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How Many Acerola (*Malpighia emarginata* DC.) Fruit Are Required for Reliable Postharvest Quality Assessment?

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Abstract

Acerola (*Malpighia emarginata* DC.) is a tropical fruit known for its high vitamin C (ascorbic acid) content. This study aimed to determine the optimal sample size (OSS) required to reliably estimate postharvest quality traits in acerola. A total of 50 red-ripe fruit from four cultivars (BRS Rubra, Cabocla, Costa Rica, and Junko) were evaluated individually for their physical (weight, diameter, length, color, and firmness) and chemical (soluble solids content [SSC], titratable acidity [TA], SSC/TA ratio, and vitamin C) attributes. Bootstrap resampling and nonlinear power models were used to model the relationships between sample sizes and the width of 95% confidence intervals ($CI_{95\%}$). Three methods were applied to determine the maximum curvature point (MCP): general, perpendicular distance (PD), and linear response plateau (LRP). The PD and LRP methods led to consistent and conservative OSS estimates, which ranged from 12 to 28 fruit depending on the trait and cultivar. A sample size of 20 fruit was identified as a practical and reliable reference. Chemical traits showed greater variability and required larger samples. Cultivar comparisons indicated that 'BRS Rubra', 'Cabocla', and 'Costa Rica' are suitable for fresh consumption, while 'Junko' is ideal for vitamin C extraction. These results provide statistical support for experimental planning in acerola postharvest research.

Keywords: sample size; fruit quality; vitamin C; fresh consumption; experimental planning; sampling; maximum curvature point; nonlinear models; bootstrap

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1. Introduction

Acerola (*Malpighia emarginata* DC.) is a cherry-like tropical fruit native to the Caribbean, Central America, and Northern South America. Brazil is currently the world's leading producer, consumer, and exporter of acerola, with favorable edaphoclimatic conditions in the semiarid São Francisco Valley (SFV) enabling its commercial expansion [1]. Due to its exceptional nutritional value, particularly its high vitamin C (ascorbic acid) content—up to 100 times greater than that of orange and lemon—as well as its high levels of phenolic compounds, carotenoids, and minerals, acerola is recognized as a superfruit with a growing interest from the scientific community and food and pharmaceutical industries [2–5].

Despite its economic relevance, research on the quality of acerola has emerged mainly in the last decade [4] and remains relatively limited, especially when compared to more established fruit crops grown in the Brazilian semiarid region, such as mango (*Mangifera indica* L.) [6] and grape (*Vitis vinifera* L.) [7]. In postharvest studies, defining an appropriate

sample size is a critical step that directly impacts the reliability and reproducibility of experimental results. An adequate sample size ensures the accurate estimation of quality attributes while avoiding the unnecessary allocation of financial, physical, and human resources [8].

For various fruit species, such as apple (*Malus domestica* Borkh.) [9], mango [8], wild passion fruit (*Passiflora foetida* L.) [10], papaya (*Carica papaya* L.) [11], peach [*Prunus persica* (L.) Batsch] [12], seriguela (*Spondias purpurea* L.) [13], and cashew apple (*Anacardium occidentale* L.) [14], the optimal sample size (OSS) has been determined using a traditional frequentist approach, which involves evaluating individual fruit and using measures of dispersion (i.e., standard deviation, SD) to estimate the number of units required for future experiments.

The traditional frequentist formula for sample size estimation, $(Z_{\alpha/2}\,\mathrm{SD}/E)^2$, assumes normality of the data and requires prior knowledge of the SD and acceptable error (E), and often faces limitations when assessing biological data [15,16]. To address these issues, modern approaches which offer greater flexibility and accuracy have been proposed. The bootstrap method, for instance, is a non-parametric resampling technique that allows for the estimation of sample variability directly from observed samples without assuming a specific distribution [17]. This variability information can then be combined with the maximum curvature point (MCP) method, which identifies the point at which increases in the sample size yield diminishing returns in precision based on nonlinear models. Together, these approaches provide a robust framework to define the OSS for complex or heterogeneous biological data [18].

The combination of bootstrap resampling and the MCP method is particularly relevant for acerola postharvest research due to the high biological variability of acerola fruit. The MCP allows for the identification of a threshold sample size beyond which increases in sample units provide diminishing returns in accuracy, thus optimizing resource allocation in experimental designs [18]. This approach optimizes the efficiency of experimental planning by helping researchers to determine a balance between statistical confidence and practical constraints such as time, labor, and cost, which is especially important in studies involving tropical fruits such as acerola [2].

These approaches have been successfully applied in plant science experiments, including studies on soybean [*Glycine max* (L.) Merr.] [19], rye (*Secale cereale* L.) [20], and cauliflower (*Brassica oleracea* L. var. *botrytis* L.) [21]. In postharvest experiments, where biological variability and complex data structures are common, MCP-based methods offer significant potential. However, to the best of our knowledge, no studies to date have proposed an OSS for evaluating the quality of acerola.

Therefore, the objective of this study was to estimate the minimum number of fruit required to reliably determine the mean values of postharvest quality attributes in acerola using different approaches, with the aim of providing practical guidance for future experiments and cultivar evaluations. We assumed that sample size requirements may vary across cultivars due to the inherent biological variability in fruit traits; thus, each cultivar was evaluated individually to account for these differences. Additionally, we compared the quality traits of promising acerola cultivars intended for fresh consumption with those of a widely cultivated commercial cultivar, with the goal of identifying phenotypic advantages that may inform breeding strategies and cultivar recommendations according to the intended use of each cultivar, including vitamin C extraction, fresh consumption, and industrial processing.

2. Materials and Methods

2.1. Experimental Conditions and Plant Material

The experiment was carried out with acerola (*Malpighia emarginata* DC.) trees cultivated in an experimental field located in Petrolina, Pernambuco State, Brazil (09°09′ S, 40°22′ W; altitude: 365 m). The trees were 10 years old, spaced at 4.0 × 3.5 m, and cultivated under similar management and irrigation practices [22].

Daily climate data were obtained from an automatic weather station located near the experimental area, which are provided in the Supplementary Materials. During the fruit development period (September 2022), the average daily temperature ranged from 22.6 to 29.4 °C, with minimum temperatures between 16.8 and 23.1 °C and maximum temperatures between 29.0 and 37.4 °C. The relative humidity varied from 47% to 69%, and no rainfall was recorded during this period. Global solar radiation ranged from 16.4 to 26.6 MJ m^{-2} day^{-1}, while reference evapotranspiration (ET_0) values ranged from 4.82 to 7.93 mm day^{-1}. All plants were irrigated daily using a micro-sprinkler system, with water amounts determined based on ET_0 and crop coefficients [22].

Red-ripe acerola fruit, characterized by fully red-colored skin, from the cultivars BRS Rubra, Cabocla, Costa Rica, and Junko (Figure 1), were manually harvested in the early morning on 26 September 2022. Among these, 'BRS Rubra', 'Cabocla', and 'Costa Rica' were selected by Ferreira et al. [23] as promising cultivars for fresh consumption due to their combination of desirable traits, whereas 'Junko' is a well-established cultivar in the SFV and the most commercialized acerola variety worldwide [24]. For each cultivar, 50 fruit were selected and individually analyzed for the quality attributes detailed below. This number was defined based on practical constraints, as sample sizes greater than 50 fruit are rarely used in postharvest studies due to increased time, labor, and resource demands.

Figure 1. Representative fruit of the four acerola (*Malpighia emarginata* DC.) cultivars evaluated.

2.2. Assessment of Fruit Quality Attributes

2.2.1. Weight, Diameter, and Length

Fruit weight (g) was determined using an analytical balance with a precision of 0.001 g (model AD50, Marte Científica, São Paulo, Brazil).

Fruit diameter (mm) and length (mm) were assessed using a digital caliper with a precision of 0.02 mm (model CD-6 CS, Mitutoyo Corporation, Kawasaki, Japan).

2.2.2. Color

Skin color was determined with a digital colorimeter (model CR-400, Konica Minolta, Inc., Tokyo, Japan), recording color values in the CIE $L^*a^*b^*$ color system, where L^* (lightness) varies from 0 (black) to 100 (white), a^* represents green (negative) or red (positive) colors, and b^* represents blue (negative) or yellow (positive). For a better representation of fruit color, the hue angle (°) was calculated as $\tan^{-1}$ (b^*/a^*), where 0/360° represents red, 90° represents yellow, 180° represents green, and 270° represents blue. For acerola, the lower the hue angle, the redder the fruit.

2.2.3. Firmness

Pulp firmness was measured as the maximum force required to press 10% of the fruit diameter using a texture analyzer (model TA.XT.Plus, Extralab Brasil, Itatiba, Brazil) equipped with a P/75 pressure plate. The results are expressed in Newton (N).

2.2.4. Vitamin C

Vitamin C content was determined by titration with 0.02% 2,6-dichlorophenol indophenol (DFI), following the AOAC 967.21 method [25]. Briefly, 1 g of acerola fruit was diluted in 100 mL of 0.5% (*w*/*v*) oxalic acid; then, a 5 mL aliquot of this solution was diluted to 50 mL with distilled water and titrated until a light pink color developed. The results are expressed in mg per 100 g of fresh weight. Although chromatographic techniques (e.g., HPLC) offer greater sensitivity and specificity, DFI titration was chosen due to its practicality, low cost, and suitability for large sample sets. When properly standardized, this method provides reliable and reproducible results, and it has been widely used in postharvest studies, particularly when chromatographic equipment is not readily available.

2.2.5. Soluble Solids Content (SSC) and Titratable Acidity (TA)

The SSC and TA were measured using a portable refractometer and acidity meter (model PAL-BX ACID3, ATAGO CO., LTD., Tokyo, Japan). SSC was determined for undiluted acerola juice, while TA was measured from a 1:50 (*v*/*v*) juice dilution prepared with distilled water. The SSC/TA ratio was calculated by dividing SSC by its respective TA for each sample.

2.3. Statistical Analysis

Descriptive statistics, including the minimum, mean, maximum, median, standard deviation (SD), and coefficient of variation (CV% = SD × 100/mean), were calculated for each variable in each cultivar. The Shapiro–Wilk test was applied to assess the normality of the data. As some variables did not meet the normality assumption ($p < 0.05$) (Table S2), the use of bootstrap resampling as an appropriate approach for this study was reinforced.

The OSS (i.e., number of fruit) required to estimate the means of acerola quality attributes for each cultivar was estimated through bootstrap resampling [26]. A total of 100 sample sizes were planned, starting with 1 fruit and incrementally increasing the number by 1 up to 100 fruit.

For each planned sample size, 10,000 resamples with replacement were generated, and the minimum, 2.5th percentile, mean, 97.5th percentile, and maximum values were calculated. The amplitude of the 95% confidence interval ($CI_{95\%}$) was calculated as the difference between the 97.5th and 2.5th percentiles. A smaller $CI_{95\%}$ indicates more accurate mean estimates.

A nonlinear power model was applied to fit the dependent variable [$CI_{95\%}$] as a function of the independent variable (number of fruit) (Equation (1)):

$$CI_{95\%} = \alpha \times n^{\beta} + \varepsilon \quad (1)$$

where α is the intercept coefficient, n is the OSS, β is the exponential decay rate, and ε is the random error term.

The MCP, representing the OSS, was estimated using three methods: general curvature function (GCF), perpendicular distances (PD), and linear response plateau (LRP).

For each quality attribute, the data were submitted to analysis of variance (ANOVA), and the four cultivars were compared using Tukey's multiple comparison test ($p < 0.05$), applied after the bootstrap resampling procedure [27]. All statistical analyses were performed using the R statistical software, version 4.5.1 (R Core Team, Vienna, Austria).

3. Results

3.1. Descriptive Statistics and Comparison of Fruit Quality Attributes Among Cultivars

The descriptive statistics for the postharvest quality attributes of the four acerola cultivars are presented in Table 1. Significant differences were observed among the cultivars for all evaluated parameters ($p < 0.0001$).

Table 1. Descriptive statistics of postharvest quality attributes in four acerola (*Malpighia emarginata* DC.) cultivars.

Quality Attribute	Cultivar	Minimum	Mean [1]	Maximum	Median	SD [2]	CV [3]
Weight	BRS Rubra	3.00	5.05 b	6.76	5.14	0.81	16.12
	Cabocla	5.02	6.78 a	9.96	6.50	1.11	16.36
	Costa Rica	4.78	6.52 a	8.87	6.33	1.08	16.50
	Junko	3.81	5.39 b	7.57	5.30	0.89	16.51
Diameter	BRS Rubra	17.5	21.1 b	24.3	21.3	1.7	7.91
	Cabocla	20.3	23.0 a	26.5	23.2	1.3	5.75
	Costa Rica	20.0	22.6 a	25.7	22.7	1.3	5.51
	Junko	17.5	20.1 c	23.4	20.4	1.4	6.71
Length	BRS Rubra	14.8	18.1 b	21.1	18.2	1.5	8.05
	Cabocla	17.7	20.2 a	22.1	20.1	1.0	4.85
	Costa Rica	17.6	19.7 a	22.2	19.7	1.1	5.45
	Junko	14.9	18.0 b	21.5	17.9	1.3	7.43
Color	BRS Rubra	17.5	23.5 b	30.8	23.1	3.3	14.01
	Cabocla	23.1	28.4 c	37.3	27.5	3.5	12.38
	Costa Rica	19.1	29.0 c	39.5	28.7	4.2	14.49
	Junko	14.2	19.1 a	27.1	18.8	3.2	16.88
Firmness	BRS Rubra	7.2	11.5 b	18.0	11.4	2.2	19.18
	Cabocla	8.1	11.7 b	18.2	11.8	2.0	17.15
	Costa Rica	11.1	15.1 a	21.2	14.7	2.5	16.69
	Junko	4.7	8.6 c	12.2	8.7	1.6	18.08
Vitamin C	BRS Rubra	537	749 d	1141	752	153	20.45
	Cabocla	1184	1748 b	2315	1736	262	15.02
	Costa Rica	712	1187 c	1741	1201	260	21.92
	Junko	1253	2119 a	3102	2162	377	17.80
SSC	BRS Rubra	8.8	10.9 b	13.8	11.0	1.2	10.58
	Cabocla	9.4	11.8 a	14.6	11.6	1.4	11.46
	Costa Rica	8.5	10.4 b	14.5	10.3	1.1	10.64
	Junko	5.5	7.0 c	8.3	6.9	0.6	9.03
TA	BRS Rubra	0.48	0.68 c	0.97	0.66	0.11	16.60
	Cabocla	0.57	0.80 b	1.08	0.80	0.12	14.41
	Costa Rica	0.56	0.84 b	1.21	0.83	0.15	18.00
	Junko	0.84	1.03 a	1.31	1.04	0.10	9.51
SSC/TA ratio	BRS Rubra	10.5	16.4 a	25.2	16.0	3.1	18.64
	Cabocla	9.1	15.0 b	21.9	15.1	2.3	15.50
	Costa Rica	7.8	12.8 c	20.5	12.5	2.4	19.01
	Junko	5.3	6.8 d	8.4	6.7	0.7	10.54

[1] Means followed by the same lowercase letter in a column do not differ statistically from each other, according to the Tukey test ($p < 0.05$) applied after a bootstrap resampling procedure (10,000 resamples) [26]. [2] Standard deviation. [3] Coefficient of variation, expressed as a percentage.

In terms of fruit weight, 'Cabocla' and 'Costa Rica' exhibited the highest mean values (6.78 and 6.52 g, respectively), differing statistically from 'BRS Rubra' and 'Junko', which

recorded lower mean weights of 5.05 g and 5.39 g. Similar trends were observed for fruit diameter and length, with 'Cabocla' (23.0 and 20.2 mm) and 'Costa Rica' (22.6 and 19.7 mm) presenting significantly larger fruit, while 'Junko' consistently exhibited the smallest size (20.1 and 18.0 mm) (Table 1).

Regarding skin color (as expressed by the hue angle), 'Junko' fruit were the reddest (19.1°), significantly differing from the other cultivars. In contrast, 'Costa Rica' (29.0°) and 'Cabocla' (28.4°) displayed higher hue values, indicating a less intense red hue. For pulp firmness, 'Costa Rica' stood out with a mean of 15.1 N, followed by 'Cabocla' (11.7 N) and 'BRS Rubra' (11.5 N), while 'Junko' showed the lowest firmness value (8.6 N) (Table 1).

Vitamin C content varied widely among the cultivars, with all cultivars differing statistically from each other. 'Junko' presented the highest concentration (2119 mg 100 g^{-1}), followed by 'Cabocla' (1748 mg 100 g^{-1}), 'Costa Rica' (1187 mg 100 g^{-1}), and 'BRS Rubra', which had the lowest value (749 mg 100 g^{-1}) (Table 1).

The SSC ranged from 7.0% in 'Junko' to 11.8% in 'Cabocla'. These cultivars differed significantly from 'BRS Rubra' (10.9%) and 'Costa Rica' (10.4%), which exhibited intermediate SSC values. For TA, 'Junko' also exhibited the highest mean (1.03% malic acid), while 'BRS Rubra' showed the lowest mean (0.68% malic acid). Consequently, the SSC/TA ratio—an important indicator of flavor—was highest in 'BRS Rubra' (16.4) and lowest in 'Junko' (6.8), suggesting markedly contrasting taste profiles between these cultivars (Table 1).

The CV for the analyzed traits ranged from 4.85% (length in 'Cabocla') to 21.92% (vitamin C content in 'Costa Rica') (Table 1), reflecting the biological variability typically observed in the postharvest studies of tropical fruits.

3.2. Determination of OSS Based on the MCP

The relationships between sample size and the $CI_{95\%}$ amplitude were described using nonlinear power models for all quality attributes (Table 2). The power models demonstrated an adequate goodness of fit, as confirmed by the high coefficient of determination ($R^2 \geq 0.9910$), low root mean square error (RMSE ≤ 9.7784), and high d index (≥ 0.9977).

Table 2. Coefficient of determination (R^2), root mean square error (RMSE), and d index of the power models for postharvest quality attributes in four acerola (*Malpighia emarginata* DC.) cultivars.

Quality Attribute	Cultivar	Power Model	R^2	RMSE	d Index
Weight	BRS Rubra	$CI_{95\%} = 3.2632 \times n^{-0.5106}$	0.9988	0.0149	0.9997
	Cabocla	$CI_{95\%} = 4.2581 \times n^{-0.4984}$	0.9990	0.0176	0.9998
	Costa Rica	$CI_{95\%} = 3.9015 \times n^{-0.4800}$	0.9984	0.0207	0.9996
	Junko	$CI_{95\%} = 3.2514 \times n^{-0.4816}$	0.9975	0.0217	0.9994
Diameter	BRS Rubra	$CI_{95\%} = 6.1982 \times n^{-0.4871}$	0.9992	0.0227	0.9998
	Cabocla	$CI_{95\%} = 5.0317 \times n^{-0.4937}$	0.9995	0.0153	0.9999
	Costa Rica	$CI_{95\%} = 4.8549 \times n^{-0.5022}$	0.9998	0.0099	0.9999
	Junko	$CI_{95\%} = 5.3321 \times n^{-0.5052}$	0.9995	0.0150	0.9999
Length	BRS Rubra	$CI_{95\%} = 5.6467 \times n^{-0.5005}$	0.9998	0.0105	0.9999
	Cabocla	$CI_{95\%} = 3.7081 \times n^{-0.4925}$	0.9995	0.0108	0.9999
	Costa Rica	$CI_{95\%} = 3.8000 \times n^{-0.4722}$	0.9949	0.0361	0.9987
	Junko	$CI_{95\%} = 5.7841 \times n^{-0.5348}$	0.9949	0.0534	0.9987
Color	BRS Rubra	$CI_{95\%} = 12.529 \times n^{-0.4944}$	0.9995	0.0361	0.9999
	Cabocla	$CI_{95\%} = 13.1691 \times n^{-0.4905}$	0.9996	0.0355	0.9999
	Costa Rica	$CI_{95\%} = 18.0854 \times n^{-0.5342}$	0.9946	0.1718	0.9987
	Junko	$CI_{95\%} = 12.1620 \times n^{-0.4925}$	0.9995	0.0353	0.9999

Table 2. *Cont.*

Quality Attribute	Cultivar	Power Model	R^2	RMSE	d Index
Firmness	BRS Rubra	$CI_{95\%} = 8.2023 \times n^{-0.4852}$	0.9983	0.0445	0.9996
	Cabocla	$CI_{95\%} = 8.0114 \times n^{-0.5066}$	0.9997	0.0191	0.9999
	Costa Rica	$CI_{95\%} = 9.3704 \times n^{-0.4873}$	0.9992	0.0344	0.9998
	Junko	$CI_{95\%} = 5.6593 \times n^{-0.4818}$	0.9968	0.0428	0.9992
Vitamin C	BRS Rubra	$CI_{95\%} = 586.4827 \times n^{-0.4972}$	0.9997	1.2452	0.9999
	Cabocla	$CI_{95\%} = 1012.465 \times n^{-0.4984}$	0.9998	1.5869	0.9999
	Costa Rica	$CI_{95\%} = 990.848 \times n^{-0.4952}$	0.9998	1.9818	0.9999
	Junko	$CI_{95\%} = 1378.287 \times n^{-0.4813}$	0.9972	9.7784	0.9992
SSC	BRS Rubra	$CI_{95\%} = 4.4027 \times n^{-0.4938}$	0.9996	0.0110	0.9999
	Cabocla	$CI_{95\%} = 4.9162 \times n^{-0.4788}$	0.9974	0.0335	0.9993
	Costa Rica	$CI_{95\%} = 4.5390 \times n^{-0.5181}$	0.9992	0.0165	0.9998
	Junko	$CI_{95\%} = 2.4248 \times n^{-0.4979}$	0.9996	0.0067	0.9999
TA	BRS Rubra	$CI_{95\%} = 0.4387 \times n^{-0.4980}$	0.9998	0.0009	0.9999
	Cabocla	$CI_{95\%} = 0.4706 \times n^{-0.5166}$	0.9974	0.0032	0.9993
	Costa Rica	$CI_{95\%} = 0.5801 \times n^{-0.4967}$	0.9997	0.0013	0.9999
	Junko	$CI_{95\%} = 0.3715 \times n^{-0.4904}$	0.9985	0.0019	0.9996
SSC/TA ratio	BRS Rubra	$CI_{95\%} = 12.3633 \times n^{-0.5141}$	0.9985	0.0617	0.9996
	Cabocla	$CI_{95\%} = 8.3038 \times n^{-0.4742}$	0.9910	0.1059	0.9977
	Costa Rica	$CI_{95\%} = 8.9859 \times n^{-0.4843}$	0.9973	0.0616	0.9993
	Junko	$CI_{95\%} = 2.7115 \times n^{-0.4931}$	0.9993	0.0096	0.9998

As expected, the $CI_{95\%}$ for all traits and cultivars showed an exponential decline with increasing sample size, reaching a point of stabilization (Figures 2 and 3). In other words, using a sample of just 1 fruit resulted in a much broader $CI_{95\%}$ (and much lower precision) than when sampling 100 fruit within each experimental unit.

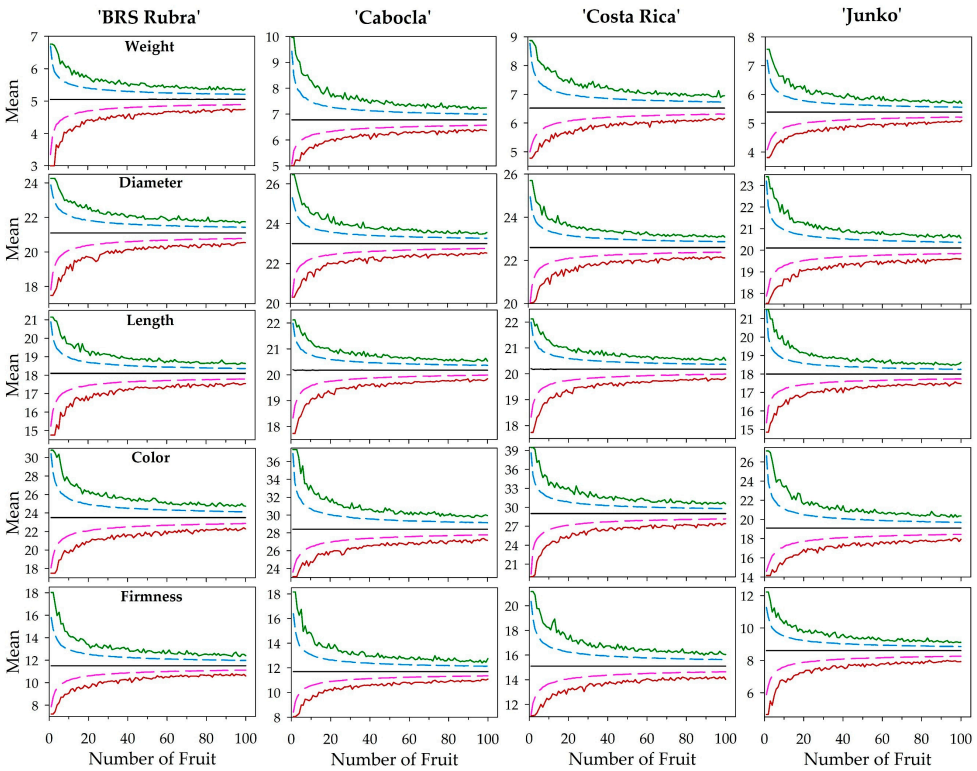

Figure 2. Bootstrap-based estimates for physical attributes in four acerola (*Malpighia emarginata* DC.) cultivars. Lines represent maximum (green), 97.5th percentile (blue), mean (black), 2.5th percentile (pink), and minimum (red) values.

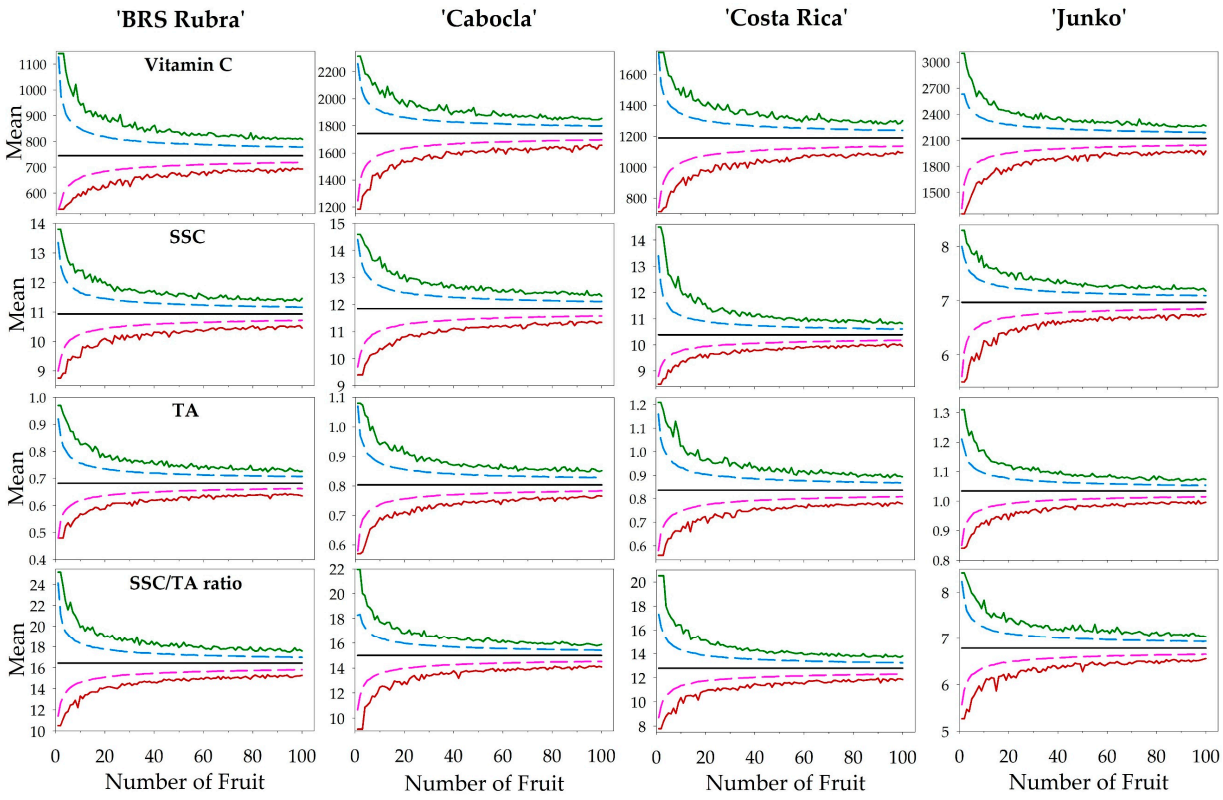

Figure 3. Bootstrap-based estimates for chemical attributes in four acerola (*Malpighia emarginata* DC.) cultivars. Lines represent maximum (green), 97.5th percentile (blue), mean (black), 2.5th percentile (pink), and minimum (red) values.

The OSSs based on MCP and estimated via the three methods are presented in Table 3. The estimation of OSSs based on the behavior of the $CI_{95\%}$ revealed consistent patterns across methods, although the OSSs varied depending on the measured attribute and cultivar.

Table 3. Optimal sample sizes (OSSs) determined via the maximum curvature points (MCPs) for estimating the means of postharvest quality attributes in four acerola (*Malpighia emarginata* DC.) cultivars.

Quality Attribute	Cultivar	General Curvature Function Method		Perpendicular Distances Method		Linear Response Plateau Method	
		Maximum $CI_{95\%}$	Sample Size	Maximum $CI_{95\%}$	Sample Size	Maximum $CI_{95\%}$	Sample Size
Weight	BRS Rubra	2.2500	2	0.9000	12	0.7718	17
	Cabocla	2.9101	2	1.1677	13	1.0289	18
	Costa Rica	2.8650	2	1.1022	14	0.8460	25
	Junko	2.4300	2	0.8259	17	0.8195	18
Diameter	BRS Rubra	4.4800	2	1.5306	17	1.4430	20
	Cabocla	3.6200	2	1.3140	15	1.2042	19
	Costa Rica	3.4400	2	1.3247	13	1.0855	20
	Junko	3.8601	2	1.3460	15	1.2483	18
Length	BRS Rubra	3.9750	2	1.4093	15	1.4056	16
	Cabocla	2.6300	2	1.0385	13	0.7838	24
	Costa Rica	2.3533	3	1.0427	15	1.0253	17
	Junko	3.7800	2	1.4362	13	1.1601	20

Table 3. *Cont.*

Quality Attribute	Cultivar	General Curvature Function Method		Perpendicular Distances Method		Linear Response Plateau Method	
		Maximum $CI_{95\%}$	Sample Size	Maximum $CI_{95\%}$	Sample Size	Maximum $CI_{95\%}$	Sample Size
Color	BRS Rubra	9.1000	2	3.0335	17	2.9585	19
	Cabocla	9.1900	2	3.4947	15	2.8609	23
	Costa Rica	11.7700	2	4.4723	13	3.8190	19
	Junko	8.6701	2	3.3101	14	2.7182	21
Firmness	BRS Rubra	6.0200	2	1.9956	18	1.9837	19
	Cabocla	5.7302	2	1.9960	15	1.7230	21
	Costa Rica	6.8500	2	2.3560	17	2.7732	13
	Junko	3.4267	3	1.5387	15	1.2068	25
Vitamin C content	BRS Rubra	409.5100	2	158.5673	14	120.8430	24
	Cabocla	716.1900	2	252.1048	16	219.8540	22
	Costa Rica	694.9400	2	257.1668	15	211.3904	23
	Junko	1033.8350	2	371.7634	15	357.5112	17
SSC	BRS Rubra	3.1704	2	1.1131	16	1.2250	14
	Cabocla	3.6500	2	1.3468	15	1.0000	28
	Costa Rica	3.2000	2	1.2250	12	0.9650	20
	Junko	1.7500	2	0.6267	15	0.5062	24
TA	BRS Rubra	0.3100	2	0.1127	15	0.1005	20
	Cabocla	0.3100	2	0.1267	12	0.1207	14
	Costa Rica	0.4050	2	0.1450	16	0.1146	26
	Junko	0.2750	2	0.1046	13	0.0796	24
SSC/TA ratio	BRS Rubra	8.5250	2	2.9953	15	2.9557	16
	Cabocla	5.1802	3	2.2407	16	1.8931	23
	Costa Rica	6.6561	2	2.1861	18	2.1285	20
	Junko	1.9850	2	0.7080	15	0.5918	22

The GCF method consistently suggested smaller OSSs (2–3 fruit) compared to the other methods, accompanied by the highest $CI_{95\%}$ width (Table 3), suggesting that this method may underestimate the required sample size for a reliable inference. In contrast, PD and LRP provided more conservative estimates for the MCP. The PD method estimated OSSs between 12 and 18 fruit (Table 3), effectively balancing the precision and sampling effort by identifying the 'elbow' point on the $CI_{95\%}$ width curve where further increases in the sample size yield diminishing returns in precision (Figure 4).

The most conservative method was the LRP, which recommended larger sample sizes, particularly for SS (14–28 fruit), firmness (13–25 fruit), vitamin C (17–24 fruit), and the SSC/TA ratio (16–23 fruit) (Table 3). This method reflects a more rigorous criterion, defining the OSS as the point where further increases in sample size yield negligible reductions in $CI_{95\%}$ width.

In general, chemical traits (vitamin C content, SSC, TA, and SSC/TA ratio) required larger sample sizes to reach acceptable $CI_{95\%}$ widths (Table 3 and Figure 4). The SSC in 'Cabocla' required the largest sample size (28 fruit) among the evaluated parameters, despite exhibiting a relatively low CV (11.46%). This may be explained by its high $CI_{95\%}$ (Figure 4), which resulted in cultivar-specific increases in the number of samples required to achieve the desired level of estimation precision.

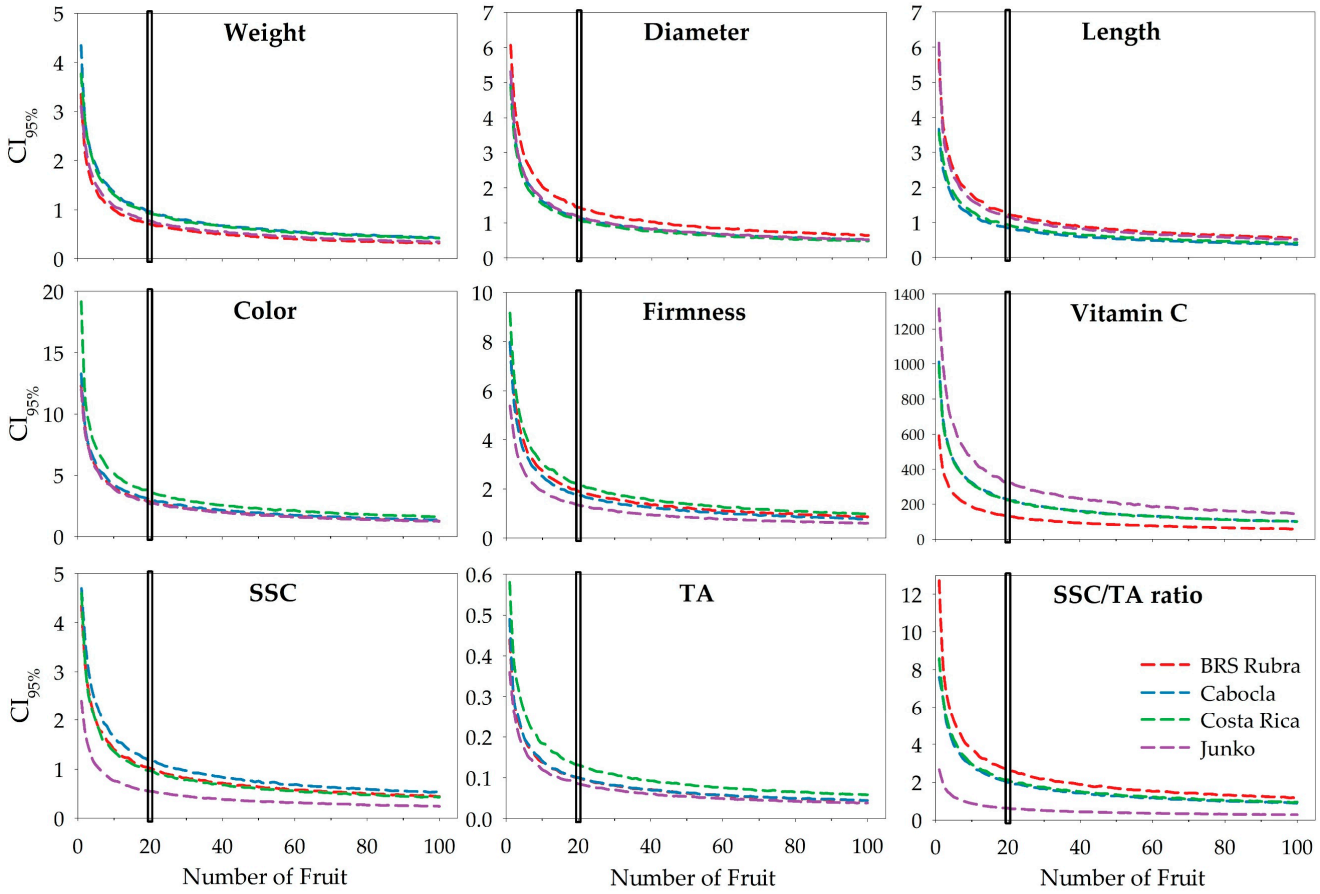

Figure 4. The 95% confidence interval ($CI_{95\%}$) widths for quality traits of four acerola (*Malpighia emarginata* DC.) cultivars. The vertical line represents the proposed OSS (20 fruit).

Differences in OSSs were also observed among the cultivars, especially with the LRP method. For instance, the cultivar Cabocla generally required larger sample sizes for several attributes based on the LRP method—particularly in terms of length (24 fruit), color (23 fruit), SSC (28 fruit), and SSC/TA ratio (23 fruit)—possibly due to greater heterogeneity among its fruit. In contrast, 'BRS Rubra' required comparatively smaller sample sizes for the same attributes—16, 19, 14, and 16 fruit, respectively—suggesting a higher degree of uniformity among its sampled fruit (Table 3).

Considering the OSS values estimated for the different traits and cultivars, a sample size of 20 fruit was highlighted as a practical reference point (Figure 4). This value falls within the range of OSSs recommended for all traits and cultivars when using the PD method and for most traits and cultivars when using the LRP method (Table 3), providing a balance between statistical precision and experimental feasibility. From this number onward, increasing the sample size yields diminishing returns in precision, as reflected by the $CI_{95\%}$ values (Figure 4), thus making the use of larger sample sizes unnecessary.

4. Discussion

SSC, vitamin C, TA, and fruit weight are among the most frequently evaluated quality traits in studies involving acerola, as identified in a recent systematic review [28]. These parameters are considered primary indicators of fruit quality, as they are essential for classifying acerola fruit for two main purposes: (1) vitamin C extraction, which prioritizes fruit harvested at the green maturity stage due to their higher ascorbic acid content; and (2) fresh consumption or industrial processing for frozen pulp or juice, which typically involves fruit harvested at the red-ripe stage [4,29].

Our study focused on red-ripe fruit intended for fresh consumption and industrial processing. These fruit are generally characterized by a higher SSC combined with lower TA and reduced vitamin C concentrations [23]. Besides vitamin C being a desirable nutrient, studies have shown that it is positively correlated with the TA in acerola [23,30,31]. Thus, acerola fruit rich in vitamin C tend to present a sourer taste. From a sensory perspective, lower acidity combined with a higher sugar content enhances the perception of sweetness, which is a key factor influencing consumer acceptance [32]. In addition to flavor, other traits such as larger fruit weight, diameter, and length are desirable, as they are associated with a higher pulp yield and improved efficiency during industrial de-pulping and processing [31]. High pulp firmness is also a valuable attribute, as it contributes to the resistance to mechanical damage, extended shelf life, and improved fruit handling and transport [33].

As the acerola is naturally acidic, cultivars must present a minimum SSC/TA ratio of around 10 to be considered suitable for fresh consumption, which is often used as a threshold for acceptable flavor balance [28]. In this context, our results confirmed that the 'BRS Rubra', 'Cabocla', and 'Costa Rica' cultivars—all previously selected by Ferreira et al. [23] as promising cultivars for fresh consumption—exhibited SSC/TA ratios above this threshold; particularly, 'BRS Rubra' stood out with the highest ratio (16.4), indicating a more favorable sweetness-to-acidity balance. Notably, the 'BRS Rubra' has previously demonstrated a high consumer acceptance in sensory evaluations involving multiple acerola cultivars [34], a finding that is supported by our results regarding its favorable SSC/TA ratio, low acidity, and acceptable levels of firmness and fruit size.

In contrast, 'Junko'—which is the most widely cultivated acerola in the SFV [24]—presented the highest vitamin C content, which is consistent with previous reports [29,35–37]. Despite the high nutritional value of 'Junko' acerolas, their pronounced acidity and lower SSC suggest a less favorable flavor profile for direct consumption, especially when compared to the other cultivars [29]. Therefore, this cultivar is more suitable for the industrial extraction of vitamin C, meeting the demands of the food and pharmaceutical industries that require natural sources of ascorbic acid. These findings highlight a common trade-off between nutritional and sensory attributes in acerolas, which should be carefully considered depending on the target use of the fruit.

While the selection of cultivars for fresh consumption or industrial use should be guided by their sensory and nutritional attributes, it is equally important to consider the inherent biological variability of these traits. In this regard, the precision of quality assessments depends on the adequacy of the sampling strategy. However, sample sizes have generally been determined empirically in the numerous studies on acerola published in recent years [4], revealing a clear lack of standardization in this regard within the scientific literature. In some studies, the number of fruit evaluated per experimental unit was relatively high, as observed in the studies of Magalhães et al. [30], who assessed 60 fruit per replicate, and Nogueira et al. [38], who used 50 fruit per plot. Matsuura et al. [39] adopted an intermediate sample size, evaluating 30 fruit per replicate. In contrast, Lima et al. [40], Ferreira et al. [23], and Farinelli et al. [41] each assessed only 10 fruit per replicate. This inconsistency in sampling strategies across studies underscores the need for a more systematic and statistically grounded approach.

To address this issue, our study is the first to propose an OSS for acerola fruit based on nonlinear power models and MCP methods, offering a robust approach for improving the accuracy and efficiency of postharvest evaluations. The application of nonlinear power models proved effective for modeling the relationships between sample size and $CI_{95\%}$ width, with all models presenting excellent fits ($R^2 \geq 0.9910$). These results confirm the suitability of this approach for sample size estimation in fruit quality studies, as supported

by previous studies in the field of plant science [19–21]. The power model-based approach allows for the identification of the point of diminishing returns in precision, which is critical for balancing accuracy with labor and cost in experiments.

Among the approaches for determining the MCP, the PD and LRP methods were more conservative and robust than the GCF, which is in agreement with previous findings [21]. The GCF tended to underestimate the required sample sizes, suggesting values as low as 2–3 fruit—a number that is unrealistically low and should not be adopted in practice due to the high risk of compromising the representativeness and reliability of the estimates. The OSS estimated using the PD method ranged from 12 to 18 fruit, while the LRP method was more conservative, recommending sample sizes between 17 and 28 fruit for evaluating fruit quality traits in acerolas. Both methods provided representative and reliable OSS estimates, with only small differences in the maximum $CI_{95\%}$ values. Therefore, although the LRP provided slightly narrower confidence intervals, the gain in precision was relatively small, suggesting that either approach can be appropriately used for estimating the OSS in this context.

The chemical traits assessed (vitamin C, SSC, TA, and SSC/TA ratio) required the largest sample sizes. Previous studies focused on other tropical fruits such as mango [8], wild passion fruit [10], and seriguela [13] have also reported larger OSSs for TA and the SSC/TA ratio while, in the cashew apple, a high OSS was observed for vitamin C [14]. In contrast to trends typically described in the literature [8–13], SSC in the 'Cabocla' cultivar required a large sample size of 28 fruit when using the LRP method, which is unusual for this variable given its typically low CV (11.46%). This result may be explained by its high confidence interval, which may have contributed to increased sampling variability in this specific cultivar.

While Bittencourt et al. [21] aimed to estimate the ANOVA-based overall experimental mean for each trait of cauliflower seedlings using different sample size determination methods, their approach did not account for genotypic differences, as the evaluation was performed across a single cultivar. In our study, we adopted a different strategy by determining the OSSs individually for each acerola cultivar. This approach allowed us to identify inter-cultivar variability in the magnitude of the sample size required for the accurate estimation of postharvest quality traits. The LRP method revealed that 'Cabocla' consistently demanded more fruit for the stable estimates of most traits, suggesting greater within-cultivar variability, while 'BRS Rubra' exhibited more uniformity and, consequently, required fewer samples.

From a practical standpoint, the findings suggest that adopting a sample size of 20 fruit per cultivar represents a reasonable compromise. This value falls within the range recommended by both the PD and LRP methods for most traits and cultivars, ensuring adequate precision without incurring excessive labor or resource demands. Such guidance is particularly valuable for experimental trials, breeding programs, and quality control, where the precise estimation of fruit quality traits must be balanced with the need for cost-effective methodologies.

Considering that the presented findings are well supported by robust statistical methods, the results on OSS could inform future postharvest research standards for acerola, particularly in terms of quality trait evaluations, the assessment of fruit shelf life and responses to postharvest treatments, comparison and selection of cultivars for different purposes (fresh consumption and industrial processing), determination of harvest timing on the farm, industrial quality control through standardized sampling for pulp yield or vitamin C extraction, and sensory research to ensure representative sample sizes in consumer acceptance trials.

As the estimation of the OSS depends on the modeling of trait variability and confidence interval behaviors rather than species-specific factors, this approach provides a robust framework to guide sample size determination across different fruit species. Nonetheless, due to intrinsic differences in variability patterns among fruit types, it is recommended that OSS estimations be conducted for each species or cultivar individually to ensure precise and reliable quality evaluations. Thus, while the statistical approach is broadly applicable, the exact sample size requirements should be validated in each case.

Although our study provides a robust framework for determining cultivar-specific sample sizes in acerola, some limitations should be acknowledged. The analyses were based solely on genotypic variation without accounting for other influential physiological and harvest-related factors such as fruit maturity stage or harvest season, which may also affect the sampling variability and should be considered in future studies. In addition, this study evaluated only four cultivars, and future research should expand the number of genotypes assessed to enhance the generalizability of the findings.

While we employed the MCP to define OSSs for individual genotypic means, further investigations could explore the sample size estimation based on precision statistics within the framework of the analysis of variance (ANOVA), as demonstrated in previous studies [42,43]. These additional approaches may offer complementary insights and increase the applicability of the sampling recommendations to a broader range of experimental conditions and objectives. Additionally, emerging computational approaches such as machine learning could be incorporated to improve the sample size prediction performance, particularly in studies involving complex interactions among multiple factors or high-dimensional datasets [44]. Integrating machine learning-based evaluation criteria can enhance both the accuracy and adaptability of experimental designs in postharvest research, thereby optimizing resource use and increasing the reliability of outcomes.

5. Conclusions

This study provides practical guidance for determining the minimum number of acerola fruit required to reliably estimate postharvest quality traits, recommending a sample size of 20 fruit as a balanced compromise between precision and feasibility. This recommendation is supported by robust nonlinear power models and MCP methods, with the PD and LRP approaches yielding consistent estimates. Notably, chemical traits such as vitamin C, SSC, TA, and the SSC/TA ratio demanded larger sample sizes, reflecting their inherent biological variability. For most traits, 'Cabocla' required the highest OSS, whereas 'BRS Rubra' required the lowest, suggesting inter-cultivar variability in OSSs.

Our comparative analysis revealed that 'BRS Rubra', 'Cabocla', and 'Costa Rica' exhibit phenotypic advantages for fresh consumption and industrial processing, including higher SSC/TA ratios and favorable physical attributes, while 'Junko' remains the most suitable cultivar for vitamin C extraction due to its high ascorbic acid content.

Importantly, this study introduced a novel and statistically sound framework for designing postharvest experiments focused on tropical fruits, addressing a methodological gap in the existing literature. Integrating bootstrap resampling, nonlinear modeling, and the MCP approach, the proposed methodology enhances the reliability and efficiency of sample size determination in postharvest research.

The presented findings offer insights that may support future applications in postharvest quality assessments and contribute to the development of studies focused on human nutrition and the food and supplement industries. Future research may further expand the applicability of these recommendations by accounting for seasonal and maturity-related variability. In addition, the use of machine learning approaches could be explored to

enhance the prediction of optimal sample sizes in postharvest studies, particularly when dealing with complex or high-dimensional datasets.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11080941/s1, **Table S1**. Climatic data in the experimental area during September 2022. Source: Agrometeorological Station of Bebedouro, Petrolina, Pernambuco, Brazil (09°09′ S, 40°22′ W). **Table S2.** *p*-values from the Shapiro–Wilk normality test for postharvest quality attributes in four acerola (*Malpighia emarginata* DC.) cultivars.

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Abbreviations

The following abbreviations are used in this manuscript:

OSS	Optimal sample size
SFV	São Francisco Valley, Brazil
SD	Standard deviation
MCP	Maximum curvature point
SSC	Soluble solids content
TA	Titratable acidity
$CI_{95\%}$	95% confidence interval
GCF	General curvature function
PD	Perpendicular distances
LRP	Linear response plateau

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