

Studies on Preparation of Custard Apple Vinegar

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Abstract— Custard apple (*Annona squamosa*) is highly susceptible to spoilage, softens very rapidly during ripening, and becomes squashy and not easy to consume fresh. The custard apple has nearly 24% sugars; it was hypothesized that this can be further processed by fermentation into a valuable product such as vinegar. The present study was carried out to develop vinegar from custard apple and it was compared with market vinegars sample for sensory evaluation. The physiochemical properties of custard apple vinegar were analysed. The custard apple vinegar production process took 30 days and had physiochemical characteristics of 1.019 gm/ml specific gravity, 1 % alcohol content, 5.39% (v/v) acetic acid, 2.0°Brix, and pH of 2.8 which complied with the standard ranges of brewed vinegar after complete fermentation. The sensory evaluation of vinegar samples was done by ten panel members. The overall acceptability of the market vinegar samples were rated as like extremely on the nine point hedonic scale, while custard apple vinegar was rated as like very much on the nine point hedonic scale.

Keywords— acetic acid fermentation, Custard apple, custard apple vinegar, sensory evaluation.

I. INTRODUCTION

The custard apple (*Annona squamosa*) is one of the important dry land fruit grown in waste land on rain water cultivated throughout the country. A relatively less moist soil and temperate environment will yield the custard apple fruit with good nutritional constituent (Shaha, 1959). Custard apple is popularly known as Sitaphal is grown in 40,000 Ha and the production is 136000 MT in 2012-2013 in India. The fruit is not indigenous to India, but it first originated from Caribbean region but has spread across the central and South America as well as Africa and Asia. It is exported in large quantity from India to UAE, Saudi Arabia, Bangladesh and Kuwait (Chadha, 1995). Fruits have an edible, soft, granular, juicy and sugary pulp with mild flavor and with slight acidity (Butani, 1976). Fruits are considered for their medicinal value besides their general use in juice, milk shakes and

soft drink (Luciana *et al.*, 2010) ice cream, confectionery and certain milk products (Broughton, 1979).

Custard apple is considered as one of the delicious and nutritionally valuable fruit (Mariappan and Saxena, 1983). 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, 12.4-18.15% sugar, 0.26-0.65% acidity and with caloric value of 105K.Cal/100g (Nissen *et al.*, 1988; Popale *et al.*, 2012 and Sravanthi *et al.*, 2014). Custard apple is highly susceptible to spoilage, softens very rapidly during ripening, and becomes squashy and not easy to consume fresh (Okigbo and Obire, 2009; Pareek, *et al.*, 2011). Hence there is greater need to processing the ripe custard apple fruits in to suitable products to minimize the post-harvest losses. Storage of the fresh fruits of *A. squamosa*, has limitations, since it is perishable, and cold storage is not promising because of the development of an unattractive brown colour on the skin which decreases the market value (Purohit, 1995). Canning of the custard apple pulp is problematic because of the development of bitterness and browning on heating beyond 55°C (Bhatia, Sastry, Krishnamurthy, Nair, and Lal, 1961; Martinez, Medina, Fans, and Gill, 1988; Salunkhe and Desai, 1984). In addition, an unpleasant off-flavour develops in the pulp when heated beyond 65 °C (Nanjunda Swamy & Mahadevaiah, 1993). Vinegar, from the French *vin aigre*, meaning “sour wine,” can be made from almost any fermentable carbohydrate source, including wine, molasses, dates, sorghum, apples, pears, grapes, berries, melons, coconut, honey, beer, maple syrup, potatoes, beets, malt, grains, and whey. Initially, yeasts ferment the natural food sugars to alcohol (Naseem Ullah *et al.*, 2014). This paper reports an attempt to utilize custard apple for the production of vinegar. Vinegar is produced by fermentation of alcohols to acetic acid by bacteria (Anchanarach *et al.*, 2010). The acetic acid bacteria break down the sugars or starch in the substrate converting it to alcohol and then further to acetic acid by different enzymes (Anchanarach *et al.*, 2010). The vinegar can then be used in dressing salads, manufacture of useful

medicines, preservation of food stuffs, provision of antioxidants or as an antibacterial agent (Johnston *et al.*, 2004; Shizuma *et al.*, 2011; Soltan and Shehata, 2012). Vinegar is commonly obtained from good wine, cider, fruits and starchy foods (Silva and Swarnakar, 2007; Krusong and Assanee, 2010). These undergo fermentation by acetic acid bacteria during the process of secondary fermentation.

Since the custard apple has nearly 24% sugars, it was hypothesized that this can be further processed by fermentation into a valuable product such as vinegar. The production of vinegar from the custard apple can be of great value to the country both economically (by increasing the economic value of custard apple, providing locally made vinegar on the market, creating jobs and reducing seasonal losses of the fruits) and will also provide an avenue to utilize the underutilized or culinary fruit.

II. MATERIALS AND METHODS

Custard apples (*Annona squamosa*) fruits were procured in bulk from the local market. Chemicals used in experimentation and analysis were of analytical grade, purchased from standard Indian companies. Media and chemicals used for microbial analysis were also from standard companies.

2.1 Extraction of pulp

Fully ripened fruits were selected and the pulp was extracted manually under hygienic conditions. The seeds and pulp were separated from each other by rubbing the mixture on a 30 mesh sieve leaving the seeds and the covering sheath of the capillary pulp (Seema *et. al.*, 2008 and Mysore *et. al.*, 2008).

2.2 Preparation of custard apple must

The physicochemical properties of the extract were measured and recorded in Table 1. The brix of the extract was adjusted to 22°Brix and ammonium sulphate (111ppm) added to stabilize the must and to provide a nitrogen source for yeast. The pH of the must was adjusted to 4 by adding 120 ml of vinegar. The treated must was pasteurized at 63°C for 30 minutes and then allowed to cool to room temperature of 25°C.

2.3 Preparation of the yeast starter culture

A small amount of must (20 ml) was inoculated with viable wine yeast (*Saccharomyces cerevisiae*) at a rate of 0.3g/L and left to incubate in a water bath for approximately 20 minutes.

2.4 Must inoculation for alcoholic fermentation

Approximately 3.8 liters of standardized must was poured into a 10 liter sterile plastic jerry-can and inoculated with 20 ml of the yeast starter culture. The jerry-can was tight fitted with an air lock filled with distilled water. The inoculated must was subjected to primary fermentation at ambient temperature for 7 days to produce custard apple

wine, which was then filtered using a sterile folded muslin-cheese cloth after complete primary fermentation. After alcoholic fermentation alcohol content must checked only 0 – 1 % TSS must remain. Fermentation was started for sedimentation and strained through cloth and clarified supernatant is taken in bottle up to ¾ capacities.

2.5 Must preparation and Acetic acid fermentation

The wine obtained after the alcoholic fermentation contained 11.50% (v/v) alcohol. It was filtered and alcohol wort added for vinegar production. The addition of alcohol wort for vinegar production was conducted by taking out 4 L of unpasteurized vinegar with about 6% (w/v) acidity and adding 2.8 L of alcohol wort with 11.50% (v/v) alcohol content. For vinegar fermentation the alcohol content of the fermented liquor was adjusted to 7 – 8 % by diluting with water. (Byarugaba-Bazirake *et al.*, 2014)

2.6 Acetic acid fermentation

The acetic fermentation was conducted by seeding the wine obtained at alcoholic fermentation with acetic acid bacteria @ 10 % v/v stirred and the bottle mouth was closed by cork with the two holes in it for proper aeration as *Acetobacter aceti* is aerobic and their activity is greatly reduced by light so kept in dark.

2.7 Aging

As the vinegar prepared was turbid and does not possess a good taste. It was stored in container during which the vinegar develops a good aroma and flavour and becomes mellows.

2.8 Pasteurization

It was poured in previously sterilized bottles, corked air tight and the bottles were heated in hot water at 71 to 77°C for 15 – 20 min so that growth of vinegar bacteria was stopped and the strength of vinegar was maintained during storage.

2.9 Routine analyses

A sample of vinegar was analysed every two days for the ethanol content, pH, and total acidity. Monitoring of the per cent alcohol production (%v/v) was done by gravimetric Analysis, Percent acetic acid production (w/w) was done by neutralizing samples at pH 7.2 with 0.1N NaOH; it was assumed that all medium acidities were due to acetic acid, pH was estimated using pH meter; (Hanna) and total soluble solids as degrees Brix using RHB-32 (ATC) refractometer; ATAGO) (Ranganna, 1986)acquired over a period of 30 days at room temperature.

2.10 Sensory evaluation of vinegar

The quality attributes like taste, flavour, color and overall acceptability were judged by panel members on the basis of nine point's hedonic scale. A single portion of vinegar preparation was served in a glass bowl. The judges were

instructed to fill the nine point hedonic score card for each vinegar.

III. RESULTS AND DISCUSSION

Table 1 depicted the physiochemical properties of the custard apple extract, and was found that the total sugars

were quite high as 22.5 %, TSS 28 °Bx and acidity 0.45%. The results were compared to those obtained by Sravanthi *et. al.*, (2014), i.e. 28 °Bx and 21.42% TSS, total sugars, respectively and similar results were also reported by Kolekar and Tagad, 2012.

Table.1: Physiochemical Properties of Custard Apple Extract

Sr. No	Parameters	Content
1.	Edible portion(g)	45
2.	Moisture (%)	70
3.	Total sugars (%)	22.5
4.	Protein (%)	1.5
5.	Fat (%)	0.3
6.	Minerals (%)	0.9
7.	Acidity (%)	0.45
8.	TSS(°Bx)	28
9.	Vitamins(mg)	37
10.	Ascorbic acid (mg/ 100g)	9.20

Primary fermentation was carried out at room temperature (25°C). The process undergoes to form various intermediate products through glycolysis in anaerobic condition. This resulted in a light brown colored custard apple wine with an alcohol content of 11.50% (v/v).The custard apple wine obtained (Table 2) was then subjected

to a two-step fermentation system using a batch process. This involved enzymatic oxidation where the ethanol substrate was first oxidized to acetaldehyde and subsequently oxidized to the final product, acetic acid. This process was carried out over a period of 30 days.

Table.2: Physiochemical properties of wine and vinegar*

Sr. No	Parameters	Content	
		Wine	Vinegar
1.	TSS (°Bx)	6±1.00	2±0.00
2.	Alcohol content (%)	11.50	1.0
3.	Specific gravity (gm/ml)	0.984±0.00	1.019±0.001
4.	pH	3.5±0.00	2.8±0.01
5.	Total acidity (%)	0.16±0.001	5.39±0.00

* Values are means ±SD of triplicate determinations.

Before the initial mixing of the inoculum and fresh wine a very short lag phase with no significant acid production was observed. This may be due to the sudden change in the medium conditions at the initial mixing that affected fermentative microorganisms. According to Brock and Madigan (1991), the observed microorganisms response can be explained as an adaptation phase in which the required enzymes for substrate degradation are synthesized. During the lag phase, acetic acid bacteria use the main proportion of their energy resources in this synthesis. It is therefore not surprising that no net production of acetic acid was produced.

The decrease in alcohol concentration was corresponded to the gradual rise in acetic acid concentration (T.T.A)

which accumulated from 11.50 to 1.0% (v/v) over a fermentation progress period of 30 days. The ethanol content in garlic vinegar was nil and onion vinegar have 2g/l (Horiuchi *et al.*, 1999). Alcohol induces stress in yeast cells causing their death and flocculation, but the stress of yeast is more related to acetaldehyde which is the first intermediate product of ethanol biological oxidation by *Acetobacter aceti*. This acetaldehyde disrupts the enzymatic activity of yeast. The beginning of acetic acid formation is related to maximum cellular growth and sufficient biomass density to start the acetification process (Seyram *et al.*, 2009).

The pH of the vinegar during the secondary fermentation was recorded to decrease slightly from pH 3.5 to pH 2.8.

The result obtained was similar to recommendations of Sassou *et al.*, 2009 who recommended that pineapple vinegar has a pH 2.8. This slight initial increase in acidity provided optimal growth conditions to initiate acetification. This fall in pH can be accredited to accumulation of acetic acid and other volatile short chain organic acids such as propionic, tartaric and butyric acids, which are important in development of the flavor and aroma of vinegar (Seyram *et al.*, 2009).

The alcohol content continued to decrease with time from 11.50% to about 1% by the 30th day. This deduces that the alcohol conversion to acetic acid reaches one when acetic acid reaches to the maximum in the medium. The vinegar produced from the custard apple extract contained 5.39% (v/v) acetic acid and was comparable with 6.33% (v/v) and 6.11% (v/v) vinegar obtained by Torija *et al.*, (2010) in their study of two vinegar plants; Laguinnelle (B,Banyuls,France) and Viticultors Masd'en gil(P,bellmunt del priorat, Tarragona,Spain).

There was also a significant amount of sugar recorded (2.0 °Brix) in the custard apple wine vinegar by the end of the fermentation. This denotes that there is better utilization of sugar in the production of custard apple wine vinegar than custard apple wine (see Table 2). Therefore, the presence of fermentable sugars in custard apple can make them ideal substrates for alcoholic fermentation of fruit juice and subsequent secondary fermentation into vinegar.

Table 3 describes the sensory evaluation of custard apple vinegar. The sensory evaluation plays an important role in the quality of food. The overall acceptability of custard apple vinegar was 8.5 score, which were comparable to other market vinegar samples. The data obtained statistically depicts that the quality of the custard apple vinegar is very well comparable with market vinegars in terms of colour, flavour, taste and overall acceptability. It shows the market viability of the product to make it commercial.

Table.3: Sensory Evaluation of Custard Apple Vinegar*

Name of vinegar	Colour	Flavour	Taste	Overall acceptability
Custard apple vinegar	8.2±0.13	8.4±0.33	8.2±0.29	8.5±0.22
Market vinegar 1	8.1±0.11	7.8±0.22	8.8±0.29	8.8±0.21
Market vinegar 2	8.3±0.20	8.5±0.24	8.4±0.30	8.9±0.24
Market vinegar 3	8.4±0.25	8.8±0.26	8.5±0.24	8.7±0.23

* Values are means of ±SD of ten panel members

The overall acceptability of the market vinegar samples were rated as like extremely on the nine point hedonic scale, while custard apple vinegar was rated as like very much on the nine point hedonic scale. Though market vinegar 2 scored higher score (8.9±0.24) in overall acceptability on a total score of 9 than custard apple vinegar (8.5±0.22). The brownish colour of the custard apple vinegar is due to the enzymatic browning during pulping.

IV. CONCLUSION

The custard apple wine vinegar production process took 30 days and had physiochemical characteristics of 5.39% (v/v) acetic acid, 2.0°Brix, and pH of 2.8 which conformed with the standard ranges of brewed vinegar after complete fermentation. The aroma of the vinegar produced was appreciated by the consumers who were acquainted with vinegar. This study therefore, showed that custard apple can be used as an ideal substrate for production good quality vinegar. This not only increases the economical and food value of custard apple but also provides a way of utilizing custard apple in India.

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