

# Study on Production and Quality Assessment of Custard Apple Wine Using Different Varieties and Fermentation Parameters

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## ABSTRACT

The present study was carried out to develop and evaluate wine from custard apple (*Annona squamosa*) using two different varieties, namely local variety (CA1) and hybrid variety (CA2), at a pulp-to-water ratio of 1:2. The fermentation process was conducted using *Saccharomyces cerevisiae* for a period of 8 days, followed by ageing and maturation. Physico-chemical parameters such as pH, titratable acidity, total soluble solids (TSS), specific gravity, and alcohol content were analysed during fermentation.

The results indicated a gradual decrease in pH from 6.4 to 3.9 (CA1) and 6.3 to 4.0 (CA2), confirming increased acidity during fermentation. Titratable acidity showed variations, with higher values observed in CA2 compared to CA1. TSS decreased from 22°Brix to 6.5°Brix (CA1) and 7°Brix (CA2), indicating utilization of sugars by yeast. Alcohol content increased progressively, reaching 8.5% in CA1 and 7.9% in CA2, demonstrating effective fermentation.

The study concludes that custard apple pulp is a suitable substrate for wine production, with the local variety (CA1) showing slightly better fermentation efficiency and alcohol yield than the hybrid variety.

**KEYWORDS:** Custard apple (*Annona squamosa*), Fruit wine, Fermentation, *Saccharomyces cerevisiae*, Physico-chemical analysis, pH, Titratable acidity, Total soluble solids (TSS), Alcohol content, Specific gravity, Local variety, Hybrid variety, Value addition, Functional beverage.

## 1. INTRODUCTION:

### 1.1 CUSTARD APPLE:

Custard apple (*Annona squamosa*), belonging to the family *Annonaceae*, is a tropical fruit widely cultivated in India and other subtropical regions. The fruit is commonly known as *sharifa*, *sitaphal*, or sugar apple and is valued for its pleasant flavour and nutritional richness. Several species of *Annona* exist, including *A. squamosa*, *A. cherimola*, and *A. reticulata*. The fruit possesses a waxy, light-green peel with an average thickness of approximately 0.5 cm [1].

Nutritionally, custard apple pulp is rich in carbohydrates, primarily glucose and fructose, contributing to its high energy value. It contains approximately 23.9 g of carbohydrates per 100 g of pulp, with moderate protein content (1.6 g/100 g) and low-fat content (0.18 g/100 g) [4,5]. The fruit is also a good source of dietary fiber (3.1–11 g/100 g), vitamins A and C, potassium, magnesium, calcium, iron, and antioxidants

[3,6]. During ripening, the starch present in the fruit is converted into sugars, enhancing sweetness and palatability. The calorific value of custard apple (300–450 kJ/100 g) is significantly higher than that of commonly consumed fruits such as apple and orange [3].

### 1.2 WINE:

Wine is a complex mixture, consisting of both organic and inorganic compounds, including esters, high alcohols, fixed acidity (malic, tartaric and citric acid), sugars, aldehydes, tannins, pectin, vitamins and minerals. It can be defined as an alcoholic beverage made from grape juice or other fruits through fermentation of must by wine yeast [4]. Most wines have a total acidity content ranging from 0.3 to 0.55% (as tartaric acid and acetic acid). The Wine can be classified as table wine, sparkling wine, fruit wine, fortified wine, dry wine or sweet wine [5]

Wine is an alcoholic beverage prepared with different fruit juices with appropriate processing and additions [6]. Wine is produced by fermentation of raw material with the help of yeast such as *Saccharomyces cerevisiae* [7]. During wine making process the yeast utilizes raw material and alcohol production takes place [8]. *Saccharomyces cerevisiae* dominates wine fermentation and is the species that has been commercialized for starter cultures.[9] Flavonoid composition of wine is related to wine sensory properties [10].

## 2. REVIEW OF LITERATURE

Bade, V. N. (2022) reported that studies on *Annona squamosa* have emphasized the importance of understanding its physical properties for efficient post-harvest handling, processing, and equipment design. Variations in fruit weight, size, pulp yield, and seed percentage significantly influence processing efficiency and value addition. Furthermore, increasing research interest has been observed in the utilization of custard apple pulp for developing value-added products such as fruit leather, while by-products like peels and seeds have been explored as economical adsorbents for wastewater treatment. These findings highlight the fruit's potential for both nutritional applications and sustainable industrial use.

Bala et al. (2017) investigated the nutraceutical properties of *Annona squamosa* and reported that the fruit possesses significant health-promoting compounds, including antioxidants, vitamins, and bioactive components. Their study emphasized the potential of custard apple as a functional food ingredient with promising applications in the development of nutraceutical and health-oriented products.

Amerine et al. (1968) conducted studies on wine preparation using different fruit substrates and explored the influence of additional plant materials such as betel leaves on fermentation characteristics. Their work involved the development of two types of wine, with and without betel leaf extract, and evaluated parameters such as acidity, pH, sugar content, and alcohol concentration. The study demonstrated that the incorporation of plant extracts can influence fermentation behaviour, as well as the sensory and stability attributes of the final product.

Amerine, M. A., and Singleton, V. L. (1968) provided a comprehensive and practical introduction to wine science, focusing on the fundamental principles of wine composition, fermentation, and quality evaluation. Their work aimed to present scientific knowledge in a simplified manner, correct common misconceptions, and promote a better understanding of wine production and consumption.

### OBJECTIVES OF THE STUDY

- To develop custard apple wine using two different varieties, namely local (CA1) and hybrid (CA2), at a standardized pulp-to-water ratio of 1:2.

- To evaluate the effect of these formulations on key physico-chemical parameters such as pH, titratable acidity, total soluble solids (TSS), and alcohol content during the fermentation process.

### 3. MATERIALS AND METHODOLOGY:

#### 3.1 Materials

Custard apple (*Annona squamosa*) fruits local variety (CA1), Hybrid variety (CA2) , yeast (*Saccharomyces cerevisiae*), sugar and Sodium benzoate were procured from a local market.

#### 3.2 Collection and Preparation of Samples:

Fully ripened custard apple fruits of two variations (CA1), (CA2) and yeast *Saccharomyces cerevisiae* collected and transported to the laboratory for further processing. Fruit was thoroughly washed with potable water to remove surface contaminants prior to peel separation.



Figure 1: Local variety (CA1)



Figure 2: Hybrid variety (CA2)

#### 3.3 Separation of Custard Apple Peel and Extraction of pulp:

Peel separation was carried out manually due to the soft nature of the pulp and the leathery texture of the peel for both the variations (CA1), (CA2). The washed fruits were gently split open, and the pulp along with seeds was scooped out. The peel, which was loosely attached to the pulp, was carefully separated and collected for starch extraction.



**Figure 3 and 4: Separation of Custard Apple Peel and Extraction of pulp**

### **3.4 Homogenization of pulp, adjustment of Total Soluble Solids and sterilization:**

The pulp was homogenized in a grinder for uniform consistency. The pulp obtained for both the variations (CA1), (CA2) were subjected to dilution in the ratio namely 1:2 using distilled water maintaining uniform consistency.

Total Soluble Solids (TSS) of pulp were maintained to the level 22 °Brix by addition of sugar that helps in growth of microorganisms that are favourable for fermentation.

After addition of sugar, the pH of the pulp was monitored and adjusted to the value of 0.3%. Add water to the pulp in known volume of custard apple was taken separately and Sodium benzoate was used as a preservative in a concentration of 300 ppm per litre of the product was added to avoid growth of wild yeast and Diammonium orthophosphate at a rate of 0.2% was added as a source of nitrogen (N<sub>2</sub>) and phosphorus to yeast. The pulp was subjected to sterilization to avoid contamination and spoilage.

### **3.5 Activation of Yeast:**

*Saccharomyces cerevisiae* of 2gm was dissolved in warm water for 5 minutes and mixed uniformly to avoid suspensions.

### **3.6 Inoculation of yeast:**

The activated growing culture of Yeast was inoculated in the sterilized juice for conversion of sugars into alcohol.

### **3.7 Fermentation:**

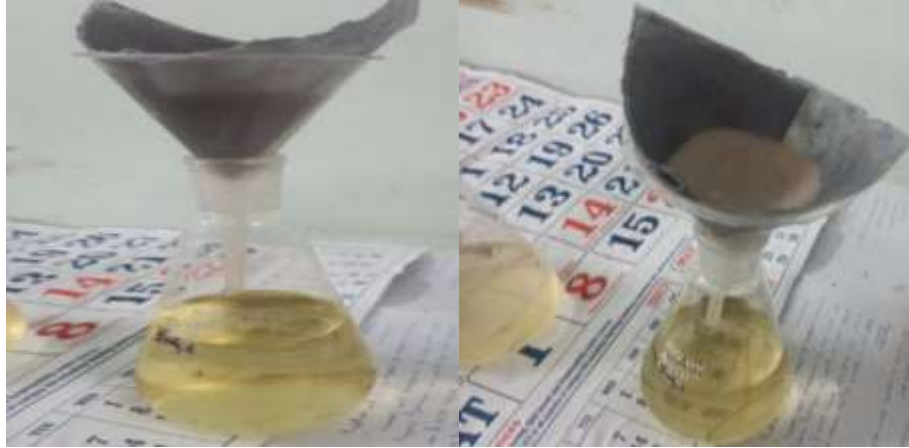
Fermentation: The formulated mixture was combined with the starter culture, and sodium benzoate was added to prevent contamination. The jars were sealed airtight and stored in darkness at room temperature for a period of 8 days, during which fermentation occurred. These jars were then set aside for aging. After a subsequent 60-day aging period, the jars were opened, and further analyses were conducted.



**Figure 5 & 6: Fermentation of local variety (CA1) and Hybrid variety (CA2)**

### 3.8 Filtration:

Following the 8-days fermentation period, the contents were filtered using a muslin cloth, with the solid residue being discarded and the supernatant collected in a separate jar.



**Figure 7 & 8: Filtration of wine of local variety (CA1) and Hybrid variety (CA2)**

### 3.9 Ageing:

Separation may not immediately occur (especially for red wines), since skins and stems are an important source of 'tannins' which affect wine's taste and maturity through ageing.



**Figure 9: Ageing of wine of local variety (CA1) and Hybrid variety (CA2)**

### 3.10 Maturation and Bottling:

The fermented wine is further stored for 6-8 months for maturation. Once the mature wine is ready, it is stabilized through cold treatment. After testing the stability of the wine, it is then filtered to screen the balance fine particles. The filtered wine is then packed in bottles, which are washed internally and externally with double filtered water to remove bacteria and germs.



**Figure 10 & 11 : Maturation and bottling of local variety (CA1) and Hybrid variety (CA2) of custard apple**

**3.11 Packaging and storage:** The product is traditionally packed in glass bottles with corks made from the bark of the cork oak (*Quercus suber*). The bottles should be kept out of direct sunlight. During storage, wines are prone to non-desirable microbial changes. Yeasts, lactic acid bacteria, acetic acid bacteria and fungi can all spoil or taint wines after the fermentation process is completed.

### 3.12 Testing Methods

#### 3.12.1 Physico chemical analysis:

##### 3.12.1.1 Test for pH:

The pH was determined directly during fermentation using a digital pH meter (model pHS -25) [11]

##### 3.12.1.2 Titrable acidity:

Acidity was estimated by the Titration method by the use of sodium hydroxide as titrant against the diluted fermented juice. Add phenolphthalein as an indicator in the fermented juice.

Total acidity = Volume of alkali used (ml) X Normality of alkali X 7.5 Weight of sample in gram

##### 3.12.1.3 Total Soluble Solids:

Total soluble solids (TSS) of the wine samples were determined using a digital refractometer. The instrument was calibrated with distilled water at  $20 \pm 1$  °C to obtain a zero reading. A few drops of the prepared sample were placed on the clean prism surface, and the °Brix value was recorded after stabilization. Measurements were carried out in triplicate, and results were expressed as °Brix (mean  $\pm$  standard deviation). Since ethanol present in fermented wine influences refractive index, appropriate correction factors were considered where necessary.

##### 3.12.1.4 Specific Gravity (S.G):

This was carried out using relative density bottle, where the bottle was wash with tap water and dried. The surface of bottle was further clean with ethanol and allowed it to be dried again. Using an electronic balance, the empty weight of the bottle was determined (M<sub>0</sub>). The weight of the bottle plus 5ml of the wine sample (M<sub>1</sub>) was determined, followed by the weight of the empty bottle plus 5ml of distilled water (M<sub>2</sub>), and the specific gravity then calculated from these values as follows:

Specific Gravity=  $\frac{\text{Weight of volume of sample}(M1-M0)}{\text{Weight of equal volume of water}(M2-M0)}$

**3.12.1.5 Alcohol Content:**

This was determined using the %Sugar/Specific gravity/Brix/PA equivalent table according to AOAC (2000).

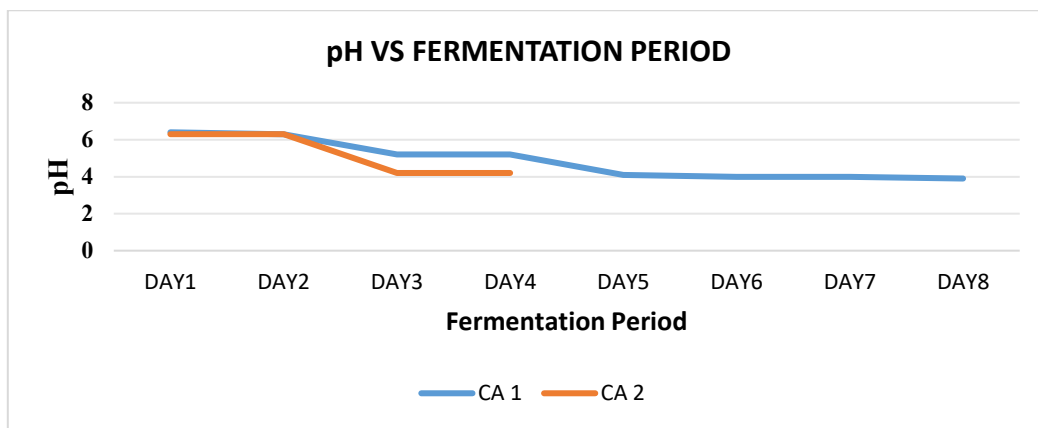
**4. RESULTS AND DISCUSSION:**

**4.1 pH:**

The variation in pH of custard apple wine formulations (CA1 and CA2) during the 8-day fermentation period is presented in Table 1.

**Table 1: pH values of custard apple wine (CA 1 & CA2 Variation)**

S. No	Formulation	Fermentation Time							
		Experimental values of pH							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
1	CA 1	6.4	6.3	5.2	5.2	4.1	4.0	4.0	3.9
2	CA2	6.3	6.3	4.2	4.2	4.1	4.1	4.0	4.0



**Graph 1: pH values of custard apple wine (CA 1 & CA2 Variation)**

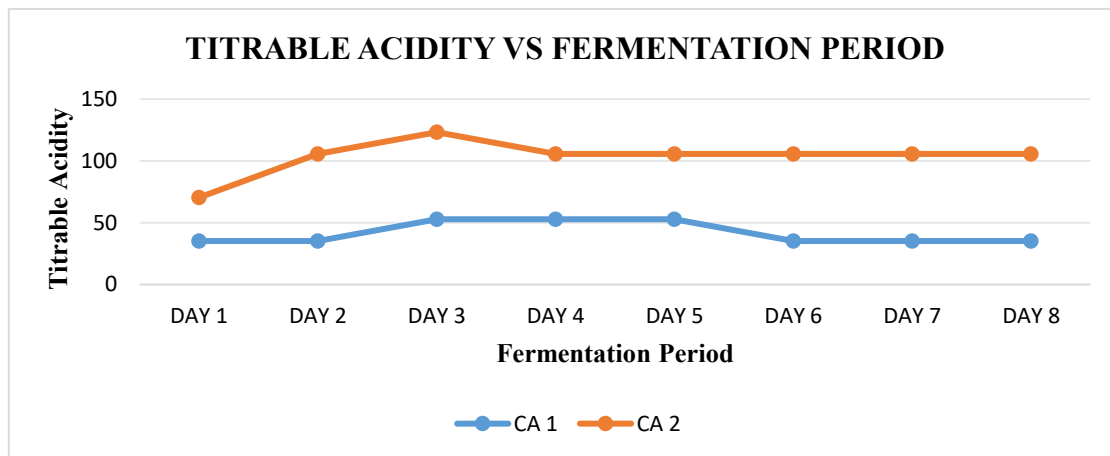
The pH values of custard apple wine formulations (CA1 and CA2) were monitored during the 8-day fermentation period, as shown in Table 1. Initially, both formulations exhibited relatively higher pH values (6.4 for CA1 and 6.3 for CA2 on Day 1), indicating a slightly less acidic nature. As fermentation progressed, the pH values gradually decreased in both formulations. In CA1, the pH decreased from 6.4 on Day 1 to 3.9 on Day 8, while in CA2 it decreased from 6.3 to 4.0 over the same period. The reduction in pH during fermentation is mainly due to the production of organic acids by fermentative microorganisms, which increases the acidity of the wine. This decrease in pH is an important indicator of fermentation progress and contributes to improved flavour, stability, and prevention of microbial contamination in the final product. During course of fermentation pH of the wine decreased due to two main reasons that is presence of organic acids (citric acid, malic acid and tartaric acid) in fruits and production of alcohol [13]. Hence, the pH readings were recorded after fermentation (Table. 1). According

to pH values the wine before fermentation was alkaline in nature but after fermentation the pH values decreased making it slightly acidic. This acidic condition makes the wine tastier and prevents contamination.

**4.2 Titratable Acidity:** The changes in titratable acidity during fermentation of custard apple wine are presented in Table 2.

**Table 2: Titratable acidity values of custard apple wine (CA 1 & CA2 Variation)**

S.No	Formulation	Fermentation Period							
		Experimental values Titratable acidity							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
1	CA 1	35.2±0.81	35.2±0.81	52.8±0.72	52.8±0.72	52.8±0.72	35.2±0.81	35.2±0.81	35.2±0.81
2	CA2	35.2±0.81	70.4±0.69	70.4±0.69	52.8±0.72	52.8±0.72	70.4±0.69	70.4±0.69	70.4±0.69



**Graph 2: Titratable acidity values of custard apple wine (CA 1 & CA2 Variation)**

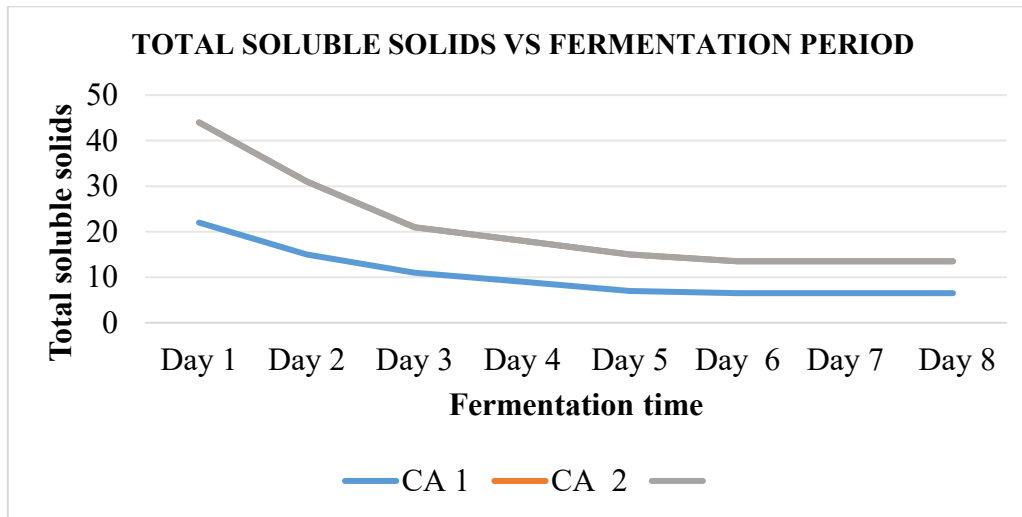
The table 2 presents the changes in titratable acidity of two formulations (CA1 and CA2) during an 8-day fermentation period. Both formulations showed an initial titratable acidity value of 35.2 on Day 1, indicating similar starting conditions. In CA1, the acidity gradually increased to 52.8 from Day 3 to Day 5, suggesting active microbial fermentation and production of organic acids during this stage. After Day 5, the acidity decreased and stabilized at 35.2 from Day 6 to Day 8, indicating a possible reduction in microbial activity and stabilization of the fermentation process. In contrast, CA2 showed higher acidity levels, reaching 70.4 on Day 2 and Day 3, followed by slight fluctuations during the later days of fermentation. The overall increase and variation in titratable acidity in both formulations indicate the progression of fermentation and the formation of organic acids, which play an important role in flavour development and product stability.

**4.3 Total Soluble Solids:**

The total soluble solids (TSS) of custard apple wine formulations CA1 and CA2 were presented in table 3.

**Table 3: Total Soluble Solids values of custard apple wine (CA1&CA2 Variation)**

S.No	Formulation	Fermentation Period (In Days)							
		Experimental values Total Soluble Solids (° Brix)							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
1	CA 1	22	15	11	9	7	6.5	6.5	6.5
2	CA2	22	16	10	9	8	7	7	7



**Graph 3: Total Soluble Solids values of custard apple wine (CA1&CA2 Variation)**

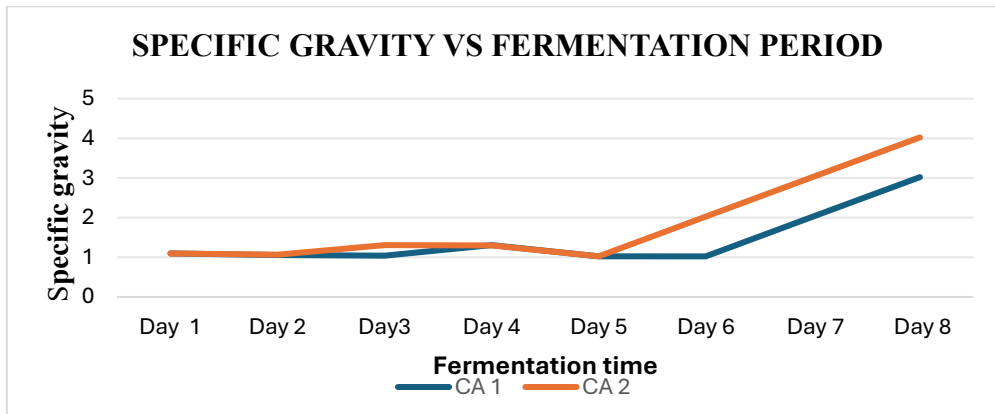
The total soluble solids (TSS) of custard apple wine formulations CA1 and CA2 were monitored during the 8-day fermentation period, as presented in the table. Initially, both formulations showed a higher TSS value of 22°Brix on Day 1, indicating the presence of high sugar content in the must. As fermentation progressed, the TSS values gradually decreased in both samples. In CA1, the TSS reduced from 22 to 6.5°Brix by Day 8, while in CA2 it decreased from 22 to 7°Brix during the same period. The continuous reduction in total soluble solids indicates the utilization of sugars by fermenting microorganisms to produce alcohol and other metabolites. This decrease in TSS confirms the progress of fermentation and the conversion of sugars into alcohol, leading to the stabilization of the final fermented product.

#### 4.4 Specific Gravity (S.G):

The specific gravity values of the custard apple wine formulations during fermentation are shown in Table 4.

**Table 4: Specific Gravity of custard apple wine (CA 1 & CA2 Variation)**

S.No	Formulation	Fermentation Period							
		Experimental values Total Soluble Solids							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
1	CA 1	1.095	1.060	1.040	1.309	1.025	1.023	1.023	1.023
2	CA2	1.095	1.065	1.309	1.30	1.025	1.025	1.025	1.025



**Graph 4: Specific Gravity of custard apple wine (CA 1 & CA2 Variation)**

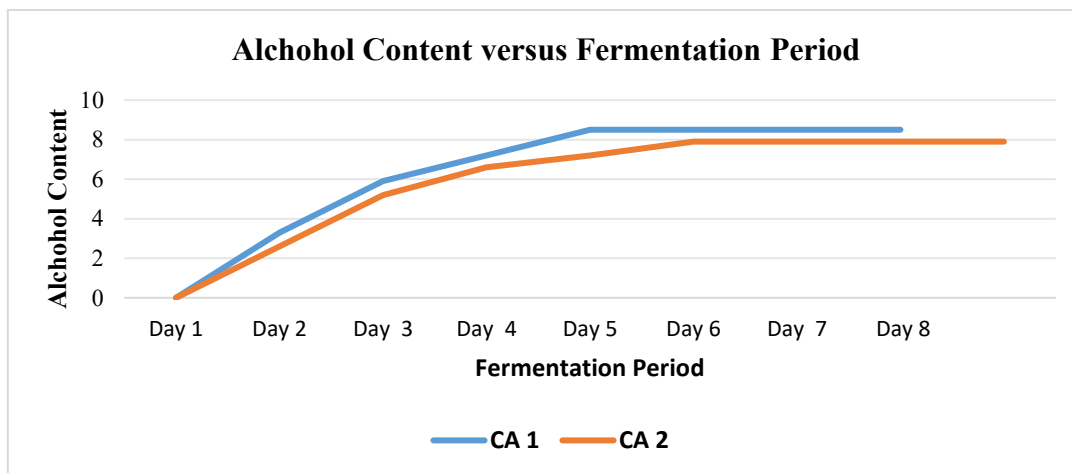
The graphical representation highlights this decline, confirming the progress of fermentation. Lower final specific gravity values indicate reduced residual sugar content and successful fermentation. The slightly lower final value in CA1 suggests more efficient sugar conversion compared to CA2, which is consistent with its higher alcohol yield.

**4.5 Alcohol content:**

The alcohol content of custard apple wine formulations (CA1 and CA2) was measured during the 8-day fermentation period, as shown in the table 5.

**Table 5: Alcohol content of custard apple wine (CA 1 & CA2 Variation)**

S.No	Formulation	Fermentation Period							
		Experimental values of Alcohol content							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
1	CA 1	0	3.3	5.9	7.2	8.5	8.5	8.5	8.5
2	CA2	0	2.6	5.2	6.6	7.2	7.9	7.9	7.9



**Graph 5: Alcohol content of custard apple wine (CA 1 & CA2 Variation)**

The alcohol content of custard apple wine formulations (CA1 and CA2) was measured during the 8-day fermentation period, as shown in the table. Initially, both formulations showed 0% alcohol on Day 1,

indicating the beginning of fermentation. As fermentation progressed, the alcohol content gradually increased due to the conversion of sugars into ethanol by yeast activity. In CA1, the alcohol content increased from 3.3% on Day 2 to 8.5% by Day 5 and remained stable until Day 8. Similarly, in CA2, the alcohol level increased from 2.6% on Day 2 to 7.9% by Day 6, after which it remained constant. The gradual increase in alcohol content indicates the active fermentation process and efficient utilization of sugars by microorganisms, resulting in the formation of ethanol in the final wine product.

## 5. Conclusion:

The study successfully demonstrated the feasibility of producing wine from custard apple pulp using both local (CA1) and hybrid (CA2) varieties. Fermentation resulted in significant biochemical changes, including a decrease in pH and total soluble solids, along with an increase in titratable acidity and alcohol content. These changes confirm active microbial fermentation and conversion of sugars into ethanol. Among the two formulations, the local variety (CA1) exhibited comparatively better performance in terms of alcohol production (8.5%) and sugar utilization than the hybrid variety (CA2). The final product showed acceptable physico-chemical characteristics, indicating its potential as a novel fruit wine. Thus, custard apple, an underutilized fruit, can be effectively used for value addition through wine production, contributing to reduced post-harvest losses and enhanced economic utilization.

## 6. Future Scope:

Further research on custard apple wine can focus on comprehensive sensory evaluation to assess consumer acceptance in terms of taste, aroma, color, and overall quality. Optimization of fermentation parameters such as temperature, yeast concentration, and pulp-to-water ratio can be carried out to enhance product quality and consistency. Detailed nutritional and functional analysis, including antioxidant activity and phenolic content, may help establish its potential as a functional beverage. In addition, shelf-life studies are essential to evaluate stability and microbial safety over extended periods. The process can also be scaled up for commercial production and industrial applications. Moreover, blending custard apple with other fruits or herbal extracts could improve sensory attributes and diversify product range. Finally, effective utilization of by-products such as seeds and peels can be explored for the extraction of valuable bioactive compounds, contributing to sustainable and economical processing.

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