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Effect of natural preservatives on pineapple juice

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Abstract
Pineapple juice is generally preserved by use of chemical preservatives. As an alternative to certain disadvantages associated with chemical preservatives, natural preservatives could be used. In present investigation, efforts were made to judge the suitability of Nisin, thyme oil and clove oil synergistically in preservation of pineapple juice. Pineapple juice was prepared with application of different concentrations of pectinase to analyze its effect of yield of product. Further, Different combination of essential oils *viz.* thyme oil and clove oil, and nisin were assessed to optimize the dosages with minimum effect on the organoleptic characteristics of pineapple juice. Further the treated juice was storage at refrigerated conditions and change in chemical properties, microbial quality and sensorial characteristics were determined. On the basis of obtained results it could be concluded that pineapple juice could be preserved with incorporation of natural preservatives and amongst studies combinations, 0.1 ml of clove oil with 200 ppm of nisin observed to be superior.

Keywords: pineapple, preservatives, nisin, essential oil

Introduction
Pineapple (*Ananas comosus*) is leading edible member of family *Bromeleaceae* and it is one of the commercially important fruit crops of the tropical world. It is extensively grown in India. Pineapple is second important harvest fruit after banana and its production during 2008-09 was around 1305800 tonnes (FAOSTAT, 2008). As is the case with most of the fruits and vegetables, their availability is limited during the year. Many techniques have been developed to make available seasonal fruits as well as vegetables even during off-season. Canned pineapple slices and juice are such products. Pineapple is consumed as dessert, in fruit salads, while making cakes and pastries etc. Thus there are large numbers of consumers who would like to consume pineapple slices or juice (Anon, 2005) [3]. Pineapple juices are generally preserved by use of chemical preservatives. However, a growing awareness among consumers towards health aspects has increased their interest on natural preservatives. As an alternative to certain disadvantages associated with chemical preservatives, industries are paying more emphasis on the use of natural preservatives. Utilization of natural preservatives has rendered foods with high nutritional value, free from chemical preservatives and adequate microbiological safety (Meena and Sethi, 1997; Shelef, 1983; Subbalakshmi and Naik, 2002) [11, 15, 17]. Various natural preservatives including cinnamon, albizia, Echinacea, Garlic, tea tree oil, turmeric, Thyme, nisin, neem could be used as preservatives in food (Anupama *et al.*, 2010) [2]. The ideal natural preservative should not cause any change in colour, appearance or flavour profile of the product where it is used in. Hence, it is always necessary to judge the suitability of natural preservative for specific type of food.

Nisin is a natural, toxicologically safe, antibacterial food preservative. It is regarded as natural because it is a polypeptide produced by certain strains of the food-grade lactic acid bacterium *Lactococcus lactis* subsp. *lactis*, during fermentation. Nisin exhibits antimicrobial activity towards a wide range of Gram positive bacteria, and is particularly effective against spores (Delves-Broughton *et al.*, 1999) [7].

The use of nisin is a potentially useful way of controlling this organism in fruit juices and fruit juice containing products. Thyme (Thymus *Vulgaris*) oil shown to possess preservative effect due to presence of volatile oil, thymol, eugenol, flavonoids, rosmarinic acid, etc. It has antibacterial and antifungal activity (Kalemba and Kunicka, 2003; anon, 2003) [9, 3]. Clove oil shows antibacterial activity against Gram-negative anaerobic bacteria (Arora and Kaur, 1999; Rajkumar and Berwal, 2003) [5, 13].

Although essential oils have strong antimicrobial activity (Lambert *et al*. 2001) [10], their application as food preservatives is limited by their strong flavor when added in large amounts, which negatively affects the organoleptic properties of food. So, it is necessary to combine these main components of essential oils with other natural preservatives such as nisin, to produce the desired antibacterial effect at a concentration that does not produce undesirable

changes in the flavor or aroma (Burt, 2004) [6]. Nisin showed to act synergistically with carvacrol, eugenol or thymol against B. cereus and/or L. monocytogenes (Pol and Smid, 1999; Yamazaki *et al*. 2004) [12, 18].

In present investigation, efforts were made to judge the suitability of Nisin, thyme oil and clove oil synergistically in preservation of pineapple juice. Pineapple juice was prepared with application of different concentrations of pectinase to analyze its effect of yield of product. Further, Different combination of essential oils *viz.* thyme oil and clove oil, and nisin were assessed to optimize the dosages with minimum effect on the organoleptic characteristics of pineapple juice. Further the juice was storage for long term storage and change in edible quality characteristics of pineapple juice were further assessed.

Materials and Methods

The present investigation was carried out in the food technology laboratory at the department of chemical technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS) India.

Materials

Fresh, ripe good quality pineapples free from any blemishes, clove oil and thyme oil were purchased from the local market of Aurangabad city. The bacteriocin Nisin was obtained from Himedia Pvt. Ltd. Mumbai. All other chemicals used during present investigation were of analytical grade and purchased from Himedia Pvt. Ltd. Mumbai.

Methods

Processing of pineapple juice

Crown of outer skin of pineapple was separated by using stainless steel knife. The peeled pineapples were then sliced. The pineapple slices were crushed by using a mixer. Different enzyme treatments were given to crushed pineapple juice to maximize the yield and clarify the juice and pectinase enzyme was used for this purpose. The essential steps for the extraction and preparation of pineapple juice are shown in Fig-1.

Chemical analysis of pineapple juice

Different chemical parameters viz. acidity, TSS, and ascorbic acid of pineapple juice samples were determined by standard method (Ranganna, 1986) [14].

Microbial analysis

Microbial quality recorded using standard plate count and yeast and mold count. The nutrient agar media was used as growth media for estimation of total plate count and potato dextrose media was used for yeast and mold count. Preparation of media (Nutrient agar media and Potato dextrose media) carried out by standard microbial procedures (Ranganna, 1986) [14].

Sensory analysis

The sensory evaluation of pineapple juice was carried out by a 10 member trained panel comprised of students and academic staff members of the faculty who had some previous experience in sensory evaluation of fruit and vegetable products. The panel members were requested in measuring the terms identifying sensory characteristics and in use of the score. Judgments were made through rating products on a 9 point Hedonic scale with corresponding descriptive terms ranging from 9 'like extremely' to 1 'dislike extremely' (Amerine *et al.*, 1965) [1].

Pineapple
↓
Removal of Crown and eyes
↓
Slices
↓
Crushing
↓
Enzyme Treatment
↓
Juice Extraction
↓
Addition of Nisin
↓
Addition of essential oils
↓
Addition of stabilizer (sodium alginate 0.1%)
↓
Mixing
↓
Bottling
↓
In Bottle Pasteurization (62oC for 20 min)
↓
Refrigerated Storage (4oC)

Fig 1: Flow sheet for preparation of pineapple juice

Preservative treatments

Prepared juice was treated with different composition of essential oils and nisin as summarized in Table-1.

Table 1: Different treatment of natural preservatives for pineapple juice

Samples	Concentration of Essential oils	Concentration of Nisin
A	Nil	Nil
B	0.1ml Clove Oil	200ppm
C	0.2ml Clove Oil	200ppm
D	0.1ml Thyme Oil	200ppm
E	0.2ml Thyme Oil	200ppm

Results and Discussion

The prepared samples were then studied for their properties at the interval of seven days. The results obtained are discussed under suitable subheadings

Effect of pectinase treatments on yield of pineapple juice

Pectinase treatment to pineapple at different concentrations and different time period showed to have marked effect on yield of juice. The results related to yield of pineapple juice at different treatments is summarized in Table-2.

Table 2: Effect of different pectinase treatments on yield of pineapple juice

Sample	Pectinase concentration (ppm)	Treatment Time (hr)	Weight of Pulp (g)	Volume of juice (ml)	Yield (%)
T1	--	2	200 gm	105ml	52.5
T2	100	1	200 gm	132ml	66.0
T3	100	2	200 gm	126ml	63.0
T4	200	1	200 gm	150ml	75.0
T5	200	2	200 gm	133ml	66.5

The results revealed that pectinase enhances the yield of pineapple juice. The yield of juice found to increase with increase in concentration of pectinase. Surprisingly, antagonistic results were observed with increase in time period. On the basis on above stated results, it was observed that maximum yield of pineapple juice could be obtained at the concentration of 200 ppm of pectinase enzyme with the treatment time of 1 hr. Further, this sample was considered for further studied.

Effect of natural preservatives on chemical characteristics of pineapple juice

As stated in table-1, different combinations of essential oils and nisin were used to optimize their suitability in pineapple juice. Different treated samples of pineapple juices analyzed for their pH, acidity, total soluble solids and ascorbic acid content during the storage period of 21 days. The results related to change in chemical properties in different treated pineapple juice at different storage periods is summarized as follows.

Effect of pH: The change in pH during storage period is shown in Fig-2.

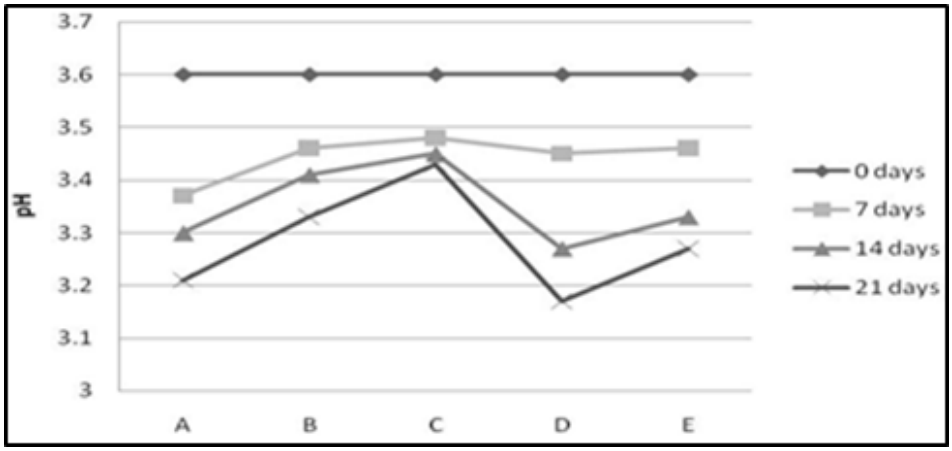

Fig 2: Effect of different treatments on pH of pineapple juice during storage

It can be seen that the pH decreases gradually. The decrease in pH during storage may be due to the acid from fructose, glucose and other carbohydrates (Splittstoesser 1996) [16]. Change in pH in Sample C was found to be minimum compared to other samples.

Effect of Acidity: The change in acidity of pineapple juice during storage is visualized in Fig-3. The low rate of acidity decrease was an indication of spoilage of fruit juice sugars into acid. The low rate of acidity decrease was an indication of spoilage of fruit juice.

Effect of ascorbic acid content

The change in ascorbic acid content of pineapple juice during storage is pictorially represented in Fig-4.

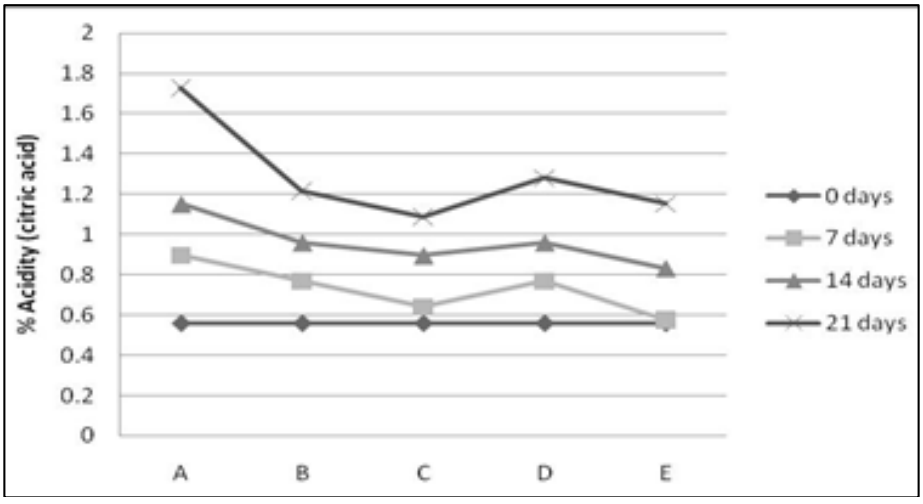

Fig 3: Effect of different treatments on acidity of pineapple juice during storage

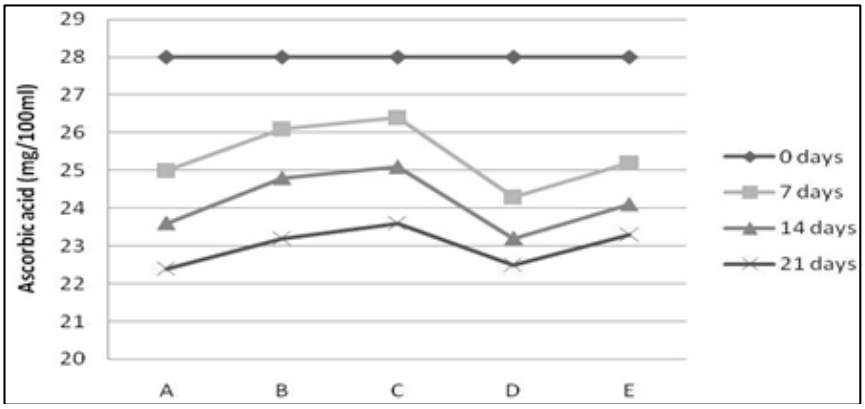

Fig 4: Effect of different treatments on ascorbic acid content of pineapple juice during storage

It was observed that during storage, there is decrease in the level of ascorbic acid content in all treatments. The destruction of ascorbic acid and discoloration of juice occur together (Frazier, 1967) [8].

Effect on total yeast and mold mount

Data representing the total yeast and mold mount of pineapple juice in terms of log (CFU/g) with respect to different treatments and storage period are presented in Fig-5. It has been observed that there was gradual increase in total yeast and mold count with increase in storage time. Storage period has significant effect on the rate of increase in microbial count irrespective of the treatments.

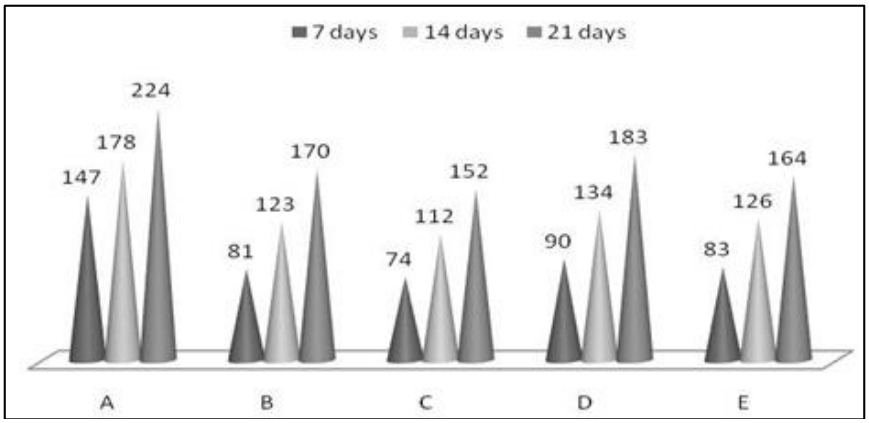

Fig 5: Effect of storage period on total yeast and mold count of pineapple juice

The yeast and mold content was found to increase during prolonged storage period this can be due to the contamination on the equipments and due to the microorganisms that are already present in the juice. Sample C> Sample E> Sample B>Sample D> Sample A.

Effect on total bacterial count

The data pertaining to total bacterial count during storage is summarized in Fig-6. It has been observed that there was significant effect of different treatments on the bacterial microbial count of the pineapple juice.

The total bacterial count for treatment C was found to be lowest whereas for sample A, it was found to be highest. This effect might be due to the destruction of micro-organisms by application of natural preservatives. In all, during the storage period the bacterial count increased. Sample C was effective in having the lowest microbial count. Entire sample were found superior to the control sample. Sample C> Sample E> Sample B>Sample D> Sample A.

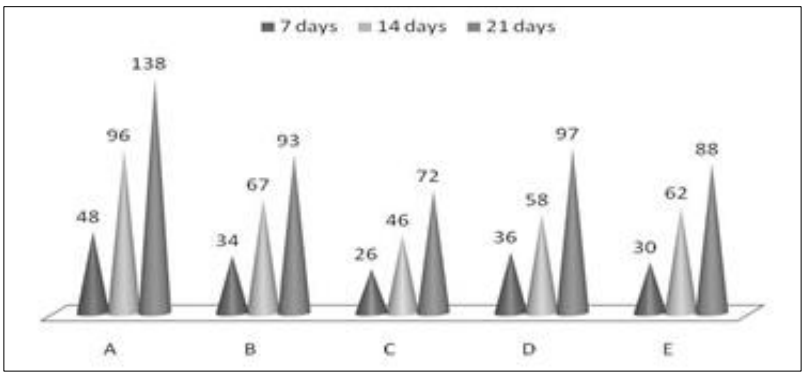

Fig 6: effect of different treatments on total bacterial count during storage

Sensorial quality characteristics of pineapple juice after 21 days storage period: The data representing the effect of different treatments on sensorial quality of pineapple juice is presented in Table 3. The pineapple juice subjected to treatments and stored at refrigerated condition for the period of 21 days and evaluated for sensorial properties by a trained panel. The scores for different sensorial characters were given on a 9 point hedonic scale and mean values are presented.

Table 3: Sensorial evaluation of pineapple juice

Sample code	Color	Aroma	Taste	Mouth feel	Overall acceptability
A	6.3	5.7	5.8	5.8	5.9
B	7.8	6.4	6.3	6.5	6.75
C	7.2	6.1	5.9	6.1	6.4
D	7.5	6.2	6.2	6.4	6.5
E	7.4	5.9	6.1	6.2	6.4

* Each value is an average of 10 determinations

The average score for color of pineapple juice exposed to different treatments ranged from 6.3 to 7.4; whereas the minimum score 6.3 observed in control sample. It was observed that colour characteristics of pineapple juice increased with the treatment of natural preservatives and sample containing 0.1 ml of clove oil and 200 ppm of nisin showed superior results compared to other treatments. Sample B also secured superior results in terms of aroma, taste and moth feel. Essentially on the basis on observed sensorial qualities, it seems that 0.1 ml of clove oil with 200 ppm of nisin resulted in higher overall acceptability. The Sample B scored the highest in the organoleptic evaluation and it was followed by Sample D, C, E and A, respectively.

Conclusion

The essential oils and Nisin can be used as preservatives. The addition of essential oils has certain limitations as it hampered the aroma and flavour of the product. In comparison among the essential oils, clove oil was found superior to thyme oil. It showed better resistance to micro-organisms, especially yeast and molds which are main spoilage organisms in fruit beverages. Nisin inhibited the growth of Lactic acid bacteria Clove oil was found superior to thyme oil as preservative for pineapple juice.

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