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Optimization of juice extraction from custard apple and fermentation to ethanol

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ABSTRACT

Custard apple is a most popular tropical fruit known for its sweet taste, rich flavour and pleasant aroma with creamy juicy flesh. The fresh fruit is enriched with precious nutrients which have great potential for nutraceutical. Though the fruit is nutritionally balanced, it is highly perishable in nature due to high respiration rate and therefore there is a great need to explore alternate methods for its utilization. Preparation of beverages could be an alternative to value addition and its economic utilization. In this study, an attempt was made to optimize the process juice juice extraction from custard apple and its further processing to ethanol production. Different concentrations of enzyme pectinase and time of incubation were explored to achieve the desired yield of ethanol. The juice thus obtained was evaluated for total soluble solids, titratable acidity, ascorbic acid, pH and sugars and used as raw for ethanol production using *Saccharomyces cerevisiae* var. *ellipsoideus*. The inoculum of yeast was also optimized during the value addition. The maximum production of ethanol (10.05 %,v/v) was achieved at 10 % (v/v) inoculum level with low reducing sugar (2.01 % w/v), total sugars 5.96 % (w/v), titratable acidity (0.61%, w/v) and pH (3.6), respectively. The ethanol so produced was without amelioration as the juice contained total soluble solids of 24° B.

Key words: *Annona squamosa*, pectinase, *Saccharomyces cerevisiae* var. *ellipsoideus.*

INTRODUCTION

Custard apple (*Annona squamosa* L.) is a deciduous tropical plant which can better acclimatizes in heat and drought conditions. In India, custard apple is grown in Maharashtra, Gujarat, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh, Bihar, Jharkhand, Assam, Rajasthan, Orissa and Tamil Nadu (Shailja *et al*., 13) It is one of the delicious fruits relished by many people for table purposes. Pleasant flavour, mild aroma and sweet taste of this fruit have a universal acceptance. It contains about 28-55% of edible portion; consisting of 73.30% moisture, 1.60% protein, 0.30% fat, 0.70% mineral matter, 23.90% carbohydrates, 0.20% calcium, 0.40% phosphorus, 1.0% iron, 12.40-18.15% sugar with 0.26 to 0.65% acidity and a total calorific value of 105 calories per 100g of edible pulp (Srivastava *et al.,* 14). This fruit is climacteric fruit and ripen very fast due to high respiration rate and ethylene production (Mallikarjuna *et al.,* 8). The short shelf life of 3-4 days of this fruit coupled with the absence of appropriate preservation and value addition techniques, leads to its glut in market during the season, resulting in low prices, ultimately causing losses to the producers. Therefore, it becomes necessary to develop standard techniques to minimise the postharvest losses.

Preparation of unfermented and fermented beverages like juice and alcohol is one technique that fulfils the requirement of standard technique to minimize the postharvest losses, but it needs proper standardization of juice extraction method with suitable recipe. Traditional method of juice extraction like mechanical press provides a very less yield of juice. For increasing yield, the traditional methods can be combined with various pre-treatment viz., cold, hot and enzymatic extraction (Chadha *et al*., 3). Enzymatic treatment resulted significant increase in juice recovery compare to cold and hot extraction. Enzymatic treatment of fruits helps in cell wall hydrolysis which increases the yield, titratable acidity, reducing sugars and soluble dry mater of the extracted products (Joshi *et al*., 5). Further, the treated and clarified juice results in viscosity reduction and cluster formation, which facilitates separation through centrifugation or filtration. As a result, the juice presents higher clarity, as well as more concentrated flavour and colour. Enzymatic degradation of the biomaterial depends upon the type of enzyme, incubation time, incubation temperature, enzyme concentration, agitation, pH and use of different enzyme combinations (Lee *et al*., 7). Fermentation of fruit juices is an effective and simple avenue for minimizing post harvest losses of mainly perishable fruits, hence perishable fruits can be used for production of ethanol. This technique is relatively low energy preservation process which increases the shelf life and decreases the need of refrigeration or any other forms of food preservation methods.

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Ethanol production has been practiced with various fruits such as apple, banana, pineapple, oranges, pear, strawberry, cherries, guava, plum, cucumber, watermelon etc. The species of *Saccharomyces cerevisiae* can convert the sugar in the fruit juices into alcohol and organic acids which can be utilized for various purposes or can be used as a wine (Grohmann *et al.,* 4).

Studies on production of ethanol from custard apple juice was not explored yet and hence, the present work deals with optimization of extraction juice by varying concentration of pectinase followed ethanol fermentation at optimized size of yeast inoculums. The approach could be an alternative method of minimizing post harvest wastes and value addition.

MATERIALS AND METHODS

Fully matured and ripened custard apples free from blemishes were procured from the local market of Ranchi (Jharkhand). Fruits were washed with tap water and then treated with 200 ppm of sodium hypochlorite solution for 15 minutes, followed by thorough rinsing with water to remove traces of chemicals. The fruits were cut into two halves and scooped with stainless steel spoon. Subsequently, the seeded pulp was passed to electronic pulper machine for separation of seeds from pulp. The extracted pulp was used for chemical analysis and processed for juice extraction.

Extracted pulp was analysed for various chemical parameters. Total ascorbic acid and total titratable acidity were determined according to the methods described by AOAC (1). Total soluble solids (TSS) was determined by using Erma hand refractometer of range 0-40°Brix and pH by using pH meter (Ranganna, 12). Reducing sugars and total sugars were determined by the method of Ranganna (12).

The pulp was treated with pectinase enzyme (Hi Media) in a concentration of range of 0.025-0.150% (w/w) and mixed thoroughly followed by incubation in water bath at a temperature 40°C (Yusof and Ibrahim, 16). The enzyme treated pulp was removed at every 1, 2 and 3 hour of time interval for measuring the yield of juice followed by heating to 80°C for 30 seconds to stop enzyme activities. All the experiments were carried out in triplicate and mean value was recorded. Enzyme treated samples were centrifuged at 3000 rpm for 15 minutes for removal of suspended material and juice clarification. After centrifugation the samples were filtered again through cheese cloth to collect the clarified juice. Clarified juice was pasteurized by heating to 85°C for 20 minutes in a pre sterilized bottles followed by cooling to room temperature.

The clarified juice samples attained by this approach were subjected to chemical analysis for total and reducing sugars, total acidity, ascorbic acid pH and total soluble solid contents by standard protocols.

A pure culture of *Saccharomyces cerevisiae* var. *ellipsoideus* was procured from the Institute of Microbial Technology (IMTECH) Chandigarh, India. The pure culture of yeast was subcultured in growth medium no. 5 for 48 hour. This culture was used for preparation of mother culture for further fermentation process as described by Matheson (9). The custard apple juice was mixed with 125 ppm of potassium metabisulphite (KMS) to prevent browning of the juice and to suppress growth of undesirable microorganisms. The juice was not ameliorated because the TSS of juice was recorded as 24°B. The sterilized fermentation bottles were filled with must and closed with cotton plugs followed by pasteurization at 85°C for 30 minutes.

The must was inoculated with 5.0, 7.5, 10.0 and 15.0% (v/v) active pure yeast mother culture and fermented at 28- 30°C temperature. After completion of fermentation, the mixture was siphoned off and filtered through a clean sterilized muslin cloth and collected in sterile glass jars. Clarified ethanol was packed in glass bottles and pasteurized (62 ± 1°C for 20 min) and stored for chemical analysis.

Various physico-chemical characteristics of wine were analysed during fermentation study. Total Soluble Solids (TSS) was determined by using ERMA hand refractometer and expressed as °B and alcohol content was measured using spectrophotometer at 600 nm (Natu *et al.*, 10) Rate of fermentation (°B/24 hours) was estimated with following formulas:

$$\text{Rate of fermentation} = \frac{\text{Initial TSS} - \text{Final TSS}}{\text{Time}}$$

RESULTS AND DISCUSSION

Mature custard apple fruits were used for pulp extraction and 43% (w/w) pulp was recovered using electronic pulper. The extracted pulp was evaluated for proximate analysis. The pulp was further utilized for juice extraction by using different concentration of pectinase enzyme.

The chemical composition of custard apple pulp was presented in Table 1 which showed the moisture content of 74.6 g. $100g^{-1}$ pulp and total soluble solids (TSS) of 26 °B. The reducing and total sugars content was found to be 16.32 and 20.9 g $100g^{-1}$ pulp, respectively. The pH of extracted pulp was 5.48 with a titratable acidity of 0.29 g $100g^{-1}$ and concentration of vitamin C was found to be 32.5 $mg.100^{-1}$.

Table 1. Chemical constituent of custard apple pulp.

S. No.	Constituents	Pulp
1.	Moisture (g $100g^{-1}$)	74.6
2.	Total soluble solids (°Brix)	26
3.	pH	5.48
4.	Titratable scidity ($g.100g^{-1}$)	0.29
5.	Total sugars ($g.100g^{-1}$)	20.9
6.	Reducing sugars ($g.100g^{-1}$)	16.32
7.	Vitamin C ($mg.100g^{-1}$)	32.5

Table 2 showed the effect of enzyme concentration and incubation time on yield of custard apple juice. A temperature of 40 °C was used for incubation of enzyme treated pulp for 1, 2 and 3 hours of incubation. As enzyme concentration and incubation time increased, the yield of juice was also increased. The untreated pulp of custard apple gave a very low yield of juice as 29% (w/v). The maximum amount of juice was achieved with 0.10 % (w/w) enzyme concentration at 3 hours of incubation period. The figure 1 showed the effect of enzyme concentration and incubation time on yield of custard apple juice.

High concentration of enzyme (0.15 %, w/w) with same incubation time did not give high yield. The time of incubation was considered as an important factor for juice extraction. It was evident from Fig. 1, that 200 g of untreated custard apple pulp resulted

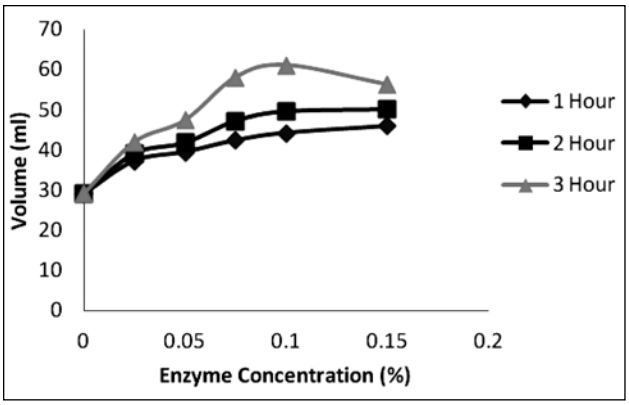

Fig. 1. Effect of enzyme concentration and incubation time on yield (ml) of custard apple juice.

only 58 ml of juice while; treated pulp resulted 122 ml of juice which was 34% higher than the untreated sample. The increase in yield was due to pectinase enzyme which may possibly break down all polymeric carbohydrates such as hemicelluloses, pectin and starches, and thus increasing the yield of juice by enabling better pressing of the pulp. This result was found in accordance with Yusof and Ibrahim (16) where 41% increase in juice recovery was observed with enzymatic treatment as compared to the untreated sample of sour sop (*A. muricata*). They further examined that the higher the amount of enzyme and the longer the time of incubation, the

Table 2. Effect of enzyme concentration and incubation time on yield of custard apple juice.

Enzyme conc. (%)	Incubation Time (h)	TSS (°B)	Yield (%)	Ascorbic Acid ($mg.100g^{-1}$)	Titrable acidity ($g.100g^{-1}$)	pH
Nil	-	24	29.05	16.41	0.49	5.02
0.025	1	23	37.23	15.16	0.47	4.96
	2	23	39.05	16.14	0.49	4.12
	3	24	**41.89**	17.04	0.51	3.85
0.050	1	23	39.43	17.20	0.48	4.23
	2	23	41.71	14.13	0.50	4.01
	3	23	**47.30**	17.01	0.53	3.97
0.075	1	23	42.33	16.14	0.50	4.19
	2	23	47.14	17.01	0.52	3.85
	3	24	**58.00**	16.96	0.53	3.82
0.100	1	23	44.16	16.32	0.51	4.11
	2	24	49.52	16.93	0.53	3.81
	3	24	**61.05**	17.04	0.53	3.72
0.150	1	23	45.85	16.56	0.47	4.09
	2	24	50.05	16.83	0.48	3.76
	3	24	**56.25**	17.01	0.47	3.71

greater was the yield of juice from sour sop. Upon enzyme treatment, degradation of pectin leads to reduction in water holding capacity of pectin so that free water is released into the system and hence, the juice yield increases (Lee *et al.,* 7). From above observation of data recorded during the present study, it was concluded that an enzyme treatment of 0.075 % (w/w) for 3 hours was found to be most efficient and economical for custard apple juice extraction.

It was noticed that ascorbic acid content in custard apple pulp was found to be 32.5 $mg.100g^{-1}$ (Table 1) and in untreated juice 16.41 $mg.100g^{-1}$ (Table 2). The significant low content of ascorbic acid in juice (~45%) was probably because of processing and pasteurization at high temperature. However, the enzyme treatment and incubation time did not affect the content of ascorbic acid significantly. Brasil *et al*. (2) also reported loss in ascorbic acid content (~27.4 %) during pasteurization along with increase in °Brix and colour index during extraction of guava juice.

From the observations, it was found that total soluble solids (TSS) of pulp was 26 °B, which decreased on enzymatic treatment to 23 °B but increased slightly by increasing the time of incubation from 1 hour to 3 hours due to the enzyme action on the pectic substances of juice pulp causing hydrolysis of these substances and release of dissolve material. The titratable acidity of untreated juice was 0.49 $g.100g^{-1}$ which varies in a range of 0.47 - 0.53 $g.100g^{-1}$ with increase of enzyme concentration and incubation time however, it remains unchanged for enzyme concentration of 0.15 % (v/w). This result showed increase of titrable acidity in custard apple fruit juice was due to enzymatic action. Unlike other constituents, ascorbic acid, total sugar and reducing sugar showed similar type of reduction in their content when pulp was treated with different enzyme concentration. The amount of total sugars and total acidity are major factor which influence the ethanol production from any fruits.

The extracted juice was used for fermentation with different strengths of yeast (5 - 15 % v/v). Initial soluble solids of custard apple juice were 24 °B, and there were no addition of extra sugar and acidity. Fermentation behaviour of different treatments on total soluble solids has been presented in Table 3. Among all these treatments, the soluble solids changes significantly with increase of incubation period of fermentation. In case of treatment 1, where no yeast inoculum was added (control), negligible fermentation and change in soluble solid was observed (23.5°B) as compared to treatment T 2 which contained only 5% (v/v) yeast inoculums, the obtained TSS was 12.83 °B. Lowest soluble solids 11.1 °B was observed for treatment T4 (10 % inoculum) and was at par with treatment T 5 with 15% (v/v) inoculum (11.5 °B). For all treatments except control, the rate of fermentation ranged from 1.2 - 1.33 °B/24 hours. However, treatment with 5% (v/v) inoculums gave least fermentation rate (1.2 °B/24 hours).

The volume of inoculums on ethanol production is of great significance for completing fermentation process. Different volume of inoculums 5, 7.5, 10 and 15 % (v/v) were used to inoculate the fermentation mixture and the effect on ethanol production was presented in Table 4.

It was observed that ethanol production increased with increase in inoculum concentration up to 10 % (v/v). The alcohol content of custard apple juice ranged from 8.76 -10.05 % (v/v) for all treatments except the control which was observed to be very less 0.91 % (v/v). The highest concentration of ethanol was found to be 10.05% (w/v). Increasing inoculum level beyond 10 % (v/v) did not enhance the ethanol production, which could be because of nutrient depletion in the fermentor. It was therefore, concluded that 10 % inoculums was favourable for higher ethanol production from custard apple juice. The concentration of ethanol was reported after 15 days of fermentation process. The observation of ethanol fermentation from custard apple juice was in accordance with Vyas and Joshi (15) who worked on fermentation of plum wine. Patharkar *et al*. (11) also reported that 10 % (v/v) inoculums of yeast produce a maximum ethanol content of 8.5 % (v/v) from orange juice. The fermentation efficiency in all treatments except control ranged from 72.65 - 78.51 %.

Table 3. Change of TSS (°B) during fermentation period of custard apple.

Treatments	Days of Fermentation						Mean
	0	3	6	9	12	15	
T1 (control)	24	24	24	24	22	22	23.5
T2 (5% Yeast)	24	19	13	8	7	6	12.83
T3 (7.5% yeast)	24	18	13	7	5	5	12.0
T4 (10% yeast)	24	16	10	7	6	4	11.17
T5 (15% yeast)	24	17	11	8	5	4	11.5

Table 4. Effect of inoculums size on ethanol production from custard apple juice.

Treatments	TSS (°B)	Reducing sugars % (w/v)	Total sugars % (w/v)	Titratable acidity %	Ethanol % (w/v)	pH
T1 (control)	23.5	10.37	11.5	0.45	0.91	4.98
T2 (5% yeast)	12.5	2.72	5.75	0.77	8.76	3.96
T3 (7.5% yeast)	12.0	2.56	6.1	0.72	9.01	4.0
T4 (10% yeast)	11.33	2.01	5.96	0.61	10.05	3.6
T5 (15 % yeast)	11.67	2.32	5.2	0.65	9.3	3.7

Table 4 also depicts that for all treatments reducing sugar and total sugar decreased drastically during fermentation of ethanol. Among all treatment control have the highest value of reducing sugar 10.37 % (w/v) and total sugar 11.5 % (w/v) over rest of the treatments. In other treatments, T4 was recorded with minimum reducing sugars (2.01 % (w/v) followed by T5 (2.32 % (w/v), where as in case of total sugars, treatment T5 recorded total sugars content of 5.2 % (w/v) and T3 recorded the highest content (6.1 % (w/v). The titratable acidity was found to be increased while the pH of fermentation broth was decreased with increase in inoculums size. The increase in acidity during ethanol formation may be due to formation of carbonic acid in the fermentation medium (Patharkar *et al.,* 11). The results showed that the maximum alcohol was achieved at pH of 3.6. With increase in pH, the ethanol production was also reduced gradually, which may be because yeast grows well in acidic environment (Kulkarni *et al.,* 6).

Processing of custard apple based products like juice and ethanol is a way to exploit exotic flavour, aroma and valuable nutrients. The study concluded that treatment of fruit pulp with pectinase enzyme was found to be beneficial in increasing yield of juice up to 34 %(w/v) as compared to untreated sample. The optimum yield was achieved at the level of enzyme 0.075 % (w/w) with incubation period of 3 hours. Thus extracted juice had a TSS of 24 °B which can be further used for fermentation without amelioration. The ethanol produced from custard apple juice showed a moderate range of production from 8.76 to 10.05 % (w/v).

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