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Formulation and analysis of fermented soda from *Averrhoa* carambola with ginger bug as a fermenting agent

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Abstract
Averrhoa carambola is an underutilized fruit in food processing industry owing to its high moisture content. The research aimed to utilize the underutilized fruit and formulate and analyse a fermented soda from Averrhoa carambola with ginger bug as a fermenting agent. Ginger bug is a slurry of ginger, sugar and water that is fermented for several days until ready to use. Three samples were prepared: the standard with no addition of ginger bug, variation 1 and variation 2 with the addition of ginger bug but with higher concentration of sugar in variation 2(200gms/litre) compared to variation 1(100gms/litre). The variations were allowed to ferment and were tested for it's physicochemical properties such as titratable acidity, pH, total phenols, brix, Vitamin C, alcohol percentage and colour measurements. Sensory and statistical analysis was also performed. TA increased and pH decreased with fermentation. Phenols were released on fermentation and Brix increased with sugar concentration. Fermentation did not impact Vitamin C levels. 4.5% alcohol was found in variation 1 and 5.5% in variation 2.Correlation coefficient 1 suggested a perfect relationship between sugar concentration and alcohol formation. Sensory analysis revealed variation 2 as the most acceptable sample.The research formulation has shown that acceptable fermented soda can be prepared from Averrhoa carambola. The fermentation has also enhanced the quality and preserved the characteristics of the fruit.

Keywords: averrhoa carambola, ginger bug, lacto-fermentation, brix, underutilized fruit, probiotic, lactic acid

Introduction

An 'Oxalidaceae family' member- *Averrhoa carambola* which is also known popularly as star fruit, is a golden-yellow to green coloured fruit (Kumar Hitesh *et al*., 2016) [2]. Carambola is available in two varieties, the unripe green is more sour and the ripe yellow fruit is more sweeter. The carambola fruit is an economically important commodity. Most carambola fruits are marketed in processed forms. It is edible and has numerous uses. The star fruit tree is used as a traditional medicine. It has many medicinal properties which include anti-inflammatory, analgesic, hypotensive, anthelmintic, anti-oxidant, anti-ulcer, hypocholesterolemic and hypolipidemic, antimicrobial, anti-tumor activities, but owing to its high moisture content it is underutilized. The ripe fruit may be processed into fermented or unfermented drinks, preserves, jams or jelly (Napahde S *et al*., 2010) [6].

Ginger bug is a product of wild fermentation, it is used as a starter culture for producing fruit sodas or ginger beer. It is a mixture of grated ginger, sugar and water that is allowed to be fermented over a period of days or until it becomes bubbly or foamy. Brewers utilise the bug to produce probiotic tonics and drinks like root beer, ginger beer or probiotic lemonade. The wild bacteria native to the skins of the ginger and in the environment consume the nutrients present, they proliferate and grow. They consume the added sugar and produce carbon dioxide as a result. When the ginger bug is added to the fruit juices or tea, it consumes the sugars added and evolves carbon dioxide thus producing a mild fizz to the drinks. The ginger bug are rich in probiotics produced by lacto fermentaion (Mcgruther Jenny, 2019) [13].

Fermented foods can be considered as a highly nutritious and digestible food. Fermentation process involves pre-digestion of foods, this makes the nutrients more bioavailable, and in many cases fermentation generates more nutrients or removes anti-nutrients or toxins. Ferments with live lactic-acid-producing bacteria intact have a positive impact on the digestive health, immune function, and general well-being. Wild fermentation is the type of fermentation that relies on the naturally occurring yeast and bacteria to ferment food. No particular strain is selected and added. A wild ferment is one that occurs spontaneously. It usually takes longer time, as the lactobacillus takes some time to come to life. This usually works with almost any fruit and vegetable as it will not rot before there's enough bacteria living to transform it. This fermentation occurs with the lactobacillus bacteria and hence can be considered as lacto-fermentation.

General Objectives

- To utilize the underutilized fruit *Averrhoa carambola.*
- To formulate a slightly alcoholic and probiotic rich fermented soda using ginger bug.

Specific objeectives

- To develop the fermented *Averrhoa carambola* soda as a probiotic drink using ginger bug.
- To analyze the physical and chemical parameters of the formulated soda
- To study the rate of alcohol formation in relation to the percentage of sugar addition.

- To evaluate the sensory acceptability of the formulated fermented soda.

Materials and Methods

Raw Material Procurement

The raw materials such as Ginger root, *Averrhoa carambola* and Sugar were procured from the local markets in Chennai, India.

Preparation of Ginger BUG

The skin was removed from the roots and washed in clean water. The ginger was chopped to small cubes. Sugar, ginger and water was mixed and the mixture was stored in clean, dry glassware. The bug mixture was fed everyday upto 7 days with 15 gms of ginger and 15gms of sugar. The ferment gets activated when it turns bubbly and is ready to use.

Table 1: Composition of ginger bug

Composition	Ginger BUG
Water	500ml
Sugar	30gms(+15 gms everyday upto 7 days)
Ginger root(chopped)	30gms(+15gms everyday upto 7 days)

Preparation of Avverhoa Carambola Juice

The fruit was washed in clean water and the edges and ridges were trimmed off. The fruits were then chopped to smaller pieces and blended in blender without addition of water. The blended pulp was then strained to obtain the concentrated juice.

Preparation of Samples

150 ml of the concentrated juice was diluted in 1000ml of water to give a 15% strength carambola juice. 100 gm of sugar was added to the juice and was used as standard for the tests.

Two samples were prepared using the standard juice, sugar and ginger bug, the samples varied in their composition of sugars added.

The ginger bug added samples were stored in clean dry plastic bottles to avoid bursting due to pressure. The juice was allowed to ferment for two days. It was again filtered and stored in another clean and dry plastic bottles with tight closure and were stored in refrigerator and tested further.

Table: 2 Composition of the samples

Composition	Standard	Variation 1	Variation 2
Carambola juice	150ml,concentrated juice in 1000ml water	Standard juice	Standard juice
Sugar	100gm	100gm	200gm
Ginger bug	-	100ml	100ml

Physicochemical Analysis

Titratable Acidity

The samples were analysed in triplicates. An aliquot of the sample was taken and titrated with 0.1N NaOH using few drops of 1% phenolphthalein solution as an indicator. The titre value were noted and the results were calculated as percent anhydrous citric acid by the following formula.

$$\% \text{ Total Acid} = \frac{\text{Titre} \times \text{normality of alkali} \times \text{vol made up} \times \text{equivalent weight of acid} \times 100}{\text{Volume of sample taken for estimation} \times \text{wt or volume of sample taken} \times 1000}.$$

pH

The pH meter was calibrated and standardized before it was used for measuring the pH of food samples. Calibration involves measuring the pH of commercially available calibration buffers at pH 4, 7 and 9 respectively and adjusting the pH if necessary. The pH electrode was rinsed with de-ionized water and was blotted using soft tissue paper. The electrode was then placed in the sample. The display was allowed to stabilize and the readings were noted. It was repeated for all the samples and the readings were noted.

Total Phenols

The total phenols of the samples were analysed by Folin-ciocalteau index method published in Compendium of international methods of analysis – OIV. 1 ml of the sample were taken in 100 ml volumetric flask. 50 mL of distilled water, 5 mL of Folin-Ciocalteu reagent and 20 mL of sodium carbonate solution were added to the sample in order. The volume was made up to 100ml with distilled water and mixed to dissolve. The mixture was left for 30minutes for the reaction to stabilize and the absorbance was determined at 750nm through a path length of 1 cm with respect to a blank prepared with distilled water in place of the samples. The results were expressed in the form of an index obtained by multiplying the absorbance by 20.

BRIX

The degree brix was measured using the refractometer. The glass prism on the meter was cleaned using distilled water and soft clean cloth was used to wick the water off. A few drops of the sample was placed on the glass measuring surface and was covered, any air trapped was released by gently pressing down the cover. The brix value was measured from the readings displayed through the eyepiece while holding the refractometer up to a natural light.

Vitamin C

Vitamin C of the samples were analysed by iodine titration method. The iodine solution and starch indicator and Vitamin C standard solution were prepared and kept aside. 25ml of Vitamin C standard solution was taken in a Erlenmeyer flask and 10 ml of 1% starch indicator was added to it. The iodine solution was taken in the burette from which the standard vitamin C solution was titrated to the endpoint. The titration was repeated few more times to get accurate value. The titration was repeated for the samples and the titre value was noted. The Vitamin C value of 100ml of the sample was then calculated from the titre value.

Alcohol Percentage

The alcohol percentage of the sample was tested using International standard method. The alcohol content was tested after distillation of the samples and using pyknometer for readings.

Colour Measurements

Spectrophotometric measurements were made using a 1-mm cell path length at A420, A520, A620 nm. This method was suggested by Yildirim H.K, (2006) [10]. Colour density (CD) was calculated as the sum of A420 and A520 nm.
(CD) Colour density = (A420+ A520)
Tint (T) was calculated as the ratio of A420 to A520 nm.
(T) Tint value = (A420/ A520)
Colour intensity (CI') was determined as the sum of A420, A520 and A620 nm.
(CI) Colour intensity = (A420+ A520+ A620)

Sensory Analysis

The samples were evaluated by a panel of 27 semi-trained consumer panelist. The samples were placed in a clean and dry glassware suitable for tasting the product. The samples were coded. The panelist were asked to rate the samples on the basis of colour, taste, flavor, Odour, mouth feel, after taste and overall acceptability using a 9 point hedonic scale. The judges were asked to rinse their mouth with water in between the testing of the samples. The panelist were familiar with the attributes of a fermented beverage.

Results and Discussion

The results for titratable acidity showed that both the variations had 0.32% TA whereas for standard the TA was 0.16%. Through statistical analysis it was found that there were significant difference between the standard and the variations ($p < 0.05$). The results were similar to the results reported by Garg Neeelima, (2019), where wine prepared from red mulberry variety MI-497 had 0.34 per cent acidity.
It is obvious from the results that fermentation has affected the titratable acidity. After fermentation, the titratable acidity has increased from 0.16%- 0.32%. Ogodo A *et al*., (2018) [7] also reported results that showed an increase in titratable acidity on fermentation, they produced and evaluated fruit wine from *Mangifera indica* (Mango) and the titratable acidity increased from 0.21% to 0.63% during fermentation.
The results showed a clear decrease in the pH on fermentation. The variation 2 containing more sugar concentration, showed the lowest pH value of 3.16±0.0058, and the variation 1 with lesser concentration of sugar showed pH 3.21±0.0058, the standard which did not undergo any fermentation showed a pH of 3.326±0.0058. The acidity of the samples has increased with fermentation. There were significant difference between the standard and variations ($p<0.05$). The pH value were within acceptable range and similar range of value were reported in several other similar studies. Qi Ningli *et al*., (2017) [8] reported pH 3.52 in pineapple wine, where the pH decreased from 3.81to 3.52 upon fermentation. Williams G *et al*., (2016) [9] reported pH values that ranged between 3.45 – 3.55 in fermented beverage from plums.
The total phenolic content was found to be 314±5.29 mg/l GAE for standard and for the variation1 and variation 2, it was 449.33±3.05 mg/l GAE and 470.66±3.05 mg/l GAE respectively. The results revealed that fermentation has enabled the release of phenolic compounds that has resulted in an increase in the total phenols concentration in the fermented samples. There is an increase in the concentration of phenols between the standard and the variations and there is significant difference ($p<0.05$) between the stanadard and the variations. Similar results were observed by Marković M *et al*., (2015) [5] where a range of phenol concentrations (mean value of 425.23 mgL-1 GAE) in selected wine were reported. Similarly, 449 mg/l GAE was reported as an average value for apple wines by Ljevar A *et al*., (2016) [4].
Changes in degree brix was not observed in the variation 1 sample containing 100gms of sugar, on fermentation. The standard and variation 1 had 8^{O} Brix, the variation 2 containing 200gms showed 12^{O} Brix value. The standard and variations were significantly different ($p<0.05$). The rise in sugar concentration has resulted in higher brix values. The results are comparable to results revealed by Williams G *et al*., (2016) [9] where the total soluble solids (TSS), measured in ° Brix, ranged from 8.30 to 8.95.
Fermentation did not have any effect on the Vitamin C content of the samples. All three samples showed the same value. The standard, variation 1 and variation 2 showed 72.25±0.0058mg/dl of Vitamin.C. Clearly there weren't any significant differences ($p>0.05$). The result also revealed that the samples were rich in Vitamin C content.
The standard was a non-fermented product hence there wasn't any alcohol detected. The variation 1 containing 100gms of sugar had 4.5% alcohol and the variation 2 containing 200 Gms of sugar had 5.5% alcohol. The variations were significantly different ($p<0.05$). From the results it was revealed that the product was low alcoholic and there was an increase in the percentage of alcohol with an increase in sugar concentration. The ginger bug has consumed more sugar present in variation 2 and has emitted higher percentage of alcohol compared to variation 1. The relation between sugar concentration and alcohol percentage was statistically analysed by correlation coefficient. The results showed correlation coefficient 1, the value 1 clearly indicates that there is a correlation between the sugar concentration and alcohol formation. A similar phenomenon was observed in jackfruit wine, studied by Kumoro A *et al*., (2012) [3], it was concluded that ethanol concentration increased with an increase in initial sugar concentration.

Table: 3 Physico chemical analysis of the samples.

Parameter	Standard	Variation 1	Variation 2
Titratable acidity (%)	0.16	0.32	0.32
Ph	3.326±0.0058	3.21±0.0058	3.16±0.0058
Total phenols (mg/l GAE)	314±5.29	449.33±3.05	470.66±3.05
Brix (degrees)	8^{O}	8^{O}	12^{O}
Vitamin C (mg/dl)	72.25	72.25	72.25
Alcohol Percentage (%)	0	4.5%	5.5%

The standard which did not undergo any fermentation had a slightly darker colour in appearance and had the highest colour density, colour intensity and tint value (1.7, 2.41, 1.42:1.98). The darker colour of the sample could be the result of enzymatic browning caused in the star fruit juice. The variation 1 showed least absorbance and least colour density, colour intensity and tint value (0.92, 1.56, 1.26:1.78). The variation 2 showed values for colour density, colour intensity and tint value (1.57, 2.24, 1.3:1.84) slightly higher than variation 1 but lower than the standard. The increase in the colour intensity and density in variation 2 could be a result of higher rate of fermentation. An increase in colour in fermented chalagopali fruit beverage, as a result of fermentation was observed by Flores-García A *et al*. (2019) [1], it was observed that values increased during fermentation, which indicates the samples are darker. From the results, it can be said that fermentation has inactivated the enzymes present in the starfruit, and thus have prevented enzymatic browning in the samples.

Table 4: Colour density, Colour intensity and Tint value

Sample	Colour Density	Colour Intensity	Tint Value (Ratio)
Standard	1.7	2.41	1.42:1.98
Variation 1	0.92	1.56	1.26:1.78
Variation 2	1.57	2.24	1.3:1.84

It was observed that the variation 2 that was subjected to fermentation with 200gms added sugar, obtained a higher acceptance value in color, taste, flavor, odour, mouthfeel, aftertaste and overall acceptability parameters. The fermented drink with 100gms sugar received the second highest scores in all the parameters and the standard which is the non-fermented star fruit juice received the least scores. The colour and odour parameters of the three samples were significantly different ($p<0.05$).

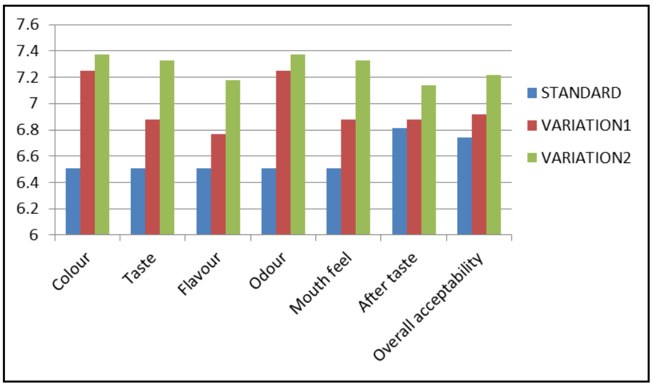

Fig 1: Sensory analysis

Conclusion

- The research formulation enabled the utilization of the underutilized star fruit. The fermentation has also enhanced the quality and preserved the characteristics of the fruit.
- It was reported by Qi Ningli *et al*., (2017) [8] that wine of low alcohol percentage has become a consumption trend, so the low-alcohol fermented soda from *Averrhoa carambola* is in line with modern consumer habits.
- The research revealed the possibility of producing a fermented drink from fruits using wild fermented products such as ginger bug.

Limitations

- Shelf life of the product has not been studied
- Microbial analysis was not performed

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