IJPPHT
e ISSN-2231-6426
Volume 5 | Issue 2 | December, 2014 | 145-150
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*International Journal of Processing and Post Harvest Technology*

RESEARCH PAPER
DOI: 10.15740/HAS/IJPPHT/5.2/145-150

# Pre-treatment of pectinase and amylase on production of banana based wine

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**Research chronicle : Received :** 05.08.2014; **Revised :** 30.10.2014; **Accepted :** 16.11.2014

SUMMARY :

Pretreatment of pectinase and amylase to hydrolyze pectin and starch of banana must prior to its use to produce a banana wine product. The Synergetic activity of this enzyme enhances hydrolyses of complex carbohydrate. A threefold increase in amount of extracted juice were obtained after incubating with 0.5 per cent (w/w) of commercial pectinolytic enzyme at $40^0$C for one hour, followed by treating with 0.05 per cent (w/w) of amylase at $50^o$C for three hours. A 15-17 per cent increase in total soluble sugar in extracted juice was achieved, respectively. After this enzyme treated must and non enzyme treated banana must was diluted with four volume of water and then fermented by yeast produce banana wine. The pretreatment of banana with enzyme before fermentation resulted in higher level of reducing sugar than control during fermentation. The clarification of enzyme treated banana wine was three fold higher than that of control at 30 days of fermentation were observed. The concentration of total soluble sugar and alcohol in the enzyme treated and control have no significance difference were observed.

KEY WORDS : Banana must, Pectinase, Amylase Clarification, Total soluble sugar

**How to cite this paper :** Zaker, Md. Aleem, Syed, K.A. and Harkal, R.S. (2014). Pre-treatment of pectinase and amylase on production of banana based wine. *Internat. J. Proc. & Post Harvest Technol.,* **5** (2) : 145-150.

Wine is one of the most recognizable high value added products from fruits. It can be also be used as a substrate for the manufacture of vinegar, a by-product of wine manufacture. Wine manufacture is challenging in the sense of obtaining a marketable product, but the processes involved in its production are relatively straight forward (Cheirsilp and Umsakul, 2008). Wine can be defined as the alcoholic product resulting from the fermentation of fresh grape, riped banana (must) etc. that have been propagated over the ages (Lustrato *et al.*, 2003). In traditional winemaking the grape juice, after the grape pressing, is put in to vats where fermentation takes place, spontaneously transforming the juice or must into wine, without any human intervention. In this juice involves, or can involve, many of wild yeasts (*Condidopichia, metschnikowia, Brettanomyces, Saccharomycodes, Zygosacchromyces* and *Honsenulo*) with the constant presence.

Banana is the largest produced fruit crop especially in India, which is available in all over the year. It is highly nutritive and very delicious containing carbohydrates, minerals, vitamin and therefore it is known as 'common mans fruit'. India ranks first amongst the banana cultivating country in the world with an annual production share of 25 per cent of the total harvest. Banana crops which has also accepted by tissue culture technique mainly in India.

The cloudiness of fruit juices and wines is mainly due to pectin's, which may exhibit various degrees of methyesterification and other plant polymer's and even

debris. The pectin's are digested by pectinolytic enzyme prepaired from *A.niger*. It includes the mixture of enzyme activities:

- Polygalacturonase (random digestion)
- Pectin esterase (removal of methyl esters and release of methanol)
- Pectinlyase (cleaves pectin in to oligosaccharides)
- Hemicellulase (degrade hemicellulose) (Sing, 2007).

In this study, pectinase and α-amylase were used to pretreated banana must, before wine fermentation, in the expectation that they would decrease the viscosity and increase clarification, increase the extracted juice, and formation of simple sugar and oligosaccharide that is increase in reducing sugar would facilitate yeast growth. The pH and temperature were set at the optimal condition for pectinase and α-amylase activities. The optimal enzyme concentration and treatment time for both enzymes we investigated. The enzyme treated banana must use then fermented by yeast to produce banana wine.

The performance of banana wine during fermented was evaluated by measuring changes of total soluble sugar (TSS), reducing sugar, clarity and alcohol (Cheirsilp and Umsakul, 2008).

## EXPERIMENTAL METHODS

The riped banana (Completely yellow in skin colour) was purchase from the local market of Hingoli, the commercial enzyme like Pectinase and α Amylase, Yeast culture, DNSA reagent, Phenol sulphuric acid reagent, Spectrophotometer, Distillation apparatus were available in the Department of Food Microbiology, M.I.P. College of Food Technology.

**Method :**

*Part - I : Pretreatment of enzymatic hydrolysis* :

- The Banana fruits were washed, peeled and fresh crushed to make a banana must in water at ratio 1:1 (40g of pulp to 40ml water).
- The pH of banana must was 4.9 (measured by pH metre).
- The must was treated with 0.0125 to 0.11 (w/w) of pectinase at 40°C (Adjusted in incubator).
- The treatment time of fruit must by pectinase varied from 30 to 120 minute.
- Followed pectinase treatment time of fruit pulps subsequently the pectinase – hydrolyzed must was treated with 0.05 to 0.5 per cent (w/w) of amylase at 50°C.
- The treatment time was varied from 3, 6, 9 and 12 h.
- The activity of enzymes in the sample was stopped by heating the suspension at 100°C for 10 minute in a water bath and cooled before analysis.

*Part-II : Wine fermentation by yeast* :

Rarely is the fruit composition ideal for wine production. The must usually requires added sugar pH adjustment with or without additional water.

- 200g of each enzyme treated and non – enzyme treated banana must was diluted with 800ml distilled water.
- Total soluble sugar measured refractometer and adjusted to 22 $^0$Bricks unit with sucrose.
- The pH was adjusted to 3.5 with citric acid.
- The yeast strain was cultured for 48 h on a medium (200g of crushed banana in 200ml water, before it was added to the fermentation).
- Then 10ml of yeast culture was inoculated to each 1L flask or bottle of banana must previously sterilize at 100$^0$C for 20 min. in water bath.
- Fermentation was performed in triplicate in 1L flask and covered with cotton cap for 1 month.
- For the measurement of extracted juice after pectinase treatment prepares a separate set of flask, which is not allowed to fermentation.

*Analytical assay* :

- The extracted juice of pectinase – treated banana was filtered through a filter paper (Whatman no.1, Whatman International Ltd., Maid Stone, England). The volume of extracted juice was measured.
- Estimation of total soluble sugar by phenol sulphuric acid reagent method.
  -The amount of total soluble sugar method was estimated by phenol sulphuric acid reagent method (Manmit *et al.*, 2008).
- Estimation of reducing sugar was determined by Dinitro-salicylic acid (DNSA) reagent of miller method.
- The clarification (transparency) of the supernatant of fermented juice was determined by measuring per cent transmittance at a wave length of 660 nm. Using spectrophotometer. Distilled water was used as a reference for 100 per cent transmittance.
- Alcohol content was measured by distillation followed by gravity bottle method.

## EXPERIMENTAL FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

**Effect of pectinase concentration and treatment time on banana must :**

The effects of different pectinase concentration and treatment times on the volume of extracted juice of banana

must are reported in the Table 1 and 2, respectively.

The non-enzyme-treated banana must was used as a control. The volume of extracted juice after treatment with 0.05 per cent (w/w) pectinase was near about 3-fold higher (89 ml) than that of control (28 ml). This could be explained by the pectinase hydrolyze the pectin molecule, reduction to a water holding capacity, and consequently, more free water is released. Akubor *et al*. (2003) investigated production and quality evaluation of banana wine.

The pectinase concentration of higher than 0.0125 per cent and up to 0.1 per cent (w/w) there was no significant difference in the volume of extracted juice as shown in Table 1 ($p>0.05$). On the other hand, as the pectinase concentration increased in double amount that is from 0.05 to 0.1 per cent (w/w) but only a 16 per cent increase in juice. Thus if we use the more enzyme will increase the cost of the operation, since 0.05 per cent (w/w) concentration of pectinase which yield a 3-fold increase in extracted juice (Egwim *et al.,* 2013; Alvarenga *et al*., 2011 and Dhar *et al.,* 2013).

Treatment time greater than 1h made no significant

**Table 1 : Effect of pectinase concentration on the volume of extracted juice of banana must**

| Sr. No. | Pectinase conc.(%w/w) | Volume of must (ml) | Treatment time (h.) | Volume of extracted juice (ml) |
|---|---|---|---|---|
| 1. | 0 (control) | 200 ml | 0 | 28 ml |
| 2. | 0.0125 | 200 ml | 1 | 87 ml |
| 3. | 0.05 | 200 ml | 1 | 89 ml |
| 4. | 0.10 | 200 ml | 1 | 105 ml |

**Table 2 : Effect of pectinase treatment time on the volume of extracted juice of banana must**

| Sr. No. | Pectinase conc.(%w/w) | Volume of must (ml) | Treatment time (h.) | Volume of extracted juice (ml) |
|---|---|---|---|---|
| 1. | 0.05 | 200 ml | 0.5 | 97 ml. |
| 2. | 0.05 | 200 ml | 1 | 85 ml. |
| 3. | 0.05 | 200 ml | 1.5 | 91 ml. |
| 4. | 0.05 | 200 ml | 2 | 105 ml. |

**Table 3 : Effect of amylase concentration and treatment time on total soluble sugar and reducing sugar of pectinase treated banana must**

| Sr. No. | Amylase conc.(%w/w) | Volume of pectinase treated must.(ml) | Treatment time ( t ) | Total soluble sugar (mg/ml) | Reducing sugar (mg/ml) | O.D. for total soluble sugar | O.D. for reducing sugar |
|---|---|---|---|---|---|---|---|
| 1. | Control [non-treated] | 200 | | 98.25 | 60.10 | 1.01 | 0.62 |
| 2. | '0' pectinase Treated control | 200 | '0' | 109.20 | 65.12 | 1.13 | 0.68 |
| 3. | 0.05 | 200 | 3 | 115.50 | 75.00 | 1.19 | 0.77 |
| 4. | 0.1 | 200 | 3 | 121.30 | 78.20 | 1.25 | 0.79 |
| 5. | 0.3 | 200 | 3 | 124.31 | 82.35 | 1.28 | 0.84 |
| 6. | 0.5 | 200 | 3 | 125.10 | 85.00 | 1.29 | 0.84 |

**Table 4 : Effect of amylase treatment time of total soluble sugar and reducing sugar of pectinase treated banana must**

| Sr. No. | Amylase conc. (%w/w) | Volume of pectinase treated must (ml). | Treatment time (t) | Total soluble sugar (mg/ml) | Reducing sugar (mg/ml) | O.D. for total soluble sugar | O.D. for reducing sugar |
|---|---|---|---|---|---|---|---|
| 1. | 0.05 | 200 | 3 | 116.30 | 76.10 | 1.20 | 0.78 |
| 2. | 0.05 | 200 | 6 | 118.30 | 77.00 | 1.22 | 0.79 |
| 3. | 0.05 | 200 | 9 | 117.75 | 81.33 | 1.21 | 0.83 |
| 4. | 0.05 | 200 | 12 | 118.00 | 90.58 | 1.22 | 0.94 |

**Table 5 : Comparison enzyme treated banana wine and control (Non-enzyme treated) wine with respect to total soluble sugar and alcohol after 30$^{th}$ days of fermentation**

| Treatments | Total soluble sugar (mg/ml) | Alcohol (%) |
|---|---|---|
| Enzyme treated banana wine | 3.75 | 14.5 % |
| control | 6.30 | 15.3 % |

($p>0.05$) difference to the volume of extracted juice (Table 2). Therefore, a pectinase concentration of 0.05 per cent (w/w) and 1h of treatment time were used to hydrolyses the banana must.

**Effect of amylase concentration and treatment time on banana must :**

After treatment with pectinase, amylase was used to hydrolyze starch and shorten the starch glycoside chains. Total soluble sugar and reducing sugar were measured to evaluate starch hydrolysis. The effect of different amylase concentration and treatment time on total soluble starch and reducing sugar of banana must are reported in Table 3 and 4, respectively. The no enzyme treated banana must and was used as a control. The banana must had 11 and 5 per cent increase in total sugar (from 98.10 to 109.20 mg/ml) and reducing sugar from (60.10 to 65.12 mg/ml), respectively after pectinase treatment. Subsequent treatment with 0.05 (w/w) concentration of amylase for 3h produced a 6% increase in total soluble sugar (from 109.20 to 115.50 mg/ml)) and a 10 per cent increasing in reducing sugar (from 65.12 to 75.00 mg/ml) over the pectinase treated–control. A further increase in a concentration of α–amylase concentration from 0.05 to 0.5 per cent (w/w) resulted in further increase in total soluble sugar (from 115.50 to 125.10) but no significant effect in reducing sugar (Table 3). Idise *et al*. (2011) studied wine produced from banana Fermentation of banana must.

And also increasing the time of incubation from 3, 6, 9 and 12h with constant concentration (0.05%) had no significant effect on the sugar content (Table 4). This result shows that amylase has no impact on the sugar content of the must compared with pectinase treatment. Therefore, on amylase concentration of 0.05 per cent (w/w) with 3h incubation time was used to treat the banana must after pectinase treatment subsequent pectinase and amylase treatment gave 17 and 15 per cent increase in total soluble sugar (from 98.25 to 115.50mg/ml) and reducing sugar (from 60.10 to 75.00mg/ml), respectively.

**Fermentation :**

Untreated banana must was used for wine fermentation. Anaerobic fermentation was carried out at room temperature. The concentrations of total soluble sugar and alcohol in banana wine after 30 days of fermentation are shown in Table 5. There were no significant difference in final concentration of this enzyme – treated and control.

The quality of enzyme-treated banana wine compared to that of control was evaluated by following the changes in reducing sugar level and transparency at 660nm, respectively.

The reducing sugar level in a enzyme treated banana wine increased to maximum (150mg/L) at about six days higher than that of control (48mg/L) After 6$^{th}$ and 12$^{th}$ day, in both cases reducing sugar level fell rapidly. Initially clarity of supernatant of enzyme treated banana was three fold higher (95%) than that of control (35%) (Table 6).

Then clarity of enzyme treated banana wine decreased rapidly during the first 12 days. Then again clarification of enzyme treatment sample began to increase due to precipitation of yeast cell and polysaccharides complex, but at this time very little changes in clarity of control. Thus, even though the alcohol concentration of both banana wines were same, the enzyme treated banana wine provides a much clearer wine product within a shortest period. Similar work related to the present investigation was also carried out Pandhre *et al.* (2010) and Emmanuel and Ikenna (2011).

**Table 6 : Analytical assay: During fermentation at time interval of having 0.05 (1% w/w) concentration and 3hr enzyme treatment**

| Sr. No. (days) | Reducing sugar (550 nm) | | Total soluble sugar (550nm) | | Transparency (660nm) | |
|---|---|---|---|---|---|---|
| | Sample | Control | Sample | Control | Sample | Control |
| '0' days | 0.46 | 0.40 | 45 | 39 | 0.951 | 0.356 |
| 3$^{rd}$ days | 0.78 | 0.46 | 75 | 45 | 0.934 | 0.310 |
| 6$^{th}$ days | 1.54 | 0.49 | 150 | 48 | 0.956 | 0.262 |
| 9$^{th}$ days | 0.52 | 0.59 | 50 | 57 | 0.982 | 0.293 |
| 12$^{th}$ days | 0.36 | 0.70 | 35 | 68 | 0.270 | 0.305 |
| 15$^{th}$ days | 0.10 | 0.20 | 10 | 20 | 0.233 | 0.333 |
| 18$^{th}$ days | 0.07 | 0.12 | 7 | 12 | 0.250 | 0.204 |
| 21$^{st}$ days | 0.05 | 0.08 | 5 | 8 | 0.244 | 0.182 |
| 24$^{th}$ days | 0.30 | 0.03 | 3 | 3 | 0.407 | 0.201 |
| 27$^{th}$ days | 0.00 | 0.00 | 0 | 0 | 0.423 | 0.190 |
| 30$^{th}$ days | 0.00 | 0.00 | 0 | 0 | 0.450 | 0.205 |

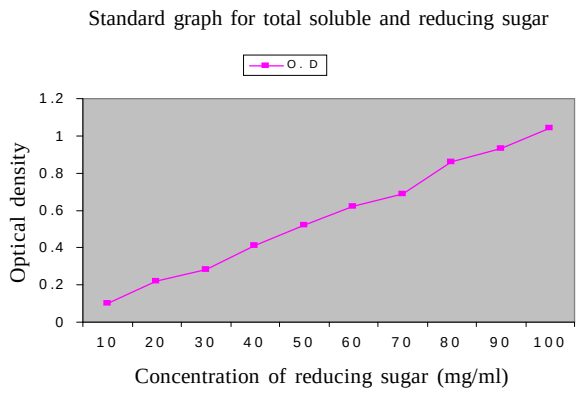


**Fig. 1 : Standard graph for reducing sugar sample, control and total soluble sugar**

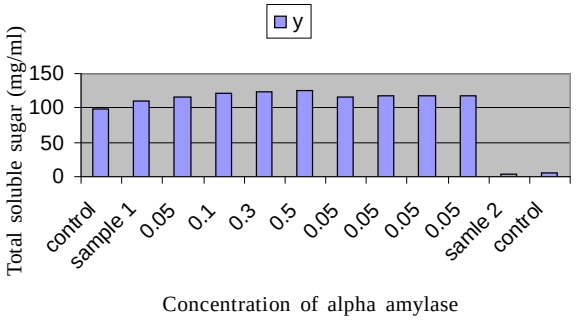


**Fig. 2 : Effect of amylase concentration and treatment time on total soluble sugar before and after fermentation**

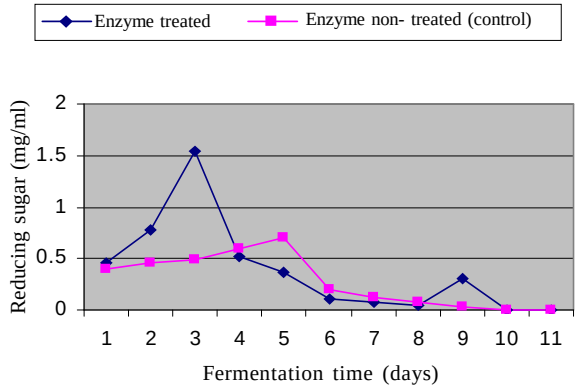


**Fig. 3 : Comparison of reducing sugar level of enzyme treated and control during wine fermentation**

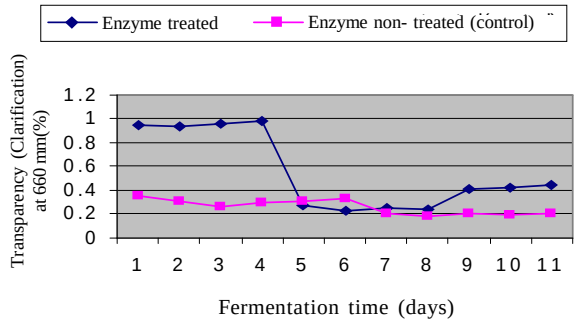


**Fig. 4 : Comparison of transparency at 660 nm of enzyme treated banana must and control during wine fermentation**

## Conclusion :

The pre-treatment of pectinase and amylase to banana must before the wine fermentation an effective method to increase the amount of the extracted juice. During this process the optimum conditions *i.e.* temperature and times for pectinase and amylase were observed. The enzyme treated banana must used to produce banana wine were shown the enhanced clarification of the wine product without affecting any other character. Thus in this study it is the objective observed that it reduce the cost of operation process and thereby reducing storage cost and time. Therefore, the use of enzyme hydrolysis with pectinase and amylase result in a threefold shorter process of clarification and greatly improve the quality of the commercial wine product.

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