



E-ISSN: 2278-4136

P-ISSN: 2349-8234

www.phytojournal.com

JPP 2020; 9(2): 2452-2454

Received: 02-12-2019

Accepted: 26-02-2020

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Assessing effect of pectinase action on guava wine properties formed using *S. cerevisiae* IARI strain and native strain

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Abstract

Guava wine was treated with pectinase enzyme and the effects of this enzymatic treatment on the physicochemical properties of guava wine including pH, ethanol yield and total soluble solid (TSS) was investigated. Two different strains of *Saccharomyces cerevisiae* - IARI IARI and native strain were used in the production of guava fruit wine. The results indicated that the enzyme treatment promoted juice clarification, decreased TSS, increased ethanol yield and reduced pH of guava wine than that without treatment with pectinase enzyme. In conclusion, the quality of guava wine can be improved by pre-treatment with pectinase enzyme.

Keywords: Guava, TSS, *Saccharomyces cerevisiae*, Fermentation, Pectinase, IARI, Wine

1. Introduction

Guava (*Psidium guajava* L.) is often considered as a “super fruit” due to its rich nutritional value. It is a member of large Myrtaceae family and is one of the most important commercial fruit in India generally consumed fresh. It is common in most tropical and subtropical regions throughout the world. These fruits have a high digestive value, and also contain Vitamin A (beta carotene) and Vitamin C (ascorbic acid) in considerable amounts. The seeds are rich in omega-3 and omega 6 fatty acids, dietary fibers and mineral salts. The antioxidant properties in guavas are due to the presence of high amounts of vitamin C (Ascorbic acid) and a carotenoid lycopene (Celso *et al.*, [3] which help in prevention of many degenerative diseases [6]. Pleasant aroma and taste of guava are highly appreciated across India and make it competent in the market, either as guava juice or as mixtures with other juices or as guava wine. The conventional guava juice processing can be made by mechanical pressing of guava mash. The obtained juice is cloudy and low in ascorbic acid due to a high content of ascorbic acids remains in the pomace [7]. The use of enzyme in a mash treatment is now essential in juice industry and it shows increases in yield and ascorbic acid and also promotes juice clarification in a short processing [10, 5]. The enzymes including pectinase, cellulase and/or arabinase assist in the hydrolysis of pectic substances, pectins, celluloses or hemicelluloses. Consequently, it is advantageous to facilitate the subsequent filtration process and increase juice yield [7]. The time to add enzyme is dependent on the type of fruits used in juice processing. Generally, the pectinase is applied during the maceration pretreatment for reducing the viscosity of fruit mash and the juice produces high yield and nutritive values [14]. The achievement of enzyme treatment in fruit juice processing is influenced by several process variables such as enzyme concentration, incubation time, incubation temperature or these interactive effects [8, 9].

2. Materials and Methods**Raw material**

Guava fruits (Chittidar) with 80-90% maturity and free from visual blemishes and bruises were purchased from local market of Meerut.

Culture

Saccharomyces Cerevisiae IARI was procured from Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agricultural Research Institute (IARI), Pusa, New Delhi. The tubes were kept at 30 degree temperature for 24 hours and a full test tube along with media is poured into one liter juice of guava and incubated at 30 °C for 24 hours under anaerobic conditions.

Guava juice preparation

Ripened guavas were washed with tap water, trimmed to remove blemishes (if any), cutin halves and deseeded. The guava halves were sliced into about 2 cm thickness and blended with appropriate amount of added water using blender for 3 min. The guava puree was filtered through a muslin cloth to obtain the juice.

Enzyme treatment (Pre-treatment)

200 g guava pulp was subjected to enzyme treatment with pectinase. The reaction was carried out in a water bath shaker ($30 \pm 2^\circ\text{C}$) with a constant stirring rate of 100 rpm, and then heated at 90°C for 5 min in order to inactivate enzyme activity. The guava puree was filtered through a muslin cloth to obtain the juice.

Determination of pH

pH is the measurement of the logarithm of inverse of hydrogen ion concentration in the solution.

$$\text{pH} = -\log [\text{H}^+]$$

Where, H^+ = hydrogen ion concentration (g/lit)

The electronic pH meter (Elico, LI -127) was calibrated using 7 pH and 4 pH standard buffer solutions. Then electrode was dipped in the test solution and the temperature knob was adjusted to temperature of test solution. The function selector switch was set to pH and reading of digital display was allowed to stabilize.

Total Soluble Solids (TSS)

TSS (total soluble solid) of guava juice was measured by hand refractometer of range of $0-32^\circ\text{Brix}$ and for measuring TSS of wine. Use of this method was recommended by Srivastava and Kumar [13] a brief description is given below.

A drop of sample was placed on the prism and the observation was taken in front of sunlight. The visible scale showed a dark line indication measuring TSS in degree Brix ($^\circ\text{B}$).

Determination of ethanol

Standard ethanol cure is obtained by using spectrophotometer. Sample and standard concentrations were prepared by distillation in potassium dichromate solutions. The ethanol was estimated by colorimetric method as described by [2].

Preparation of Reagent

Potassium Dichromate Solution: Thirty four grams of $\text{K}_2\text{Cr}_2\text{O}_7$ was dissolved in 500 ml distilled water and 325 ml of sulphuric acid was added and volume was made up to 1000 ml with distilled water to give 0.23N $\text{K}_2\text{Cr}_2\text{O}_7$.

Preparation of Stock Solution: Standard stock solution of 100 per cent pure analytical grade (containing 789 mg/ml) ethanol was prepared by dissolving 12.6 ml of ethanol in 100 ml distilled water, which results in 100 mg/ml of standard ethanol.

Procedure

One ml of representative samples from each treatment was transferred to 250 ml round bottom distillation flask connected to the condenser and was diluted with 30 ml distilled water. The sample was distilled at $74-75^\circ\text{C}$. The distillate was collected in 25 ml of 0.23 N $\text{K}_2\text{Cr}_2\text{O}_7$ reagent, which was kept at receiving end. The distillate containing alcohol was collected till total volume of 45 ml was obtained. Similarly standards (20-100 mg ethanol) were mixed with 25 ml of $\text{K}_2\text{Cr}_2\text{O}_7$ separately. The distillate of samples and standards were heated in water bath at 60°C for 20 minutes

and cooled. The volume was made up to 50 ml with distilled water and the optical density was measured at 600 nm using Systronics spectrophotometer-117. The standard curve was plotted considering the concentration against absorbance

3. Results and Discussion

Table 1 shows physicochemical properties of guava juice with and without enzyme treatment. The addition of pectinase caused an increase in ethanol yield, but low in pH and TSS values. It also improved clarity of the guava juice. This can be explained that pectinase, which include pectin methyl esterase and polygalacturonase, assist in pectin hydrolysis. Their reactions cause a release of carboxylic acids and galacturonic acids. This leads to a decrease in pH of juice, but a significant increase in titratable acidity and juice ethanol yield [15]. In addition, the enzymes react on the guava peel which is rich in ascorbic acids (Chopda and Barrett) [4], thus an increment of ascorbic acid would be due to the pectin breakdown from the peel. The pectinase-treated guava juice also demonstrated a lower absorbance value in relation to that without enzyme treatment, indicating that the juice was clearer as shown in Figure 1. This was possibly due to the agglomeration of degraded products from pectinase hydrolysis of pectin, followed with the precipitation of fine particles as the time increased [12, 15]. This finding was also confirmed by the work of Abdullah *et al.*, [1] who studied on the enzymatic clarification of carambola fruit juice.

Results reveal that the enzyme treatment had a greater effect on juice yield. The pectinase usage showed a potential to hydrolyze soluble polysaccharides (high viscosity) to soluble sugars and short chain molecules (low viscosity) [1]. It promoted the reduction of waste loss because the less viscous puree was easier for the filtration, showing a significant increase in juice yield [11]. After the pectinase treatment of the guava juice, an experiment was conducted to evaluate the two strains of *S. cerevisiae* (*S. cerevisiae* IARI and native strain) and to check the difference of fermentation behavior between pectinase treated and untreated juice by these strains. Results present in Table 1 revealed that the overall *S. cerevisiae* strain IARI performed better than native *S. cerevisiae* strain and had higher fermentation efficiency of 79% over the 65% of the native strain. All the three parameters i.e. pH, TSS and ethanol yield presented significantly different values when both strains as well as pectinase treatment were compared. A fermentation period of 15 days was found to be optimum for completion of fermentation with Brix decreasing to zero in both the strains with pectinase treated guava juice. In terms of ethanol production, *S. cerevisiae* IARI strain produced significantly higher ethanol in terms of strain variation as well as pectinase treatment.

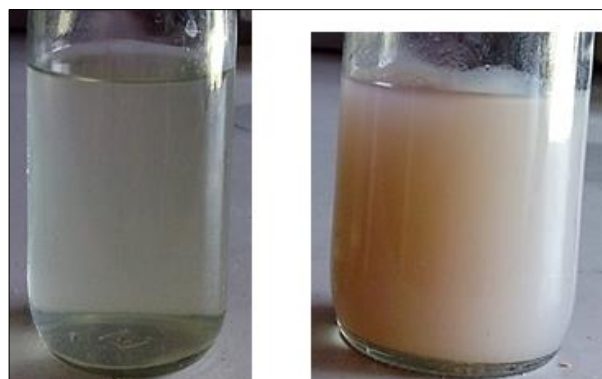


Fig 1: Shows a comparison between pectinase treated and untreated Guava juice respectively

Table 1: Shows a comparison of pectinase treated and untreated Guava juice for ethanol production by *S. cerevisiae* strain

Fermentation time (days)	Parameters									
	Brix (°B)				pH				% Ethanol	
	<i>S. cerevisiae</i> strain IARI		Native strain		<i>S. cerevisiae</i> strain IARI		Native strain		<i>S. cerevisiae</i> strain IARI	
	1.	2.	1.	2.	1.	2.	1.	2.	1.	2.
0	20.0	20.0	20.0	20.0	4.4	4.4	4.4	4.4	0.5	0.5
2	14.1	17.5	16.3	18.6	4.1	4.3	4.2	4.3	6.4	5.7
4	11.8	15.1	14.3	16.5	4.1	4.3	4.3	4.3	7.6	5.3
6	9.2	13.9	11.4	14.7	4.1	4.2	4.3	4.3	8.1	6.2
8	6.5	10.3	7.3	11.7	3.7	4.2	4.2	4.2	10.6	8.2
10	2.4	7.3	4.0	8.0	3.7	4.1	4.2	4.1	11.6	8.8
12	0.9	3.2	1.6	3.9	3.7	4.1	4.1	4.1	11.9	9.3
14	0.0	1.0	0.3	1.2	3.6	4.1	4.1	4.1	11.9	9.8
16	0.0	0.0	0.0	0.0	3.6	4.0	4.0	4.0	11.8	9.7
*F.E.(%)	<i>S. cerevisiae</i> strain IARI - 79% Native strain of <i>S. cerevisiae</i> - 65%									

IARI and Native strain

1. Pectinase treated Juice 2. Untreated Juice (Control)

$$\text{*Fermentation Efficiency (\%)} = \frac{\text{(Actual ethanol produced)} \times 100}{\text{Theoretical ethanol produced}} \quad v/v =$$

Cultural Conditions

Scale of fermentation - 200 ml

Temperature - 28±2°C

Inoculum - 4% (v/v)

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