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ANNONA SQUAMOSA FRUIT PULP AS A SUSTAINABLE BIO RESOURCE FOR PRODUCTION OF WINE LIKE PRODUCE: A CASE STUDY

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ABSTRACT Present investigations focus on possibility of *Annona squamosa* fruit pulp as a sustainable resource for wine like produce. Fruit pulp was evaluated with twenty one yeast strains revealed that the Balanagar and Saharanpur variety were showing high content of pulp as compared to the wild variety. Later, the fruit pulp was digested by pectinase treatment. Different ratios of pectinase was examined, a ratio of 3:2 ratio of pulp and enzyme was found to yield better results in terms of sugar release and wine production. Some strains producing small quantities of wine even without enzymatic treatment. In majority of the cases, positive correlation was observed between the reducing sugars relased and wine produced. The wine produced showing good organoleptic characters with low quantities of phenolic, higher alcohols and good taste and aroma. Hence, the *Annonasquamosal* fruit wine is an economically valuable and can be promoted for consumption.

KEYWORDS : *Annonasquamosa* fruit pulp, bioresource, wine production

INTRODUCTION

Most of the industrial scale fermentations are in need of cheaplyavailable raw materials as the primary substrates which can serve as the carbon and nitrogen source (Aliyu and Bala (2011)). Sometime they happened to be the derivatives of the naturally available plant materials (Balandrin, et al., (1985).). It is a proactive approach to test the raw materials in pilot scale, whether they are suitable or not for the fermentation economics and sustainability. The final choice of the raw materials is duly influencing the fermentations at a gross root level. Traditionally, several carbohydrate rich materials employed as the carbon and energy source for actual fermentations, although the other sources too (Lin and Tanaka (2006)). A cost effective feedstock is always preferable for sustainable fermentation technology (Pimentel, 1994). A variety of raw material and derivatives ranging for molasses, sugar cane bagasse, corn starch, sorghum, Jersusalem artichoke, potatoes and lignocellulose biomass are the greatest interest of biofuel production (Kim and Dale 2004). In addition to these starchy materials several naturally available fruits may use as the raw materials for wine and alcohol production (Anuradha et al., 2014). When the wine produced from the fruits is referred to as the 'fruit wine'. There are several naturally available and derivatives of fruits can used for the purpose of wine making such as Grapes, Red Currant, Black Currant Banana, Plums, Pomegranate, Pine Apple, Dandilion, Rose Hip,Sapota, Orange, Cherry, Lychee and barriers from different plant varieties (Kosseva, et al .,2016).Sometimes the fruit wine may be amended with various natural aromas derived from flowers and herbs for improving wine organoleptic characteristics, they are either dry or off dry types (Christoph and Bauer-Christoph (2007).). The main aim of the purpose to use the fruits is they rich in sugars, vitamins, antioxidants and some of them are available throughout year (Wootton-Beard et al., 2011).

Annona suqamosa is a perennial tree vernacular name is seethaphal, due to high content of sugar it is also termed as sugar apple or custard apple. *Annona* fruit contains more than 60 % of pulp which is known to contain high content of sugars. Hence, in the present investigation an attempt was made to test the possibility of the pulp for wine and alcohol production.

MATERIALAND METHODS

1.Collection of fruits: Custard apple fruits of different cultivars i.eBalanagar, Saharanpur were collected from the local markets of districts of Telangana. Ripen fruits of uniform shape and size was selected for processing.

2.*Yeast strains* and yeast culture:The yeast strains employed in the present investigations were isolated from sweet fruits which are known to harbour yeasts, like grapes, papaya, cherry, banana, date palm etc,. Some of the strains were isolated from soil, sewage water, garden soil and toddy. Bakers yeast strains were obtained from the bakery. The industrial strains included in the present investigations were obtained from National Chemical Laboratory, Pune (NCL), and from Institute of Microbial Technology (IMTECH), Chandigarh. Yeast strains isolated and purified were maintained on YEPD medium (g/l, Yeast extract,10g; Peptone, 20g; Dextrose, 20g; P^H. 5.5). In all cases of the present investigations, unless otherwise mentioned, 24 hours broth cultures in the exponential phase were used.

3.Studies on pulp liquefaction with commercial pectinase enzymes

Commercially available enzymes viz., Pectinase was employed to hydrolyze the pectin. The enzyme was obtained from Bicon India Pvt. Ltd., Bangalore.

It is an extracellular heat stable pectinase (Polygalacturonase activity, Pectin esterase activity, Pectin lyase ≥ 2200 U/g ≥ 550 U/g ≥110 U/g) derived from a selected strain of *Aspergillusniger*.

Mash preparation and ethanol fermentation:

At first pulp liquefaction was carried out by suspending individual pulp in hot water and the hydrolysis was carried out as recommended by the manufacturers. Different possible ratios of the enzyme and pulp were used.To the slurry required amount of pectinase, was added heated in water bath at 55^o for 60 minutes and then the solution was cooled and kept in a freezer to inhibit the enzyme. Now the pH was adjusted to 4.0 to 5.0. This resultant hydrolysis product was used as carbon source. To this mixture 2% of yeast culture was added, the pH was adjusted to 5.5.The flasks were steam sterilized at 121^oC for 15 minutes and cooled.

Fermentation of liquefied pulp:

One ml of the culture broth in exponential phase was inoculated aseptically into all the flaks. The flasks were incubated at 30^oC for three weeks. After the stipulated incubation period, about 1 ml of the culture broth was centrifuged to remove the cells and the supernatant was collected. From the supernatant, ethanol produced and unutilized sugars present were estimated .Experiments were carried out in triplicates and the average values were calculated.

3. Estimation of residual sugars and ethanol

The unutilized residual sugars in the fermentation broth were estimated by DNS method (Millers, 1959). Standard graph was plotted by plotting the value of optical density against the concentration of glucose. The actual amount of residual sugars was obtained by substituting the values in standard graph for glucose. Ethanol in the fermentation broth was estimated by the procedures suggested by Caputi*et al*. (1968), AOAC (1990) and Anon (1992). The results are presented in the table 1-4.

RESULTS AND DISCUSSION

Annonasquamosa is a tropical and subtropical fruit, it is available in Telangana during winter season only, it is an important fruit crop due to its taste and natural availability, due to high content of pulp, nutritional value this fruit can be used in folk medicine. The fruit is highly perishable. Hence, an attempt was made to convert the pulp in to value added wine like produce. In order to identify the ideal variety of the *Annonasquamosa* pulp concentration and peel percentage are calculated and presented in the Table-1.

Table-1 Identification of ideal variety of custard apple.

Cultivar	% Fruit peel	% Pulp
Balanagr	40	60
Akasahan	37	63
Mahaboobnagar	35	65
Sharanpur	30	70
Wild	37	53

It is evident from the Table -1, that the ideal variety of custard apple selected was Sharanpur variety because it consists of 70% of pulp and 30% of peel, followed by Mahaboobnagar Akasahan, Balanagr and wild varieties.

In the present investigation an attempt was made to evaluate the nutrient content like moisture, crude protein, fat and carbohydrates and the results were present in Table-3

Table -3 the nutrient contents of selected varieties

Cultivar	Moisture content	Crude protein	Crude fat	Carbohydrate /100g pulp
Balanagr	8.9±0.1	5.7±0.3	1.1±0.3	80.9±0.1
Akasahan	8.8±0.2	5.7±0.3	0.5±0.1	82.2±0.3
Mahaboobnagar	7.3±0.2	4.0±0.2	1.0±0.1	85.3±0.2
Sharanpur	9.0±0.2	5.3±0.2	3.2±0.3	89.6±0.4
Wild	6.7±0.3	4.9±0.1	1.0±0.1	81.0±0.3

The results presented in Table-2 explains the nutrient content of selected varieties fruit pulp and it is very interesting to state that the Sharanpur variety is possess9.0 percentage of moisture followed by Balanagr, Akasahan, Mahaboobnagar and Wild varieties. The protein content was same in Balanagr, Akasahan followed by Sharanpur, Mahaboobnagar and Wild custard apple varieties. In case of crude fat content the Sharanpurvariety shows huge quantity as compared to the other selected varieties. The carbohydrate quantity is more than 80% in all selected varieties.

The total phenolic compounds present in the pulp also play an important role in fermentation and consumption of wine. Hence, an attempt was made and the results were presented in the Table-4.

Table-4 total phenolic compounds present in the pulp

Cultivar name	TPC	TCC	GA
	mg of GA/g	µg of TCC/g	µg of GA/g
Balanagr	140.4	1634	390
Akasahan	43.4	1027	148
Mahaboobnagar	108.7	1805	163
Sharanpur	34.1	954	148
Wild	77.9	167	148

It is evident from the Table 4 total phenolic contents were more in the Balanagar and Mahaboobnagr variety whereas the Sharanpur and Akashan variety showing less Phenolics, TCC and GA contents. The findings are in correlate with the works of Nandhakumar, E., & Indumathi, P. (2013).

Selection of yeast strains.

About 21 different yeast strains were isolated from variety of fruits selected for the

Table-5 Selection of yeast strains from different sources.

S.No	Strain	Source
1	AYS1	Annona
2	AYS2	Annona
3	BYS1	Baker's yeast
4	BYS2	Baker's yeast
5	SBF5	Banana
6	SBF6	Banana
7	SBG8	Black grapes
8	SCF4	Cherry
9	SDP1	Date palm
10	SGF7	Grapes
11	NCL-3473	NCL, Pune
12	NCL-3559	NCL, Pune
13	SPF2	Papaya fruit
14	SPF3	Papaya fruit
15	SCM11	Soil (coal mine soil)
16	SCM12	Soil (coal mine soil)
17	SGS13	Soil (Garden soil)
18	SSS9	Soil (sewage)
19	SSS10	Soil (sewage)
20	TSS1	Toddy
21	TSS2	Toddy

Perusal of the Table-5 reveals that in the present investigation twenty one strains of yeasts were isolated from different natural habitats like fruits, soil and toddy and purified.

Must preparation and Clarification of juice by using and enzymatic hydrolysis by pectinases.

After must preparation the juice was clarified by treating with commercially available pectinase and the effect of incubation time and concentration of the pectinase was observed and the results were presented in the Table-6, It is noticed that 0.08 to 0.10 % of pectinase with 60 minutes of incubation was found to be optimum for clarification of the juice. The above investigations are in accordance with the findings of Ceci, L., & Lozano, J. (1998).

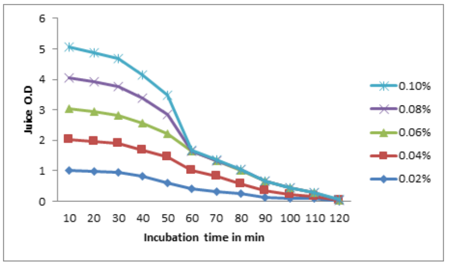

Fig.1: Effect of pectinase concentration and incubation time on pulp liquefaction.

Estimation of total sugars present in the clarified juice.

Table-7 Total sugars present, utilized by the selected strains in the clarified juice.

		Sugar utilization (in %)			
				Alcohol(v/v)	
S.No.	Strain	Residual	Utilized	Control	Pectinase
1	SDP1	32.9	67.1	5.1	17.3
2	SPF2	44.6	55.4	5	15
3	SPF3	16.4	84	5	23
4	SCF4	15	85	5	16.2
5	SBF5	16	86	6	26.1
6	SBF6	15	85	2.2	6.1
7	SGF7	17	83	5	25.5
8	SBG8	29	72	5	18.2
9	SSS9	14	86	8	24.6
10	SSS10	37	63	8	24.6
11	SCM11	32	68	5	13.8
12	SCM12	40	60	5	18.6
13	SGS13	37	63	6	15.2
14	TSS1	16	84	7	27.2
15	TSS2	24	76	5	29.5
16	AYS1	5	95	5	35.6
17	AYS2	6	94	6	35.3
18	BYS1	27	73	7	15.5
19	BYS2	16	84	8	13.4
20	NCL-3473	9	91	8	30.3
21	NCL-3559	6.5	93.5	8	31.6

After must clarification the juice was inoculated with 21 yeast stains to determine the sugar utilization and alcohol production capability and results were presented in the Table-7. It is observed that AYS1, AYS2, NCL-3473 and NCL 3559 strains were utilized more than 90% of the sugars with less than 10 % of residual sugars and >30 g/100ml of alcohol production. Hence, these four strains were selected for further studies. The above investigations are in collaborate with the Sahu, et al., 2012.

Investigations on four strains in batch fermentation was carried out and the influence of different parameters like Pulp Concentration, pH, temperature, yeast extract, peptone, Inoculum % on growth and ethanol production was carried out.

Table-8: Effect of different concentrations of Annona pulp on the growth and alcohol production by selected strains.

		Pulp Concentration (in %)									
		0.5		1		1.25		1.5		2	
S.No.	Strain	O.D	AL	O.D	AL	O.D	AL	O.D	AL	O.D	AL
1.	AYS1	0.04	23.5	0.24	28.2	0.47	33.6	0.42	35.2	0.87	32.4
2.	AYS2	0.1	21.5	0.26	28.6	0.22	33.6	0.31	35.6	0.39	32.3
3.	NCL-3473	0.11	22.5	0.27	28.2	0.58	30.5	0.94	32.1	1.08	25.2
4.	NCL-3559	0.14	23.8	0.18	28.2	0.27	30.2	0.64	33.2	1.22	23.2

Fig.2. Effect of pulp on growth and production of alcohol by selected yeast strains.

An attempt was made to optimize the pulp concentration for optimum growth and maximum ethanol production was carried out. It is noticeable from the Table-8, that about 2.0 % of pulp was found to promote more growth, whereas, more quantity of alcohol was obtained at 1.25 % of the pulp concentration. Further increase in the pulp concentration did not result in the improved alcohol production. Hence, 1.25% of pulp is found to be optimum.

Analysis of wine characteristics

Table-15: Analysis of wine characteristics and testing the consumer acceptability.

Strain	Total acidity	Volatite avdity	Total phenols	Opacty	Agreeable taste rank	pH
AYS1	4.22	0.39	4.60	0.11	5.00	4.5
AYS2	4.20	0.38	4.80	0.23	5.00	4.3
NCL-3473	5.17	0.36	5.70	0.61	3.00	3.5
NCL-3559	5.19	0.38	5.80	0.56	2.00	3.5

The reflections made from the Table-15 confirm that the wine produced by four strains varied. It was very interesting to note that AYS1 and AYS2 strains produced clear wine with good agreeable taste. The opacity was found to be 0.11 and 0.23 respectively. At the same time the total acidity was also very less as compared to the reference strains. Whereas, the pH of the wines produced by the AYS1 and AYS2 were slightly less acidic as compared to the NCL reference strains. The volatile acidity was not significantly different for all the strains. It very interesting to quote that the total phenol content of wines produced by the AYS1 and AYS2 is very less as compared to NCL reference strains. The findings are correlated with the Kumar, et al., (2011).

CONCLUSIONS

Annonasquamosa contains huge of amounts of fermentable sugars can be easily converted into valuable wine by using fermentations techniques. For effective fermentation pulp liquefaction with pectinase is a worthy and sustainable approach. The wine consists of good aroma and low amounts of phenolic compounds with good taste and agreeability. So, it will be very safe to drink and promote.

Hence, we conclude that the *Annonasquamosa* fruit pulp can be used as the cheap source of wine and alcohol production.

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