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# Biochemical characterization and quality evaluation of indigenous custard apple (*Annona squamosa* L.) Genotypes

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**Abstract**
Custard apple (*Annona squamosa* L.) is an important tropical fruit crop valued for its pleasant flavor, nutritional richness, and medicinal properties. The present investigation was undertaken to evaluate the biochemical variability among twenty indigenous custard apple genotypes collected from the Fruit Research Station, Madhidibaug, Junagadh, Gujarat, India. Moisture content varied from 65.98 to 74.78%, while total soluble solids (TSS) ranged from 20.37 to 24.89°Brix. Total sugar content ranged between 15.21 and 20.76%, whereas acidity varied from 0.163 to 0.291%. Reducing sugar and non-reducing sugar contents ranged from 11.67 to 15.45% and 3.54 to 5.31%, respectively. Among the evaluated genotypes, CA genotype 20 exhibited superior performance for total sugar, reducing sugar, and non-reducing sugar contents, whereas CA genotype 16 recorded the highest TSS. The observed variability suggests considerable genetic potential for the improvement of fruit quality traits in custard apple. These superior genotypes may be utilized in future breeding programs aimed at developing high-quality cultivars suitable for commercial cultivation and processing industries.

**Keywords:** *Annona squamosa*, custard apple, biochemical characterization, fruit quality, sugars, TSS, acidity, genetic variability

## Introduction

Custard apple (*Annona squamosa* L.) is an important tropical fruit belonging to the family *Annonaceae* and is widely cultivated in tropical and subtropical regions due to its adaptability to marginal soils and dry climatic conditions. India is one of the leading producers of custard apple, where it is commercially cultivated in states such as Maharashtra, Gujarat, Rajasthan, Madhya Pradesh, Andhra Pradesh, and Chhattisgarh. The fruit is highly valued for its pleasant aroma, sweet creamy pulp, and excellent nutritional quality, making it popular for both fresh consumption and processing. Besides its economic importance, custard apple has gained considerable attention because of its medicinal properties, including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities, which are mainly attributed to its diverse bioactive phytochemicals (Morton, 1987; Nakasone and Paull, 1998) [9, 11]. Fruit quality in custard apple is primarily determined by biochemical attributes such as moisture content, total soluble solids (TSS), sugar composition and titratable acidity. These parameters directly influence fruit sweetness, flavour, palatability, nutritional value, processing suitability, and consumer acceptance. The expression of these quality traits is largely governed by the genetic makeup of the genotype, although environmental conditions and orchard management practices also contribute to their variation. Therefore, the evaluation of indigenous germplasm for biochemical characteristics is essential for identifying superior genotypes that can be utilized in breeding programmes and commercial cultivation (Kader, 2002; Wills *et al.,* 2007) [8, 16]. Several researchers have reported substantial variability in biochemical traits among custard apple genotypes (Yadav *et al.,* 2017; Ghawade *et al.,*2018) [4, 17].

Although considerable diversity exists in indigenous custard apple germplasm, systematic information on the biochemical characteristics of many local genotypes from Gujarat remains limited. The custard apple collection maintained at the Fruit Research Station, Madhidibaug, Junagadh, represents a valuable genetic resource that has not been comprehensively characterized for fruit quality traits. Therefore, the present investigation was undertaken to evaluate the biochemical variability among twenty indigenous custard apple genotypes based on moisture content, total soluble solids, total sugar, reducing sugar, non-reducing sugar and acidity. The findings of this study will facilitate the identification of promising genotypes for crop improvement programmes and the development of superior cultivars with enhanced fruit quality.

## Materials and Methods

The present investigation was carried out during the year 2024–2025 to evaluate the biochemical characteristics of twenty indigenous custard apple (*Annona squamosa* L.) genotypes. Mature fruits were collected from healthy and bearing trees maintained at the Fruit Research Station, Madhidibaug, Junagadh Agricultural University, Junagadh, Gujarat, India. The harvested fruits were transported to the Department of Biotechnology, College of Agriculture, Junagadh Agricultural University, Junagadh, where all biochemical analyses were performed.

The experiment data for biochemical analysis was computed in a Completely Randomized Design (CRD) with three replications as described by Panse and Sukhatme (1985) [12]. Uniform, fully mature and disease free fruits were selected for biochemical analysis. The pulp was manually separated from the peel and seeds, homogenized thoroughly, and used immediately for the estimation of various biochemical parameters described below.

**Moisture Content:** Moisture content of custard apple pulp was determined by the hot air oven drying method described by Pomeranz and Meloan (1994) [13]. Approximately 5 g of fresh pulp sample was weighed accurately in a pre-weighed moisture dish and dried in a hot air oven until a constant weight was obtained. The moisture content was calculated and expressed as percentage (%).

**Total Soluble Solids (TSS):** The total soluble solids (TSS) content of fresh custard apple pulp was determined using a hand refractometer following the procedure described by Ranganna (1997) [14]. A few drops of filtered pulp juice were placed on the prism of the refractometer, and the readings were recorded at room temperature. The results were expressed as °Brix after applying the necessary temperature correction.

**Total Sugar:** Total sugar content was estimated using the Fehling's solution titrimetric method as described by Ranganna (1997) [14]. The clarified fruit extract was hydrolysed, neutralized, and titrated against standardized Fehling's solution using methylene blue as an internal indicator. The total sugar content was calculated and expressed as percentage (%) on a fresh weight basis.

**Reducing Sugar and Non reducing sugar:** Reducing sugar content was determined by the Nelson–Somogyi method following the procedure described by Somogyi (1952) [15]. The reducing sugars present in the pulp extract reduced copper ions under alkaline conditions, and the resulting cuprous oxide reacted with arsenomolybdate reagent to produce a blue-colored complex. Absorbance was measured spectrophotometrically, and the reducing sugar content was expressed as percentage (%). Non-reducing sugar content was calculated by subtracting reducing sugar from total sugar.

**Titratable Acidity:** Titratable acidity of custard apple pulp was determined by the alkali titration method described by Hammett (1928) [5]. A known quantity of pulp extract was titrated against standardized 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator until the appearance of a persistent light pink endpoint. Acidity was calculated as percentage of citric acid equivalent.

## Results and Discussion

**Biochemical variability among custard apple genotypes:** Significant differences were observed among the twenty indigenous custard apple (Annona squamosa L.) genotypes for all biochemical quality attributes, namely moisture content, total soluble solids (TSS), total sugar, reducing sugar, non-reducing sugar and titratable acidity (Table 1). The wide variation observed among the genotypes indicates the existence of substantial genetic diversity for fruit quality traits, which could be effectively utilized in future crop improvement and varietal selection programmes.

**Moisture content:** Moisture content of the evaluated genotypes ranged from 65.98 to 74.78%, with an overall mean of 69.86%. The highest moisture content was recorded in CA genotype 11 (74.78%), followed closely by CA genotypes 20, 8, 9, 16 and 19, whereas the lowest value was observed in CA genotype 17 (65.98%). Variation in fruit moisture is mainly associated with genetic differences influencing water accumulation and pulp development during fruit maturation. Higher moisture contributes to improved pulp recovery and desirable texture but may also reduce postharvest shelf life due to increased susceptibility to microbial deterioration. Similar moisture ranges have been reported by Kachhadiya and Jethva (2017) and Akhtar *et al.* (2025) [1, 7], confirming that custard apple is naturally a high-moisture fruit.

**Total soluble solids (°Brix)**: Total soluble solids varied significantly from 20.37 to 24.89°Brix, averaging 22.54°Brix. The highest TSS was recorded in CA genotype 16 (24.89°Brix), followed by CA genotypes 7 and 12, whereas CA genotype 2 exhibited the lowest value (20.37°Brix). TSS is a major indicator of sweetness and fruit maturity, largely reflecting the accumulation of soluble carbohydrates during ripening. Genotypes with higher TSS are generally preferred for fresh consumption and processing because they impart superior sweetness and flavour. The present findings agree well with earlier reports of Ghawade *et al.* (2018) [4] and Jain *et al.* (2019), who also reported considerable variability in TSS among custard apple genotypes.

**Table 1:** Biochemical characteristics of twenty indigenous custard apple (*Annona squamosa* L.) genotypes

| Genotype | Moisture (%) | TSS (°Brix) | Total Soluble sugar (%) | Acidity (%) | RS (%) | NRS (%) |
|---|---|---|---|---|---|---|
| CA genotype 1 | 69.34 | 22.84 | 16.81 | 0.24 | 13.09 | 3.72 |
| CA genotype 2 | 67.25 | 20.37 | 18.50 | 0.16 | 14.74 | 3.76 |
| CA genotype 3 | 71.58 | 24.16 | 19.17 | 0.21 | 15.42 | 3.75 |
| CA genotype 4 | 66.89 | 21.90 | 15.21 | 0.18 | 11.67 | 3.54 |
| CA genotype 5 | 68.71 | 23.48 | 20.21 | 0.24 | 14.95 | 5.26 |
| CA genotype 6 | 67.00 | 20.95 | 15.23 | 0.18 | 11.68 | 3.55 |
| CA genotype 7 | 69.80 | 24.72 | 19.13 | 0.26 | 15.33 | 3.80 |
| CA genotype 8 | 74.25 | 22.11 | 18.97 | 0.22 | 14.97 | 4.00 |
| CA genotype 9 | 73.89 | 21.07 | 18.56 | 0.17 | 14.71 | 3.85 |
| CA genotype 10 | 70.45 | 23.95 | 18.88 | 0.27 | 14.57 | 4.31 |
| CA genotype 11 | 74.78 | 20.58 | 18.99 | 0.17 | 14.78 | 4.21 |
| CA genotype 12 | 67.08 | 24.30 | 15.26 | 0.28 | 11.70 | 3.56 |
| CA genotype 13 | 66.67 | 22.67 | 15.28 | 0.25 | 11.71 | 3.57 |
| CA genotype 14 | 67.56 | 21.08 | 19.05 | 0.17 | 14.51 | 4.54 |
| CA genotype 15 | 67.59 | 23.21 | 15.29 | 0.21 | 11.69 | 3.60 |
| CA genotype 16 | 72.34 | 24.89 | 16.50 | 0.25 | 11.79 | 4.71 |
| CA genotype 17 | 65.98 | 20.74 | 15.32 | 0.17 | 11.73 | 3.59 |
| CA genotype 18 | 68.32 | 22.43 | 15.93 | 0.18 | 12.12 | 3.81 |
| CA genotype 19 | 73.45 | 21.57 | 19.05 | 0.28 | 15.06 | 3.99 |
| CA genotype 20 | 74.45 | 23.79 | 20.76 | 0.29 | 15.45 | 5.31 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Mean | 69.86 | 22.54 | 17.60 | 0.23 | 13.58 | 4.02 |
| S.Em. ± | 1.275 | 0.267 | 0.239 | 0.003 | 0.186 | 0.065 |
| CD @ 5% | 3.650 | 0.764 | 0.686 | 0.011 | 0.533 | 0.188 |
| C.V. (%) | 3.160 | 2.052 | 2.377 | 3.026 | 2.376 | 2.837 |

**Sugar composition:** Sugar composition exhibited marked variability among the studied genotypes. Total sugar content ranged from 15.21 to 20.76%, while reducing sugar varied between 11.67 and 15.45%, and non-reducing sugar ranged from 3.54 to 5.31%. Among all genotypes, CA genotype 20 consistently recorded the highest total sugar (20.76%), reducing sugar (15.45%) and non-reducing sugar (5.31%), indicating its superior sweetness and excellent eating quality. Conversely, CA genotype 4 exhibited the lowest values for all sugar fractions. The accumulation of sugars during fruit ripening results from enzymatic conversion of starch and other reserve carbohydrates into simple sugars, which directly determine fruit sweetness and consumer preference. The superiority of CA genotype 20 suggests efficient carbohydrate metabolism and greater translocation of assimilates into the developing fruit. Similar variation in sugar composition has been documented by Yadav *et al.* (2017), Ghawade *et al.* (2018), Nag *et al.* (2018) and Jain and Kumar (2024) [4, 6, 10, 17], who attributed these differences primarily to genetic variability and fruit developmental processes.

**Titratable acidity:** Titratable acidity ranged from 0.163 to 0.291%, with a mean value of 0.23%. The highest acidity was observed in CA genotype 20 (0.291%), closely followed by genotypes 12 and 19, whereas CA genotype 2 recorded the lowest acidity (0.163%). Organic acids play a significant role in determining fruit flavour by balancing sweetness and contributing to the characteristic taste of custard apple. Differences in acidity among genotypes may be attributed to variations in organic acid synthesis and degradation during fruit maturation. Comparable acidity ranges have been reported by Ghasil *et al.* (2022) and Barge *et al.* (2023) [2, 3].

**Overall evaluation of genotypes:** The present investigation clearly demonstrated considerable biochemical diversity among the indigenous custard apple genotypes. A comprehensive assessment of biochemical quality parameters identified CA genotype 20 as the most promising genotype owing to its superior sugar composition and favourable fruit quality characteristics (Table 2). CA genotype 16 ranked second because of its exceptionally high TSS, whereas CA genotype 3 exhibited a desirable combination of high TSS and sugar content. CA genotype 11 was distinguished by its maximum moisture content and favourable sweetness-to-acidity balance, while CA genotype 2 recorded the lowest acidity, indicating excellent palatability. These elite genotypes may serve as valuable genetic resources for cultivar improvement, commercial cultivation and processing industries.

**Table 2:** Overall Quality Ranking of Twenty Custard Apple Genotypes Based on Biochemical Attributes

| Overall Rank | Genotype | Major Superior Biochemical Traits | Potential Use |
|---|---|---|---|
| 1 | CA genotype 20 | Highest total sugar (20.76%), highest reducing sugar (15.45%), highest non-reducing sugar (5.31%), high moisture (74.45%), high TSS (23.79°Brix) | Excellent for fresh consumption, processing and breeding |
| 2 | CA genotype 16 | Highest TSS (24.89°Brix), high moisture (72.34%) | Excellent dessert quality and processing |
| 3 | CA genotype 3 | High TSS (24.16°Brix), high reducing sugar (15.42%), high total sugar (19.17%), | Premium table purpose and breeding |
| 4 | CA genotype 11 | Highest moisture (74.78%), high total sugar (18.99%), low acidity | Good pulp recovery and fresh consumption |
| 5 | CA genotype 2 | Lowest acidity (0.163%), high reducing sugar | Superior eating quality and consumer acceptability |

The observed variability among the evaluated genotypes is primarily attributed to inherent genetic differences affecting carbohydrate metabolism, water accumulation and organic acid biosynthesis during fruit development. Since all genotypes were evaluated under identical environmental and management conditions, the differences observed are likely to be predominantly genetic in nature. Such variability provides valuable opportunities for selecting elite genotypes with superior fruit quality traits and utilizing them in breeding programmes aimed at developing high-yielding cultivars with improved nutritional and sensory characteristics.

Overall, the results demonstrate that the indigenous custard apple germplasm evaluated, constitutes a valuable genetic resource for future varietal improvement. In particular, CA genotype 20, CA genotype 16, CA genotype 2, and CA genotype 11 may be considered promising donor genotypes for quality improvement and commercial cultivation.

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