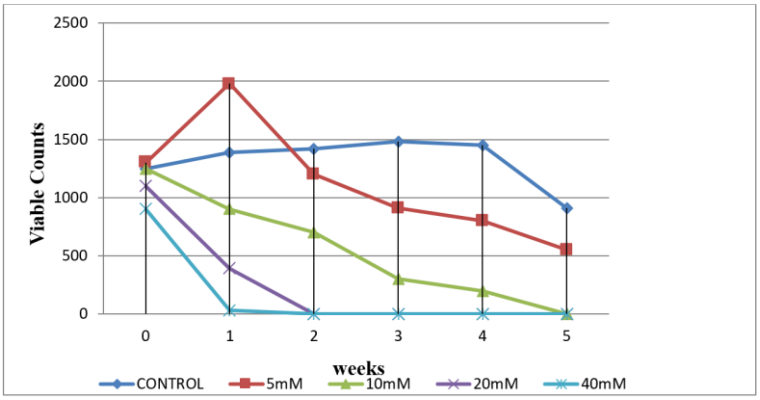

Fig 1: Effect of different vanillin concentration on viable counts (numbers/0.1 ml juice) *of S.cerevisiae* in decimally diluted pomegranate juice stored at 25 ºC

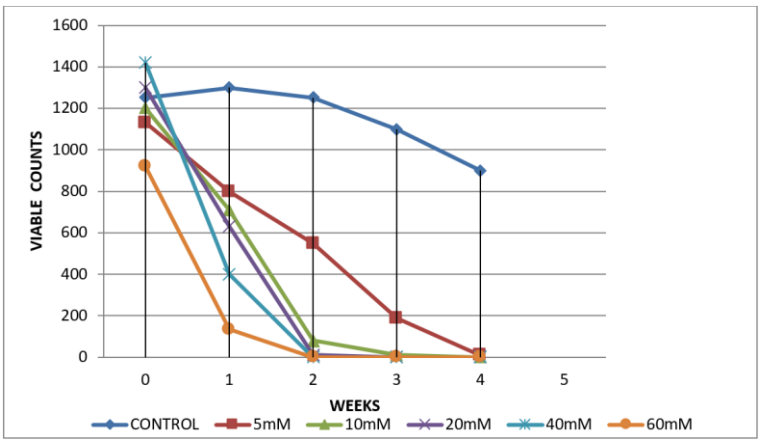

Fig 2: Effect of different vanilin concentration on viablecounts (numbers/0.1 ml juice) of *S.cervisiae* in decimally diluted pomegranate juice stored at 10 ºC.

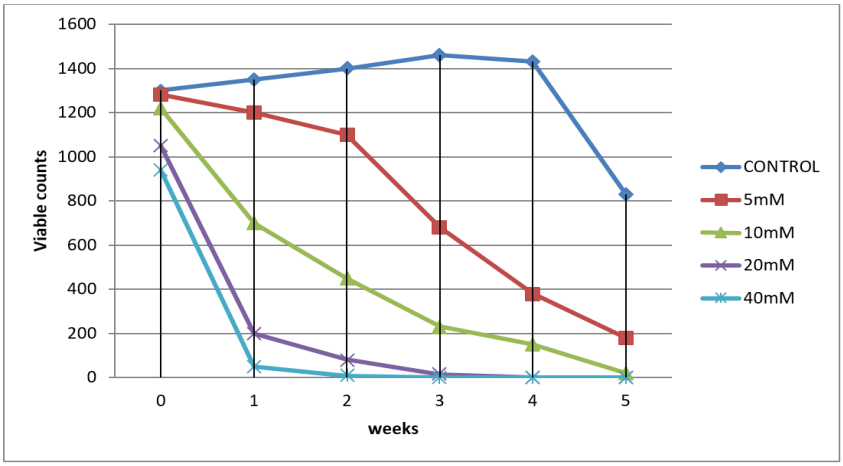

Fig 3: Effect of different vanillin concentration on viable counts (numbers/0.1 ml juice) of *C.parapsilosos* in decimally diluted pomegranate juice stored at 25 ºC

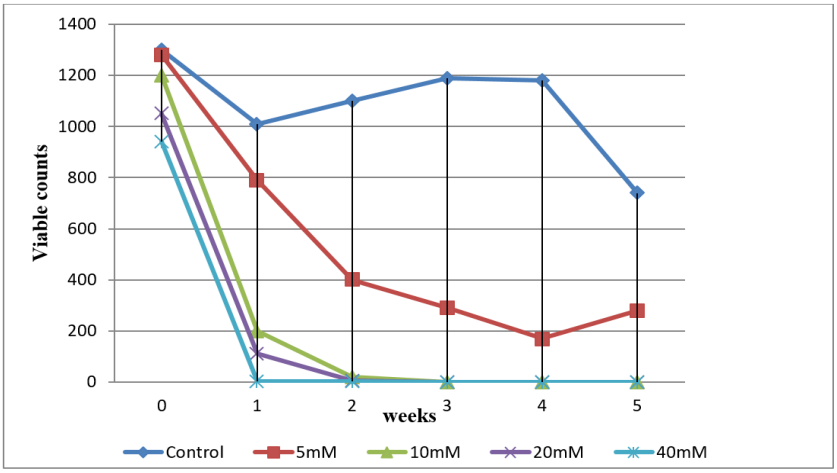

Fig 4: Effect of different vanillin concentration on viable counts (numbers/0.1ml juice) of *C.parapsilosis* in decimally diluted pomegranate juice stored at 10 ºC

4. Conclusion

In present study it was found that the concentrations of 20 and 10 mM vanillin were required to achieve complete inhibition of *S. cerevisiae* investigated in the pomegranate juice stored at 25 °C and 10 °C respectively. And also concentrations of 20 and 10 mM vanillin were required to achieve complete inhibition of *C. parapsilosis* investigated in the pomegranate juice stored at 25 °C and 10 °C respectively. The results of this investigation indicate the potential for using vanillin as an alternative to synthetic preservative agents. The vanillin levels required to inhibit growth of yeasts at the lower temperature compare favorably for practical use in fruit juices and soft drinks that are low in both lipid and protein content, which are thought to interfere with the antimicrobial activity of vanillin. However, as high vanillin concentration gives the strong aromatic flavor of vanillin, lower levels would have to be added if vanillin was to be used as a widespread preservative.

5. References

1. Alzamora SM, Tapia MS, Argaíz A, Welli J. The Application of combined methods technology in minimally processed fruits. Food Res. Int. 1993;26:125-130.
2. Beuchat LR, Golden DA. Antimicrobials occurring naturally in the foods. Food Technol. 1989;43(1):134-142.
3. Board, Gould. Future prospects, Ch. 14 in Food Preservatives; c1991.
4. Cerrutti P, Alzamora SM. Inhibitory effects of the vanillin on some food spoilage yeasts in laboratory media and fruit purees. Int. J Food Microbiol. 1996;29:379-386.
5. Cerrutti P, Alzamora SM, Vidales SL. Vanillin as an antimicrobial for producing shelf stable strawberry puree. J Food Sci. 1997;62:608-610.
6. Davidson P, Naidu AS. Phyto-phenols. In: Naidu, A.S. (Ed.), The Natural Food Antimicrobial Systems. CRC Press LLC.Boca Raton, London; c2000. p. 265-294.
7. Deak T, Beuchat LR. Yeasts associated with fruit juice concentrates. J Food Prot. 1993;56:777-782.
8. Dziezak JD. Spices. Food Technol. 43, 1989;102:1-16.
9. Gould. Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. J. Food Prot; c1996. p. 82-86
10. Hao YY, Brackett RE, Doyle MP. The inhibition of Listeria monocytogenes and Aeromonas hydrophilia by plant extracts in refrigerated cooked beef. J Food Prot. 1998;61:307-312.
11. Jay JM, Rivers GM. The antimicrobial activity of some food flavoring compounds. J Food Saf. 1984;6:129-139.
12. Juven BJ, Kanner J, Schved F. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J Appl. Bacteriol. 1994;76:626-631.

13. Lo'pez-Malo A, Alzamora SM, Argaiz A. The effect of vanillin concentration, pH and the incubation temperature on *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus* and *Aspergillus parasiticus* growth. Food Microbiol. 1997;14:117-124.
14. Russell NJ. The bacterial membranes: The effect of chill storage and food processing. An overview. Int. J Food Microbiology. 2002;79:27-34.