



**STATE UNIVERSITY OF SOUTHWEST BAHIA
GRADUATE PROGRAM IN AGRONOMY
AREA OF CONCENTRATION: CROP SCIENCE**

**ACEROLA (*Malpighia emarginata* DC.):
BREEDING FOR FRUIT POSTHARVEST QUALITY AND
CHARACTERIZATION OF BIOACTIVE COMPOUNDS**

JOÃO CLAUDIO VILVERT

**VITÓRIA DA CONQUISTA
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CHARACTERIZATION OF BIOACTIVE COMPOUNDS**

Thesis presented to the State University of Southwest Bahia, as part of the requirements of the Graduate Program in Agronomy, area of concentration in Crop Science, to obtain the title of Doctor.

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DEDICATION

To my grandpa, Vô João (*in memorian*),
the first fruit grower I met.

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“Knowledge has a beginning but no end.”

Geeta Iyengar

GENERAL ABSTRACT

VILVERT, J. C. **Acerola (*Malpighia emarginata* DC.): breeding for fruit postharvest quality and characterization of bioactive compounds.** Vitória da Conquista – BA, UESB, 2024. 214 p. (Thesis: Doctor Science in Agronomy; Area of Concentration: Crop Science).*

The acerola is a tropical berry known as one of the world's richest natural sources of vitamin C. Despite global interest in acerola as a superfruit, investigations are necessary to elucidate the genetic variability in the fruit quality traits. Additionally, understanding the genetic influence on antioxidant compounds can be very useful for the genetic improvement of acerola, focusing on enhancing the fruit nutritional value. This thesis was structured into five chapters, designed to achieve the following objectives: i) provide an overview of the influence of genetic variability on the quality traits and bioactive compounds of acerola, based on a systematic review; ii) synthesize aspects related to phenolic compounds of acerola fruits and by-products, including the identification and quantification of phenolic compounds, influencing factors, and biological properties; iii) estimate genetic parameters and the coefficient of repeatability and determine the ideal number of fruits for quality trait analyses in different acerola genotypes using different approaches; iv) evaluate the genetic diversity of acerola genotypes based on fruit quality traits at different maturity stages; and v) investigate the genotypic and seasonal effects on the quality traits, bioactive compounds, and antioxidant activity of acerolas. The systematic review revealed high variation among acerola genotypes in the physicochemical quality traits and bioactive compounds evaluated in different studies. At least 76 phenolic compounds have been previously identified in acerola, with several biological properties. Based on two experiments conducted with acerola genotypes belonging to the Active Germplasm Bank of Embrapa Semi-Arid Region, genetic parameters were estimated, indicating favorable conditions for acerola selection. A total of 17 fruits are necessary for the effective selection of acerola genotypes, with 95% accuracy. The results revealed high genetic diversity for different quality traits and maturity stages, with Okinawa being the most divergent in green fruits with greater mass and firmness, and BRS Rubra the most divergent in red fruits with higher soluble solids (SS) content and SS/titratable acidity (TA) ratio. The SS/TA ratio was the trait that most contributed to the genetic diversity of the fruits. Additionally, the fruit quality of three cultivars pre-selected for fresh consumption (BRS Rubra, Cabocla, and Costa Rica) and one commercial cultivar (Junko) was evaluated in different seasons and showed significant genotypic and seasonal influence. The bioactive compounds and antioxidant activity of these cultivars, in turn, were mainly affected by genotypic variations. Therefore, it should be feasible for genetic improvement programs focused on developing advanced acerola cultivars rich in bioactive compounds to succeed, considering the greater influence of genotype compared to seasonal conditions on bioactive components.

Keywords: plant breeding; multivariate statistics; genetic variability; phenolic compounds; fruit quality.

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RESUMO GERAL

VILVERT, J. C. **Acerola (*Malpighia emarginata* DC.): melhoramento da qualidade pós-colheita dos frutos e caracterização dos compostos bioativos.** Vitória da Conquista – BA, UESB, 2024. 214 p. (Tese: Doutorado em Agronomia; Área de Concentração: Fitotecnia).[†]

A acerola é uma fruta tropical conhecida como uma das maiores fontes naturais de vitamina C no mundo. Apesar do interesse global na acerola como superfruta, investigações são necessárias para elucidar a variabilidade genética nos atributos de qualidade dos frutos. Além disso, entender a influência genética nos compostos antioxidantes pode ser muito útil para o melhoramento genético da acerola, com foco no aumento do valor nutricional dos frutos. Esta tese foi estruturada em cinco capítulos, construídos para alcançar os seguintes objetivos: i) fornecer uma visão geral da influência da variabilidade genética nos atributos de qualidade e compostos bioativos da acerola, a partir de uma revisão sistemática; ii) sintetizar aspectos relacionados aos compostos fenólicos dos frutos e subprodutos da acerola, compreendendo a identificação e quantificação de compostos fenólicos, fatores de influência e propriedades biológicas; iii) estimar os parâmetros genéticos e o coeficiente de repetibilidade e determinar o número ideal de frutos para análises de atributos de qualidade em diferentes genótipos de acerola, usando diferentes abordagens; iv) avaliar a diversidade genética de genótipos de acerola, com base em atributos de qualidade dos frutos em diferentes estágios de maturação; e v) investigar os efeitos genotípicos e sazonais nos atributos de qualidade, compostos bioativos e atividade antioxidante de acerolas. A revisão sistemática revelou uma alta variação entre genótipos de acerola nos atributos de qualidade físico-química e compostos bioativos avaliados em diferentes estudos. Ao menos 76 compostos fenólicos já foram previamente identificados em acerola, com diversas propriedades biológicas. Com base em dois experimentos realizados com genótipos de acerola pertencentes ao Banco Ativo de Germoplasma da Embrapa Semiárido, foram estimados os parâmetros genéticos, que indicaram condições favoráveis para a seleção de acerola. Um total de 17 frutos é necessário para a seleção efetiva de genótipos de acerola, com uma precisão de 95%. Os resultados revelaram uma alta diversidade genética para diferentes atributos de qualidade e estágios de maturação, sendo Okinawa o mais divergente em frutas verdes, com maior massa e firmeza, e BRS Rubra o mais divergente em frutas vermelhas, com maior teor de sólidos solúveis (SS) e relação SS/acidez titulável (AT). A relação SS/AT foi o atributo que mais contribuiu para a divergência genética dos frutos. Adicionalmente, a qualidade dos frutos de três cultivares pré-selecionadas para consumo *in natura* (BRS Rubra, Cabocla e Costa Rica) e uma cultivar comercial (Junko) foi avaliada em diferentes estações do ano, e apresentou grande influência genotípica e sazonal. Os compostos bioativos e a atividade antioxidante destas cultivares, por sua vez, foram afetados principalmente por variações genotípicas. Portanto, deve ser viável que programas de melhoramento genético focados no desenvolvimento de cultivares avançadas de acerola ricas em compostos bioativos tenham sucesso, considerando a maior influência do genótipo em comparação com as condições sazonais nos componentes bioativos.

Palavras-chave: melhoramento de plantas; estatísticas multivariadas; variabilidade genética; compostos fenólicos; qualidade dos frutos.

[†]**Orientadora:** Profa. Dra. Cristiane Martins Veloso, UESB.

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LIST OF ABBREVIATIONS, INITIALS AND SYMBOLS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AGB	Active Germplasm Bank
ANN	Artificial neural network
ANOVA	Analysis of variance
AOX	Antioxidant activity
AsA	Ascorbic acid
C*	Chroma
CN	Condition number
CV	Coefficient of variation
CV _e	Experimental coefficient of variation
CV _g	Genetic coefficient of variation
dm	Dry mass
DPPH	2,2-diphenyl-1-picrylhydrazyl
EAE	Enzyme-assisted extraction
fm	Fresh mass
FM	Fruit mass
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic acid equivalent
h ²	Heritability
HPLC	High-performance liquid chromatography
ISSR	Inter Simple Sequence Repeats
L*	Luminosity/lightness
LOD	Limit of detection
LOQ	Limit of quantification
MCMC	Monte Carlo via Markov chains
MIC	Minimum inhibitory concentration
MJ	Megajoules
Mnt	Montmorillonite
MS	Mass spectrometry
N	Newtons
ORAC	Oxygen Radical Absorbance Capacity
PCA	Principal component analysis
PCA-Cor	Principal component analysis based on a correlation matrix
PCA-Cov	Principal component analysis based on a covariance matrix
PF	Pulp firmness
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analysis
<i>r</i>	Coefficient of correlation
R ²	Coefficient of determination
RAPD	Random Amplified Polymorphic DNA
ROS	Reactive oxygen species
RP	Reverse phase

SA-Cor	Structural analysis based on a correlation matrix
SFV	São Francisco Valley
SS	Soluble solids
SSC	Soluble solids content
SWE	Subcritical water extraction
TA	Titrateable acidity
TPC	Total phenolic compounds
TPTZ	Tripyridyltriazine
UAE	Ultrasound-assisted extraction
UPGMA	Unweighted pair group method with arithmetic mean
USD	United States dollar
UV	Ultraviolet
VIF	Variance inflation factor
η_0	Number of fruits

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GENERAL INTRODUCTION

Acerola (*Malpighia emarginata* DC.), also known as Guarani cherry, Barbados cherry, West Indian cherry, or Antilles cherry, is a tropical fruit native to Central and South America. Acerola has been cultivated in tropical and subtropical regions, including Brazil, which is the largest producer, consumer, and exporter of acerola globally (RIBEIRO; FREITAS, 2020; CARNEIRO FERREIRA et al., 2021; MISKINIS; NASCIMENTO; COLUSSI, 2023).

Since the late-1940s, acerola has been recognized as a superfruit, for being one of the largest natural sources of vitamin C in the world (about 1,500–4,000 mg/kg), with content up to 100 times higher than that found in oranges and lemons (FERNANDES et al., 2023; OŁĘDZKI; HARASYM, 2024). In addition to vitamin C, acerola contains many other phytonutrients, including amino acids, carotenoids, phenolic compounds, and minerals, possessing several health-promoting properties (CHANG; ALASALVAR; SHAHIDI, 2019; XU et al., 2020; MEDEIROS et al., 2024).

The cultivation of the acerola tree in Brazil began in 1955 when seeds imported from Puerto Rico were introduced into an experimental orchard in the state of Pernambuco, located in the Northeastern region (RIBEIRO et al., 2023). The acerola tree has adapted well to the soil and semi-arid climate conditions of this region, with up to eight production cycles per year under irrigation (CARNEIRO FERREIRA et al., 2021).

Over the last decades, Brazil has started to exploit the commercial potential of acerola fruits, establishing a structured agro-industrial based market, including the presence of multinational companies (PRAKASH; BASKARAN, 2018; POLETTTO et al., 2021). However, several commercial plantations were established by sexually propagated seedlings, resulting in a high genetic variability in these plantations, with uneven fruit quality and low productivity (RITZINGER et al., 2018).

In order to preserve the genetic variability and to prompt the assessment and indication of promising genotypes with favorable agronomic traits and fruit quality under tropical semi-arid conditions, an acerola Active Germplasm Bank (AGB) was established in 2012, by the Brazilian Agricultural Research Corporation, Brazil. Among the priority characteristics in the selection of acerola genotypes are high

fruit quality, high productivity, and resistance to diseases caused by nematodes (SOUZA et al., 2017; SANTOS et al., 2021; FERREIRA et al., 2022).

Acerola fruit quality is defined according to the market destination: green fruit for ascorbic acid extraction, or red fruit for fresh consumption and processing. Genotypes destined for industrial ascorbic acid extraction and production of pharmaceutical or concentrates need to exhibit abundant levels of this compound in fruits, harvested at the green maturity stage, known for its peak concentration. Conversely, genotypes designated for fresh consumption or the production of value-added products such as frozen pulp and juice are characterized by larger and firmer red fruits, as well as higher soluble solids content and lower acidity, ensuring the desired sweetness for consumers (MOURA et al., 2018; SANTOS et al., 2019). Although fruit sweetness varies with the cultivar, few commercial varieties of acerola are sweet, while most are acidic (PRAKASH; BASKARAN, 2018).

An acerola breeding program to improve fruit quality, as in other fruit crops, uses genetic diversity to develop new and improved crop cultivars. Genetic diversity refers to the range of genetic characteristics present within a crop species and allows plant breeders to identify superior genotypes with potential for the development of genetic stock for hybridization programs or the release of a crop variety (CRUZ; FERREIRA; PESSONI, 2020; SALGOTRA; CHAUHAN, 2023).

In an agricultural scientific research, up-to-date methodological schemes are indispensable for designing experiments and conducting statistical analyses. From the analysis of variance (RESENDE; ALVES, 2022; CARVALHO et al., 2023), developed in 1918, to recent computational models based on artificial intelligence (NAYAK et al., 2020; NIAZIAN; NIEDBAŁA, 2020), a diverse array of classical and modern statistical approaches is available for managing and analyzing data across different areas of agricultural research, including plant breeding.

Despite the importance of acerola as a superfruit, few studies focus on unraveling the genetic variations of fruit quality traits based on the genetic variability of acerola genotypes, which is essential for breeding programs. The expanding of acerola consumption is actually limited, between other factors, to low availability of cultivars for fresh consumption with high sweetness. Furthermore, even with high nutritional value of acerolas, research on the quality and concentration of bioactive compounds in acerola and its influencing factors falls short of the nutritional potential of this fruit.

In the present work, we applied different statistical approaches to the acerola breeding, aiming to improve fruit quality traits, especially those associated with fruit flavor and nutritional composition. This thesis is divided into five chapters, as follows: CHAPTER 1 consists of the accomplishment of a systematic literature review in order to explore the intra- and inter-study genotypic variations on different quality traits of acerola; CHAPTER 2 synthesizes previous researches on phenolic compounds in acerola fruits and by-products, with a detailed qualitative and quantitative investigation on the profiling and biological properties of phenolic compounds in acerola; CHAPTER 3 is a methodological approach for the assessment of the quality of different acerola genotypes in an acerola breeding program, based on the determination of repeatability using different approaches; CHAPTER 4 covers the assessment of genetic diversity in an acerola breeding program, offering insights about contrasting plants with potential for the development of new cultivars with desirable quality traits in different fruit maturity stages; and CHAPTER 5 explores the genotypic and seasonal variations that affect fruit quality traits and antioxidant compounds, providing perspectives about the selection of genotypes in an acerola breeding program based on bioactive compounds of fruits.

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ARTICLE I

Genetic diversity on acerola quality: a systematic review*

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Genetic diversity on acerola quality: a systematic review

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ABSTRACT

Acerola (*Malpighia emarginata* DC.) is a super-fruit with high ascorbic acid content and its quality can be highly affected by the genetic and environmental conditions. This systematic review aims to provide an overview of the influence of genetic variability on acerola quality traits. It was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) recommendations. PubMed, Scopus and SciELO electronic databases were searched using terms *acerola*, *selection*, and *genotypes*. 29 studies were selected after assessing articles for eligibility criteria, which reported data on 22 quality parameters. A high genetic diversity was observed for all quality traits. Soluble solids and ascorbic acid contents are the main parameters determined in studies with acerola. Titratable acidity, SS/TA ratio and pH are also evaluated in most studies. Different studies have already identified possible genotypes for use in acerola breeding programs based on fruit quality, both for launching new cultivars and for use as parents in crosses. Our review is a useful basis for acerola breeding programs and germplasm conservation. Future studies are required to further identify and quantify bioactive compounds in acerolas of different genotypes.

Keywords: *Malpighia emarginata* DC.; plant breeding; genotypes; breeding methods; fruit quality; vitamin C.

1.1 INTRODUCTION

Acerola (*Malpighia emarginata* DC.), also known as Barbados cherry and West Indian cherry, is a tropical fruit native to Central and South America and the Caribbean Islands. It is considered a super-fruit due to its high ascorbic acid (vitamin C) content, which is up to 100 times higher than the content observed in orange and lemon [1], in addition to phytochemicals such as phenolic compounds and carotenoids.

Global demand for acerola has been increasing due to the fruit attractive color, pleasant flavor, and nutraceutical potential. With high potential as a functional food, acerola may be consumed fresh or used in the processing industry [2]. Acerola has a high appealing economic prospect for growers to reach the specific markets of natural health-promoting products that maintain health and prevent degenerative diseases [3].

Fruit quality is defined by appearance, flavor, texture, and nutritional properties, which might be affected by several factors, including environmental conditions, crop management practices, maturity stage at harvest, storage conditions and genotype [4]. Fruit quality is measured through physical and chemical properties, which can also be used to characterize maturity stage and indicate when fruit are ready for consumption [5].

Brazil is the largest producer, consumer, and exporter of acerola, especially in tropical regions with high temperatures and high sunlight incidence, ideal for production of high-quality fruit. In most of the Brazilian commercial orchards of acerola, trees have been propagated by sexual methods, which result in a high genetic variability and high orchard heterogeneity, since this crop has predominantly cross-pollination [6–8].

Genetic diversity is one of main factors that defines acerola quality traits, such as color, weight, soluble solids, acidity, and ascorbic acid [9]. According to Magalhães and coauthors [10], the need for acerola genetic breeding results from the lack of established commercial cultivars with high fruit quality and yield. The high genetic variability in acerola has stimulated the development of breeding programs [11], essential for the preservation of biodiversity and the maintenance of genetic variability for future development of new genotypes.

Considering the importance of acerola as a crop species, this systematic review aims to provide an overview about the influence of genetic variability on acerola quality traits.

1.2 MATERIAL AND METHODS

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [12]. Two reviewers independently performed a bibliographic search in PubMed, Scopus and SciELO electronic databases. The literature search was performed using the keywords *acerola*, *selection*, and *genotypes*. The word acerola was accomplished by Boolean operator "OR" and its scientific name (i.e., *Malpighia emarginata*, synonyms *Malpighia glabra* and *Malpighia puniceifolia*) and it was combined one-to-one with all other terms. References comprising both titles and abstracts were exported to Mendeley.

No restriction was applied to language and time limit on search. The last search was performed on July 2nd, 2022. Studies were included in the selection if they met all of the following criteria: (1) were articles published on peer-reviewed scientific journals; (2) evaluated acerolas (whole fruits) in ripe maturity stage, i.e., fruits with the skin completely covered with red color; and (3) evaluated at least one identified genotype. Exclusion criteria for study selection consisted of: (1) reviews, systematic reviews, meta-analyses, letters, conference abstracts, book chapters, personal opinions and articles of non-peer-reviewed journals; (2) evaluates acerola by-products or fruits in early maturity stages (green or intermediate stages); and (3) do not identify the genotype.

Potentially relevant articles were screened independently by two reviewers, initially by abstract and then by full text. Disagreements among reviewers were resolved by a third reviewer. Two authors independently used a standard data form to extract the relevant study information, including: author(s), publication year, Köppen's climate in study area, number of genotypes, and main values of physicochemical and antioxidant traits evaluated in the studies.

1.3 RESULTS

1.3.1 Study selection and characterization

The initial search through three electronic databases resulted in 1462 journal articles. Records were reduced for 1182 when duplicates were discarded. Of these latter, 40 articles were selected for full-text reading, and 29 were included in final review (Figure 1.1). Journal articles excluded from reading the title were $n = 1095$ and the abstract were $n = 47$. Articles excluded after full text review were $n = 11$: six for not presenting the studied genotype, one for characterizing only the industrial waste (bagasse) and not the fruits, one for characterizing only the isoenzymatic data and not the fruits, two for studying the repeatability of characteristics of the acerola in plants of unidentified genotypes and one for evaluating unripe fruits.

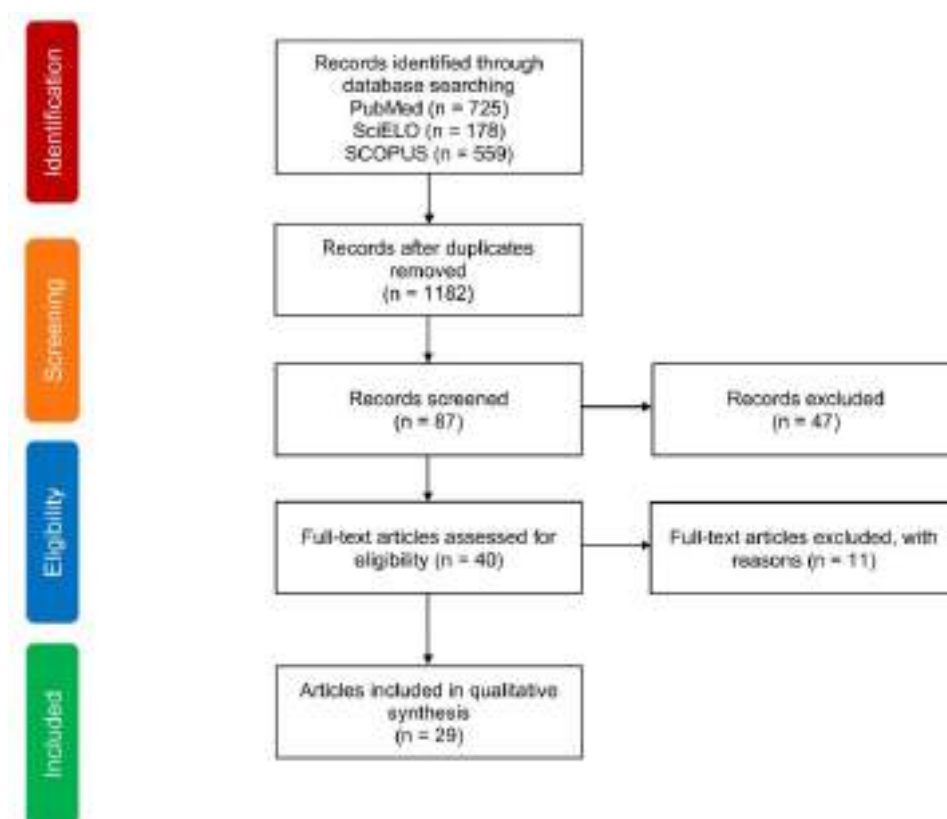


Figure 1.1. PRISMA flow chart showing the process of literature search and study selection.

Information on 22 physical, chemical and antioxidant properties of acerola were found and extracted from selected studies, namely soluble solids (SS), titratable acidity (TA), SS/TA ratio, pH, ascorbic acid, weight, diameter, length, color (expressed as lightness, a^* , b^* , chroma and hue angle), firmness, pulp yield,

moisture, reducing sugars, anthocyanins, yellow flavonoids, carotenoids, total phenolic compounds, and total flavonols (Table 1.1).

The number of genotypes identified in the studies varied between 1 and 103. ‘Flor Branca’, ‘Okinawa’ and ‘Olivier’ were the most evaluated genotypes, being present in seven, seven, and six studies, respectively. In the studies included in the systematic review, five types of climates were found, according to Koppen's classification: seven studies in As climate and six in Aw climate, both characterized as tropical savanna with drier season in summer (As) or in winter (Aw); six studies in Bsh climate (hot and dry semi-arid); six studies in Cwa climate and four studies in Cfa climate, both characterized by subtropical climates with hot summer, with dry winter (Cwa) or without dry season (Cfa).

Table 1.1. Characteristics of studies included in the systematic review assessing genetic diversity of acerola.

Reference	Study location	Climate	Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Adriano et al., 2011 [13]	Junqueirópolis, SP	Cwa	1	x ¹	x	x	x	x					x	x	x	x	x			x					
Batista et al., 2015 [14]	Petrolina, PE	Bsh	4		x	x	x	x		x	x	x	x			x	x	x							
Batista et al., 2018 [15]	Petrolina, PE	Bsh	4					x													x	x		x	x
Caetano et al., 2012 [16]	Junqueirópolis, SP	Cwa	1		x	x	x	x	x										x	x					
Carpentieri-Pípelo et al., 2000 [17]	Londrina, PR	Cfa	14		x			x	x	x	x							x							
Carpentieri-Pípelo et al., 2002 [18]	Londrina, PR	Cfa	3		x			x	x									x							
Cavalcante et al., 2007 [8]	Jaboticabal, SP	Cwa	16		x	x	x		x	x	x	x						x							
Farinelli et al., 2021 [19]	Mal. Cândido Rondon, PR	Cfa	103		x	x	x	x	x	x	x	x	x	x				x						x	
Ferreira et al. 2021 [20]	Petrolina, PE	Bsh	7		x	x	x	x	x	x		x	x	x	x	x				x	x		x	x	
Ferreira et al., 2022 [2]	Petrolina, PE	Bsh	35		x	x	x		x	x	x	x				x	x	x							
Gomes et al., 2000 [21]	Itápolis, SP	Aw	12		x			x	x	x	x	x						x							
Lima et al., 2000 [22]	Carpina, PE	Aw	6																		x		x		
Lima et al., 2005 [23]	Carpina, PE	Aw	12																					x	x
Maciel et al., 2010 [24]	Carpina, PE	As	18		x	x	x	x	x									x			x		x		
Magalhães et al., 2018 [10]	Jequitibá, MG	Cwa	24		x	x	x	x	x	x	x	x						x	x						
Mamede et al., 2009 [25]	Cruz das Almas, BA	As	3		x	x	x	x	x	x	x														
Mariano-Nasser et al., 2017 [26]	Adamantina, SP	Cwa	8		x	x		x	x			x				x	x			x		x		x	
Matsuura et al., 2001 [27]	Cruz das Almas, BA	As	12		x	x	x	x	x																
Moura et al., 2007 [6]	Fortaleza, CE	Aw	45		x	x	x	x	x	x							x				x				x
Musser et al., 2004 [28]	Carpina, PE	As	12		x	x	x	x	x												x		x		
Musser et al., 2005 [29]	Carpina, PE	As	12							x	x	x													
Nasser et al., 2018 [30]	Adamantina, SP	Cwa	7		x	x		x	x																
Nogueira et al., 2002 [31]	Paudalho, PE	As	2		x			x	x	x	x	x													
Reis et al., 2017 [32]	Petrolina, PE	Bsh	1		x	x		x	x										x						
Ribeiro & Freitas, 2020 [33]	Petrolina, PE	Bsh	2		x	x			x								x	x				x			
Rosso & Mercadante, 2005 [34]	Campinas, SP	Cfa	2																						x
Semensato & Pereira, 2000 [35]	Anápolis, GO	Aw	9		x			x	x	x	x	x							x						
Souza et al., 2014 [36]	Pacajús, CE	Aw	3		x	x	x	x	x												x	x	x		x
Viana et al., 2021 [37]	Cruz das Almas, BA	As	3		x	x	x	x	x			x					x	x		x	x			x	x

¹ The x represents quality parameters evaluated in each included study. 1. Soluble solids (SS); 2. Titratable acidity (TA); 3. SS/TA ratio; 4. pH; 5. Ascorbic acid; 6. Weight; 7. Diameter; 8. Length; 9. Lightness; 10. a*; 11. b*; 12. Chroma; 13. Hue angle; 14. Firmness; 15. Pulp yield; 16. Moisture; 17. Reducing sugars; 18. Anthocyanins; 19. Yellow flavonoids; 20. Total flavonols; 21. Total phenolic compounds; 22. Carotenoids.

1.3.2 Variation in fruit quality traits

1.3.2.1 Soluble solids, titratable acidity, SS/TA ratio, and pH

Soluble solids (SS), titratable acidity (TA), SS/TA ratio, and pH were evaluated in 83%, 66%, 52%, and 66% of the included studies, respectively (Table 1.1).

SS content of acerola genotypes varied between 5.2% and 15.8%, with mean values in each study ranging between 6.4% and 11.0% (Figure 1.2A). The lowest TA was 0.37 g of malic acid per 100 g, while the maximum TA was 3.82 g 100 g⁻¹. The mean TA in most of studies ranged between 0.78 and 1.70 g 100 g⁻¹, with exception of two studies (3.07 and 3.14 g 100 g⁻¹) (Figure 1.2B).

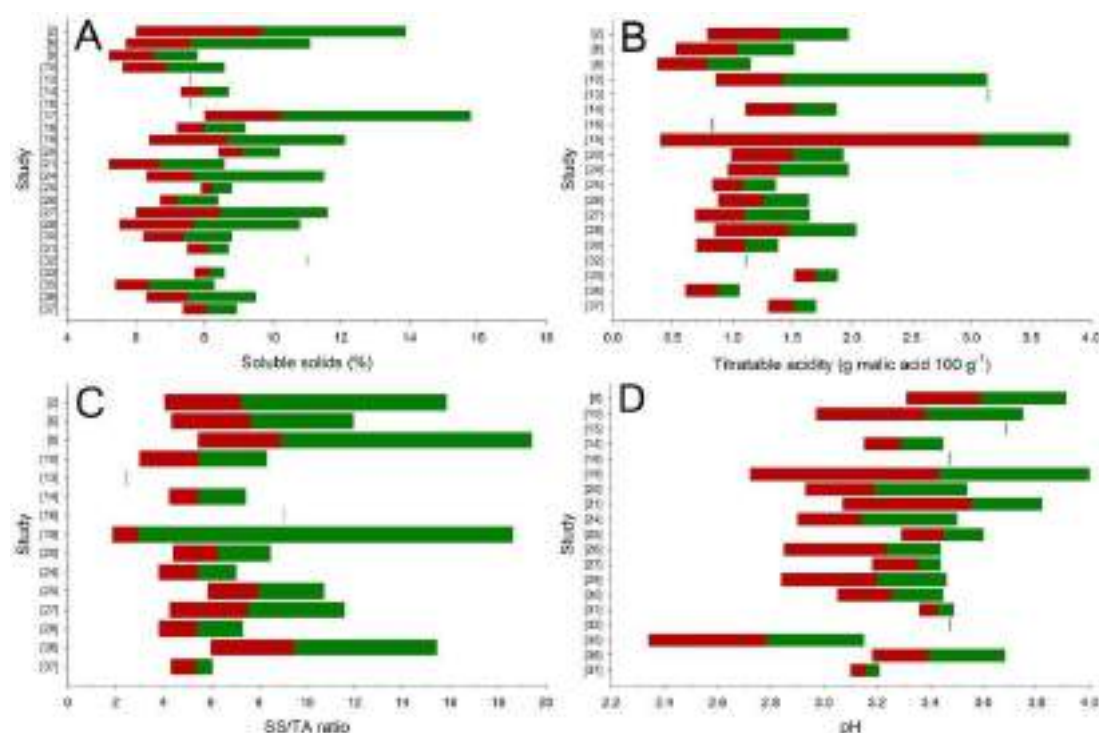


Figure 1.2. Soluble solids (SS) (A), titratable acidity (TA) (B), SS/TA ratio (C) and pH (D) of acerola genotypes as reported in selected studies.

The red and green areas in the bars represent genotypes with lower and higher values than the general mean of the physicochemical property in each study, respectively. For studies with only one genotype evaluated, the mean is represented by a vertical line.

In the 15 studies that evaluated SS/TA ratio, minimum and maximum values for this parameter were 1.86 and 19.39, respectively. The mean SS/TA ratio ranged between 2.41 and 9.46 (Figure 1.2C). The pH of acerola genotypes varied between 2.34 and 4.36 in selected studies, with a range of mean pH values of 2.78–3.68 (Figure 1.2D).

1.3.2.2 Ascorbic acid, weight, diameter, and length

The ascorbic acid content of acerolas was reported in 24 studies, which corresponds to 83% of included studies. Ascorbic acid content varied between 348 and 2800 mg per 100 g in acerola genotypes (Figure 1.3A).

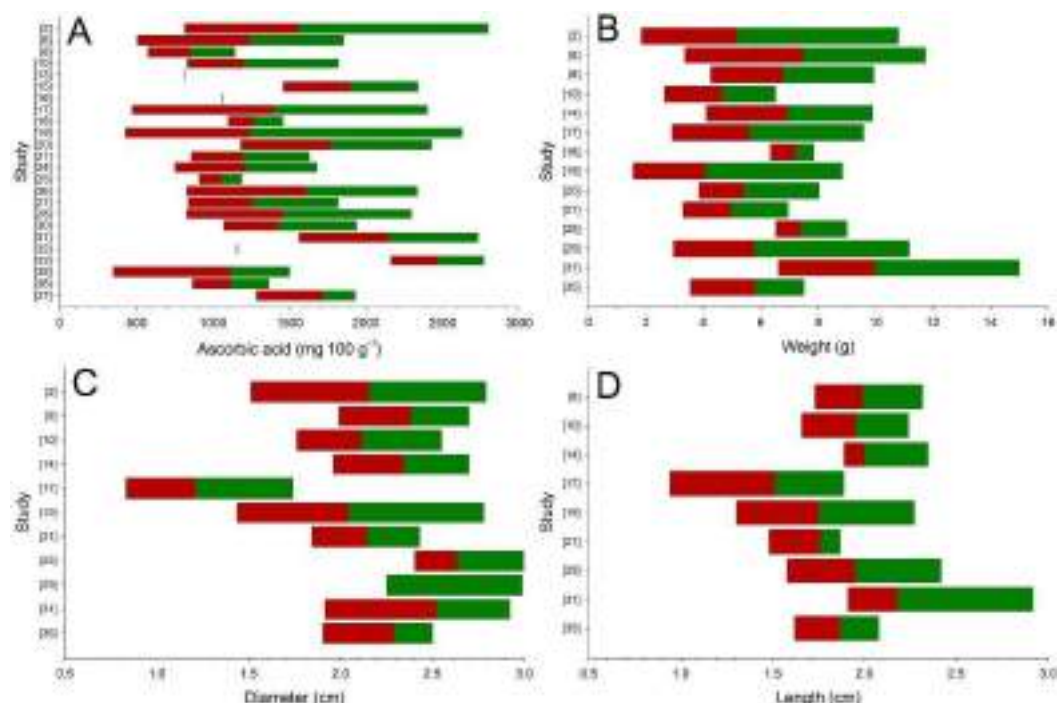


Figure 1.3. Ascorbic acid (A), weight (B), diameter (C), and length (D) of acerola genotypes as reported in selected studies.

The red and green areas in the bars represent genotypes with lower and higher values than the general mean of the physicochemical property in each study, respectively. For studies with only one genotype evaluated, the mean is represented by a vertical line.

Weight, diameter, and length were the most evaluated physical parameters in acerola genotypes, with results reported in 14, 11, and 9 included studies, respectively (Table 1.1).

Weight of acerola genotypes ranged between 1.53 g and 15.02 g, which represents a maximum weight 9.8 times greater than the minimum value. Mean weight had a variation between 4.08 g and 9.99 g (Figure 1.3B). Diameter (also called transversal diameter) had minimum and maximum values between 0.83 cm and 3.00 cm, respectively, with a range of mean values of 1.21–2.63 cm (Figure 1.3C). Length (or longitudinal diameter) varied between 0.94 cm and 2.92 cm, with mean values between 1.51 cm and 2.18 cm (Figure 1.3D).

1.3.2.3 Color, firmness, pulp yield, and moisture

The lightness was the most reported color parameter in selected studies, with seven results indicating a range between 19.40 and 55.90. Chroma and hue angle of acerola genotypes were quantified in six and seven studies, respectively, with variations of 16.36–55.84 and 9.17–60.80. The color spaces a^* and b^* were evaluated in three studies, with ranges of 11.30–46.60 and 3.00–48.90, respectively (Table 1.2).

Table 1.2. Color parameters, firmness, pulp yield and moisture of acerola genotypes as reported in selected studies.

Ref	Lightness	a^*	b^*	Chroma	Hue angle	Firmness (N)	Pulp yield (%)	Moisture (%)
[2]	32.47–51.83	-	-	27.66–53.29	19.45–45.82	5.02–20.02	-	-
[6]	-	-	-	-	-	2.56–6.48	-	-
[8]	-	-	-	-	-	-	38.58–61.21	-
[10]	-	-	-	-	-	-	67.17–81.76	90.04–92.36
[13]	40.12	37.16	21.70	43.15	30.08	-	-	-
[14]	20.48–23.68	-	-	16.36–22.33	9.17–14.04	2.14–2.62	-	-
[16]	-	-	-	-	-	-	-	92.49
[17]	-	-	-	-	-	-	61.36–86.18	-
[18]	-	-	-	-	-	-	72.10–82.50	-
[19]	19.40–55.90	11.30–46.60	3.00–48.90	-	-	-	57.20–90.10	-
[20]	33.18–44.22	36.14–46.20	12.95–29.98	38.64–55.07	19.00–32.98	-	-	-
[21]	-	-	-	-	-	-	47.80–58.90	-
[24]	-	-	-	-	-	-	53.17–72.54	-
[25]	-	-	-	-	-	-	-	-
[26]	23.30–31.80	-	-	19.45–33.70	13.10–26.10	-	-	-
[29]	-	-	-	-	-	-	-	-
[31]	-	-	-	-	-	-	-	-
[32]	-	-	-	-	-	-	-	90.75
[33]	-	-	-	-	24.90–60.80	13.60–19.20	-	-
[35]	-	-	-	-	-	-	-	90.67–93.30
[37]	34.48–37.71	-	-	43.43–55.84	21.31–30.08	-	-	91.86–93.86

Dash-separated values indicate the minimum and maximum for each parameter. In lines without a dash, the value represents the mean in studies with only one genotype evaluated.

The pulp firmness of acerolas varied between 2.14 N and 20.02 N in four reported studies. Pulp yield and moisture had ranges of 38.58–90.10% and 90.04–93.86%, respectively, in the seven and five studies that reported these parameters (Table 1.2).

1.3.2.4 Reducing sugars and bioactive compounds

Reducing sugars content of acerola genotypes was evaluated in six reported studies, with a minimum value of 2.32% and a maximum of 6.03% (Table 1.3).

Table 1.3. Reducing sugars and bioactive compounds of acerola genotypes as reported in selected studies.

Ref	Reducing sugars (%)	Anthocyanins (mg 100 g ⁻¹)	Yellow flavonoids (mg quercetin equivalent 100 g ⁻¹)	Total flavonols (mg quercetin equivalent 100 g ⁻¹)	TPC (mg 100 g ⁻¹)	Carotenoids (mg β-carotene 100 g ⁻¹)
[6]	-	1.52–28.47	-	-	-	0.34–8.41
[10]	-	-	-	-	-	-
[13]	5.73	-	-	-	-	-
[15]	-	7.03–13.80	6.80–10.73	-	850.26–1345.21 ^b	1.62–3.28
[16]	5.17	-	-	-	-	-
[19]	-	-	-	-	84.00–3196.00 ^b	-
[20]	2.57–3.80 ^a	14.99–68.23 ^a	-	3.92–8.96 ^a	1188.46–2590.40 ^b	-
[22]	-	14.06–50.98	-	9.31–20.22	-	-
[23]	-	-	-	-	737.00–1888.00 ^c	0.94–4.06
[24]	-	4.35–14.93	-	4.40–15.04	-	-
[26]	2.89–4.24	-	3.56–12.34	-	914.20–2428.30 ^b	-
[28]	-	2.40–55.80	-	5.90–22.20	-	-
[32]	-	-	-	-	-	-
[33]	-	6.79–7.28	-	-	-	-
[34]	-	-	-	-	-	3.71–18.81 ^a
[35]	-	-	-	-	-	-
[36]	2.32–6.03	5.07–12.37	7.36–9.82	-	1561.67–2631.34 ^b	-
[37]	3.56–4.97	-	-	-	410.82–764.22 ^b	0.45–1.58

TPC: Total phenolic compounds. ^aQuantified in high- performance liquid chromatography. ^bExpressed as gallic acid equivalents. ^cExpressed as catechin equivalents. Dash-separated values indicate the minimum and maximum for each parameter. In lines without a hyphen, the value represents the mean in studies with only one genotype evaluated.

For bioactive compounds, the number of studies per variable ranged from three for yellow flavonoids, to eight for anthocyanins. Even with the low number of studies reporting these variables, a large variation was observed between genotypes regarding the anthocyanins, yellow flavonoids, carotenoids, total flavonols, and total phenolic compounds (Table 1.3).

Anthocyanin content varied between 1.52 mg 100 g⁻¹ and 68.23 mg 100 g⁻¹. Contents of yellow flavonoids and total flavonols had ranges of 3.56–12.34 mg of quercetin equivalent 100 g⁻¹ and 3.92–22.20 mg of quercetin equivalent per 100 g⁻¹, respectively (Table 1.3).

Total phenolic compounds (TPC) content of acerola genotypes ranged between 84.00 and 3196.00 mg of equivalent gallic acid (GAE) per 100 grams. Carotenoids were quantified in five included studies and varied between 0.34 and 18.81 mg β -carotene 100 g⁻¹ (Table 1.3).

1.4 DISCUSSION

Among the tropical fruits, acerola is a highly attractive exotic fruit with high ascorbic acid content, adapted to different edaphoclimatic conditions with high potential for industrial use, which has been attracting the interest of fruit growers [31].

Brazil is the largest producer, consumer, and exporter of acerola. However, even with the production potential of this crop in Brazil, the implantation of commercial acerola orchards is considered recent, when compared to other fruit species, being formed mostly by plants with unidentified genetics, and which in most cases, do not present agronomic and commercial characteristics desirable for the fresh market. High genetic variability in commercial acerola orchards is a result of seed propagation, characterizing them as highly heterogeneous in terms of fruit quality and quantity, which takes place due to the lack of new genotypes with improved quality traits for fresh market [10].

Even with the importance of acerola as a crop species, this is the first time in the literature that data from quality of different acerola genotypes are gathered and discussed. From a systematic search and selection of peer-reviewed articles published in journals indexed to databases, we extracted information from 29 studies related to 22 physicochemical attributes and bioactive compounds of acerola from different genotypes.

In the selected studies, the number of evaluated genotypes varied between 1 and 103. 'Flor Branca', 'Okinawa' and 'Olivier' were the most present, in at least five studies each. 'Flor Branca' and 'Okinawa' are among the main acerola varieties produced in the São Francisco Valley (SFV) [38], which is the main acerola producing mesoregion in Brazil, occupying about 1/4 of the cultivated area with the crop in the country. Ideal edaphoclimatic conditions for acerola fruit development, including high solar radiation and high temperatures throughout the year, associated with the short cycle of 3–4 weeks between flowering and harvest, allow the production of up to eight annual crops in the SFV under irrigation [20].

'Flor Branca' is a cultivar with good productivity and high production regularity, characterized by low vigor plants and small fruits (<5 g). The fruits have low flesh firmness and high sensitivity to mechanical damage during handling and transport, as well as a very short shelf life [38]. 'Okinawa' acerolas are usually higher than other cultivars (5–9 g), with an attractive purple color and high ascorbic acid contents [39–40]; however, this cultivar presents low productivity and production irregularity as limiting factors.

Selected studies were classified in five different Köppen's climate conditions, including tropical savanna climate with a drier season in summer (As) or in winter (Aw), hot and dry semi-arid (Bsh), and subtropical climates with hot summer, with dry winter (Cwa) or without dry season (Cfa). Although the regions of the studies belong to three distinct climatic zones (A, B and C), all of them have high temperatures (≥ 18 °C) as a common factor, either throughout the year (A and B zones), or at least one month a year (C zone) [41]. As a typical species of tropical climate, acerola grows in temperatures between 15 °C and 32 °C and is well adapted to temperatures around 26 °C [42].

Soluble solids (SS) and ascorbic acid contents were the most reported quality traits, both present in 83% of selected studies. SS content represent substances that can be dissolved in water, mainly soluble sugars, influencing on sweetness perception by consumers. Thus, the selection of genotypes with high SS is essential in acerola breeding programs for fresh consumption [27]. Average SS of acerola genotypes in the selected studies varied between 6.4% and 11.0%, close when compared to other fruits rich in vitamin C, such as guava [43], lemon [44], and strawberry [45–46]. Some importing countries require a minimum SS content in acerolas, such as the European Union (7.0° Brix) and Japan (7.5° Brix) [47].

Titrateable acidity (TA) represents the organic acids that greatly affect fruit overall eating quality and flavor [48]. The major organic acid in acerolas is malic acid, although succinic, citric, and other acids are also detectable [20,49]. In selected studies, TA of genotypes ranged between 0.37 g and 3.82 g of malic acid 100 g⁻¹. Low pH values were observed in acerola genotypes, with a range of 2.34–4.36 in the selected studies. In general, acerola is a very acidic fruit, so low fruit acidity associated with high sugar content is essential for the release of successful commercial acerola cultivars for fresh consumption [3]. The combined perception of sweetness and sourness (acidity), expressed through the SS/TA ratio, is a useful practical indicative of flavor perception by consumers [8], whose minimum value of 10 is established for acerolas intended to fresh consumption [47].

A wide range of 1.86–19.39 was observed for SS/TA ratio in selected studies, but few genotypes showed a SS/TA ratio greater than 10. ‘Florida Sweet’ acerola evaluated by Souza and coauthors (2014) [36] showed a high SS/TA ratio of 15.42, in contrast to the values below 7 observed in the other evaluated cultivars (‘Flor Branca’ and ‘BRS 366’). Ferreira and coauthors [2] observed that ‘BRS Rubra’ was the only genotype among 35 evaluated in two production cycles that presented SS/TA greater than 10, confirming a previous result by Mamede and coauthors [25]. ‘Florida Sweet’ is a well-established acerola cultivar selected in Florida, USA, and available since the mid-1950s, outstanding for its high sugar and low acid contents. In 2004, the Brazilian acerola cultivar ‘BRS Rubra’ was released with the same intention to meet the demand for fruits for fresh consumption with a high SS/TA ratio.

Mean values of ascorbic acid content in acerola genotypes were higher than 1000 mg 100 g⁻¹ in all studies, except in those by Adriano and coauthors (810 mg 100 g⁻¹) [13] and Cavalcante and coauthors (852 mg 100 g⁻¹) [8]. Ascorbic acid is an essential nutrient for the human diet, playing a key role on formation of blood vessels, cartilage, muscle, and bone collagen, in addition to improving the immune system and aiding in the absorption of iron [50]. Ascorbic acid is also one of the most important water-soluble antioxidants in fruits. Acerola is the second richest natural source of ascorbic acid in the world, only comparable to the native Amazonian fruit camu-camu (*Myrciaria dubia*, Myrtaceae) [51]. Siqueira and coauthors [52] compared the costs of nutrients provided by typical foods found in the Brazilian diet and found that vitamin C is the cheapest nutrient in Brazil,

considering that with only 3.3 Brazilian Real cents ($\cong$ USD 0.0067) it is possible to supply the whole daily needs of this nutrient by consuming acerola juice.

Weight of acerola genotypes ranged between 1.53 g and 15.02 g, which represents a maximum weight 9.8 times greater than the minimum value. For fruit diameter and length, also known as transversal and longitudinal diameters, the ranges were 0.83–3.00 cm and 0.94–2.92 cm, respectively. Acerola fruit is a thin-skinned, three-lobed small globose drupe [42]. High variations in acerola physical traits are intrinsic to genetic materials and their interaction with different edaphoclimatic conditions of the crop environments [10]. Fruit dimension is an important trait for acerola selection, since the larger the fruit, the easier and faster the harvesting, reducing required labor and production costs [14].

As an important quality trait for consumers and a major criterion used to assess fruit maturity, the color of acerola changes from bright glossy green to darkish-red or purple throughout fruit development due to chlorophyll degradation concomitant with a rise in the synthesis of carotenoids and anthocyanins [42]. The presence of these pigments in ripe fruit of different genotypes was reported by the high values of color spaces a^* (11.30–46.60) and b^* (3.00–48.90) and low hue (9.17–60.80°).

Pulp firmness of acerolas varied between 2.14 N and 20.02 N, which represents a difference of 9.4 times between genotypes with minimum and maximum firmness. Acerola is considered a highly perishable fruit, with high respiration rate and ethylene production [3]. Both factors trigger an intense fruit softening after harvest that reduce fruit quality [53]. Thus, the selection of acerola genotypes with higher pulp firmness and short time between harvesting the fruit and reaching the consumer are essential for the fruit to present high quality.

Pulp yield showed a high variation between genotypes (38.58–90.10%). The low pulp yield is not a characteristic that makes it impossible to use a fruit species for fresh consumption or industrial processing, although higher values are desirable to reduce cost/benefit ratio [24]. Fruit moisture was higher than 90% in all genotypes, while reducing sugars content ranged between 2.32% and 6.03%. Reducing sugars of acerola are mainly composed of fructose and glucose and are responsible for the sweetness of the fruit. Santos and coauthors [53] quantified sugars in acerola through nuclear magnetic resonance spectroscopy and found that

glucose and fructose were predominant and accumulated in ripe fruit, while sucrose exhibited a slight increase during ripening.

Acerola is a 'superfruit' due to presence of phytochemicals with antioxidant, astringent, anti-viral, anti-carcinogenic, anti-inflammatory, antianemics, and antifungal properties [9]. Anthocyanin content had a large variation of 45 times between genotypes with minimum ($1.52 \text{ mg } 100 \text{ g}^{-1}$) and maximum ($68.23 \text{ mg } 100 \text{ g}^{-1}$) values. Large differences between studies regarding the anthocyanin content of acerola genotypes tend to be related to the procedures of laboratory analysis, since the determination of these pigments involves the use of reagents and equipment. Even so, the high intra-study variation in anthocyanin content confirms that the concentration of this pigment is highly genotype-dependent, as pointed out by Ferreira and coauthors [20]. The anthocyanin content directly impacts the color of red-ripe acerolas, which can vary from orange to dark purple, and the fruit antioxidant activity, since anthocyanins are the major phenolic compounds in ripe fruit, and therefore, can reduce oxidative damage caused by free radicals to the human organism.

High variations were also observed for contents of carotenoids ($0.34\text{--}18.81 \text{ mg } \beta\text{-carotene } 100 \text{ g}^{-1}$), yellow flavonoids ($3.56\text{--}12.34 \text{ mg quercetin equivalent } 100 \text{ g}^{-1}$), total flavonols ($3.92\text{--}22.20 \text{ mg of quercetin equivalent } 100 \text{ g}^{-1}$), and total phenolic compounds (TPC) ($84.00\text{--}3196.00 \text{ mg of gallic acid equivalent } 100 \text{ g}^{-1}$). Acerola is a remarkable functional food resource with high levels of phenolic compounds. Phenolic compounds are important for plant metabolism and have been stated as important molecules for human organism due to their health characteristics, particularly related to their antioxidant power [42].

High variability in acerola quality has been reported in Brazilian orchards, especially those propagated by seeds, which motivated the selection of superior genotypes with desired agronomic traits and fruit quality [7]. Acerola quality is highly associated with genetic factors, but also by environmental factors such as precipitation, solar radiation, and temperature, and by preharvest factors such as irrigation, fertilization, and control of pests and diseases [42].

Studies carried out with acerola clones and accessions using different molecular markers, including Inter Simple Sequence Repeats (ISSR) [54] and Random Amplified Polymorphic DNA (RAPD) [55–56] primers, revealed a high genetic variability.

1.5 CONCLUSION

With a continuous rise in global demanding of acerolas, there is a need for the characterization of their key quality traits. In the present review, we systematically identified a high genetic diversity on all acerola quality traits. Soluble solids and ascorbic acid contents are the main parameters determined in studies with acerola. Titratable acidity, SS/TA ratio and pH are also evaluated in most studies. Different studies have already identified possible genotypes for use in acerola breeding programs based on fruit quality, both for launching new cultivars and for use as parents in crosses. Our review is a useful basis for acerola breeding programs and germplasm conservation. Future studies are required to further identify and quantify bioactive compounds in acerolas of different genotypes.

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ARTICLE II

Phenolic compounds in acerola fruit and by-products: an overview on identification, quantification, influencing factors, and biological properties*

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Phenolic compounds in acerola fruit and by-products: an overview on identification, quantification, influencing factors, and biological properties

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ABSTRACT

Acerola (*Malpighia emarginata* DC) is a cherry-like tropical fruit well-known for being a rich source of ascorbic acid (vitamin C) and phenolic compounds. This review provides a complete overview on aspects related to phenolic compounds of acerola fruit and by-products, comprising the identification and quantification of phenolic compounds, influencing factors, and biological properties. Scientific evidences suggest that the acerola is a promising superfruit with great potential in the food and pharmaceutical industries. At least 76 phenolic compounds were identified using high performance liquid chromatography in acerolas, including 55 flavonoids (anthocyanins, flavan-3-ols, flavonols, flavones, flavanones, isoflavones and chalcones) and 21 non-flavonoids (phenolic acids, stilbenes and lignans). Phenolic compounds in acerola show several biological properties, including antioxidant, antibacterial, antihyperglycemic, antihyperlipidemic, anti-inflammatory, and hepatoprotective activities. However, studies are further required to assess the seasonal and genotypic influence on the phenolics of acerola and their bioaccessibility. Acerola is an anthocyanin-rich fruit with high potential for pigment extraction, but stabilization of anthocyanins in juice and pulp should be further elucidated and improved.

Keywords: *Malpighia emarginata* DC. Polyphenols. Phytochemicals. Antioxidant activity. Biological properties.

2.1 INTRODUCTION

In recent years, global demand for fruit and vegetables is steadily increasing, as a result of healthier eating behavior and lifestyle by consumers, since their consumption have been associated with lower incidence of mortality caused by non-communicable chronic diseases [1, 2]. Red fruit, also called berries, are very popular due to their attractive appearance, pleasant taste and aroma and high nutritional value, as sources of vitamins, carotenoids, phenolic compounds, fibers, phytosterols, and other bioactive compounds with well-known biological properties, including antioxidant, anti-inflammatory, anti-cancer, antimutagenic, antimicrobial, antiproliferative, anti-neurodegenerative, antiglycemic, hypolipidemic, cardio-protective, and neuroprotective properties [3–6].

In tropical countries, there are several native red fruit species that are underexploited when compared with traditional ones such as strawberry, grape, blueberry, raspberry, and blackberry, but which have high potential for use in food and pharmaceutical industries [7]. One of these species is acerola (*Malpighia emarginata* DC), a cherry-like tropical fruit that is one of the richest natural sources of ascorbic acid (AsA, or vitamin C) in the world, representing up to 100 times the levels found in orange and lemon [8].

AsA together with phenolic compounds such as flavonoids, phenolic acids, and stilbenes are the main responsible for the high antioxidant activity of acerolas, which can help reducing the oxidative damages of free radicals to human body and strengthening the immune system.

In spite of its high nutrient profile and appeal for functional food, acerola is underutilized worldwide and information related to bioactive compounds and the factors that influence the synthesis of these compounds are still scarce [9]. This review provides a complete overview on aspects related to phenolic composition of acerola fruit and by-products, comprising identification, quantification, influencing factors and biological properties.

2.1.1 Research methodology and data collection

A search was performed on the Scopus database including the keywords *acerola* OR *Barbados cherry* OR *West Indian cherry* OR *Guarani cherry* OR *Surinam cherry* OR *Malpighia* OR *Malpighia emarginata* OR *Malpighia glabra*

OR *Malpighia puniceifolia* AND phenolic compounds OR polyphenols, resulting in 171 published documents between January 2002 and December 2022 (Fig. 2.1). The main results were related to identification and quantification of phenolic compounds in whole fruit (skin and pulp), juice or residuals from pulp processing (seeds), extraction methods of phenolic compounds, and influencing factors. We addressed and discussed results from articles published in high impact scientific journals between 2013 and 2022.

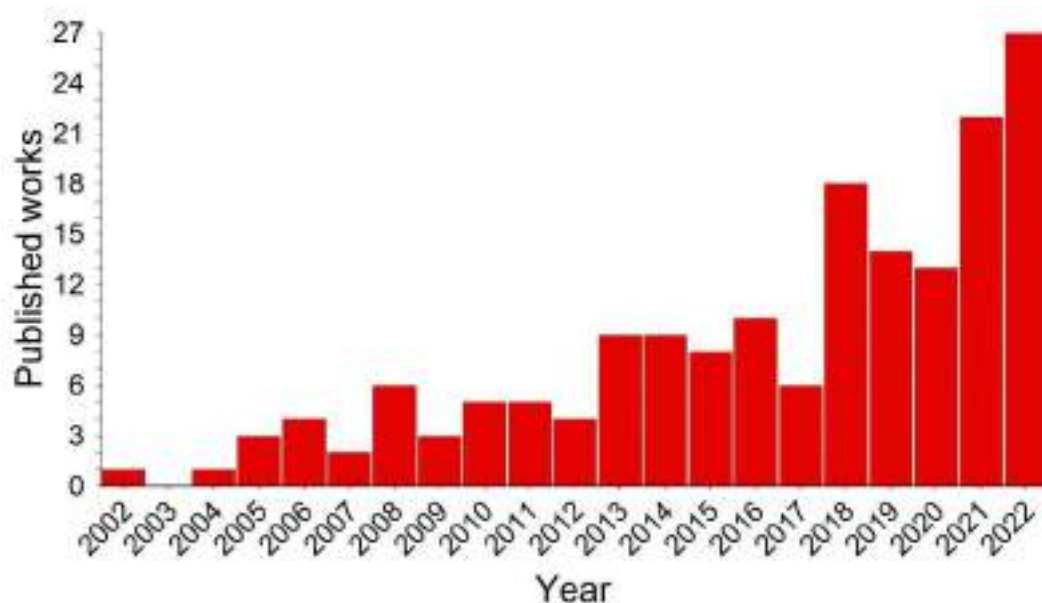


Figure 2.1. Number/year of published works on phenolic composition of acerola fruit and by-products.

Information on phenolic compounds studied in this review, as well as the 2D structures of the main phenolics in acerola fruit and by-products, were based on PubChem (<https://pubchem.ncbi.nlm.nih.gov/>).

2.2 BOTANICAL DESCRIPTION AND CULTIVATION OF ACEROLA

Acerola, also known as Barbados cherry, West Indian cherry, and Guarani cherry, is a tropical fruit belonging to the Malpighiaceae family. It is native from Central America, South American and the Caribbean islands, and is also found in tropical and subtropical areas of Asia and Africa [10].

The taxonomic classification of acerola is not well established in the literature, since some researchers defend that the acerola originates from two species, *Malpighia puniceifolia* and *M. glabra*, while others argue that both are not distinct species, but different botanical forms. In Brazil, acerola orchards were

established from *M. puniceifolia* seeds imported from Puerto Rico in the 1950s, characterized by causing strong skin irritation due to the presence of hairs on the leaves, but the presence of *M. glabra* is also confirmed due to the occurrence of glabrous plants (without hairs), which do not cause irritation [11]. Gomes et al. [12] indicate *M. emarginata* as the proper nomenclature for designating acerola. The Integrated Taxonomic Information System establishes *M. glabra* and *M. emarginata* as accepted nomenclatures for acerola, while *M. puniceifolia* is considered misapplied (non-accepted) [13].

Acerola (Fig. 2.2) tree is a shrub with small to medium height (2–3 m), the leaves are oval to elliptical-shaped with dark and glossy greenish color and height of 2.5–7.5 cm, and the inflorescences are sessile or have short peduncles, with five-fringed light or dark pink petals [10]. Ripe fruit is a globose drupe with 1–4 cm of diameter, a thin and fragile pericarp of reddish or dark purplish color, a mesocarp (pulp) that represents around 60–80% of total fruit weight, and one seed surrounded by a reticulated, tri-lobbed endocarp [14].

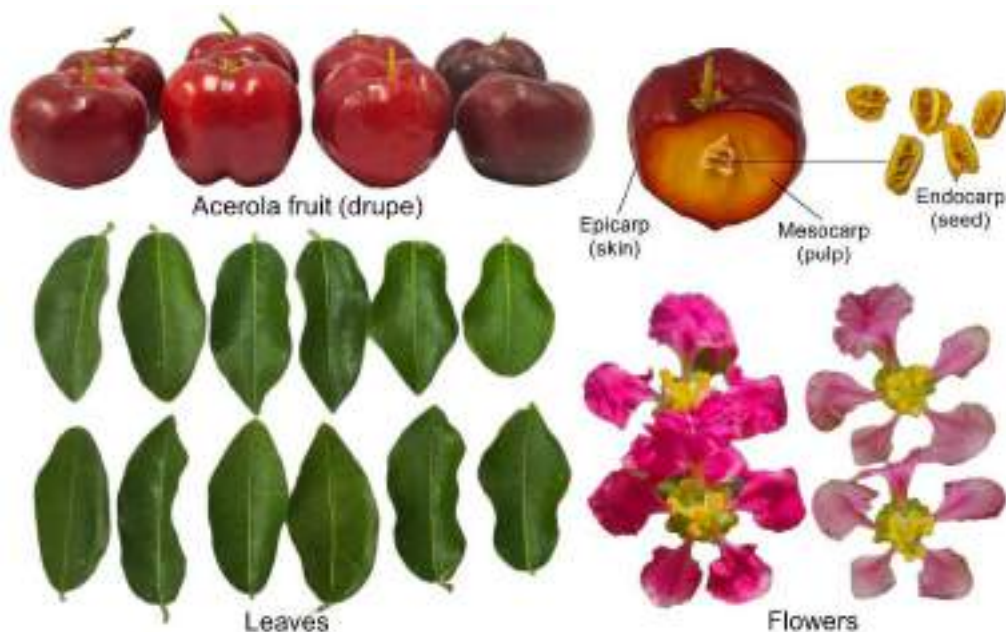


Figure 2.2. Leaves of the tree and structure of the acerola (*Malpighia emarginata* DC) fruit.

Acerola is a juicy, acid to sub-acid (0.46–1.10% malic acid, 0.05–0.09% succinic acid, titratable acidity of 0.87–1.97 g of malic acid 100 g⁻¹) and sweetish fruit (1.23–1.87% fructose, 1.24–1.83% glucose, 0.06–0.13% maltose, soluble solids of 6.03–13.87%), whose flavor is highly dependent on genotype [15, 16].

Acerola is well-known for its high AsA content, with ranges from 400 to 2,800 mg 100 g⁻¹ in red-ripe fruit and up to 4,000 mg 100 g⁻¹ in unripe green fruit [17, 18].

Acerola flowering and fruiting can occur concomitantly when temperatures are close to or greater than 26 °C and the season is rainy or water is made available through irrigation [10]. Fruit formation occurs very quickly, with an average period of 17–25 days between anthesis and harvest, which may vary depending on the genotype, temperature, solar radiation, and water availability.

Short production cycle of acerola enables the occurrence of up to eight harvests per year in irrigated orchards in the Brazilian Northeast under Semi-arid climate conditions, between August and April, when climatic conditions are favorable for fruit growth and development. Furthermore, acerola has little damage from pests and diseases when compared to other fruit species, which reduces the use of pesticides and enables the production of organic fruit, whose value is up to 50% more than that paid for fruit produced in the conventional system [11].

The commercial cultivation of acerola in Brazil has been growing every year, with a planted area of 5,753 hectares and production of 60,966 tons of fruit in 2017, according to official data by the Brazilian Institute of Geography and Statistics [19]. Acerola market is divided in two main purposes: green fruit for AsA extraction and red fruit for fresh consumption or processing.

The great AsA content in green acerolas motivated their use for the production of concentrates for food enrichment or pharmaceuticals, as an alternative to synthetic AsA in dietary supplements [20, 21]. In Brazilian Northeast, several plantations are exclusively destined for AsA extraction in green fruit through partnerships between rural producers and multinational companies [22]. The acerola extract market is steadily expanding, with an expected market value of US\$ 17.5 billion until 2026 [23], as a result of higher absorption of acerola AsA by human body when compared to synthetic AsA, besides its ability to act as a food preservative.

Acerolas at red maturity stage are commonly commercialized for fresh consumption or for processing of value-added products, including frozen pulp, juice, marmalade, frozen concentrate, jam, yoghurt, and liquor [20]. As a naturally very acidic fruit, new acerola varieties have been developed in order to reach a higher consumer acceptance of fruit, characterized by high diameter, mass, firmness and sugar content, and low acidity [16]. Even though, high natural perishability and

intense metabolic activity can limit the shelf life in acerolas, making processing an alternative to avoid postharvest fruit losses.

Scientific investigations on phenolic composition and health-promoting properties of native and exotic fruit as yet unknown or under-exploited species is important for their adding value, serving as a basis for social and economic development of local population and future source of income for small farmers [24].

2.3 PHENOLIC COMPOUNDS IN ACEROLA: IDENTIFICATION, QUANTIFICATION, AND INFLUENCING FACTORS

Phenolic compounds are phytochemicals produced by plants as secondary metabolites, i.e., compounds not required for plant growth or development but produced for its defense against biotic and abiotic stresses, such as salinity, exposure to high-energy radiation, drought, extreme temperatures, flood, attacks of insects, nematodes and phytopathogenic fungi, bacterial infection, and disease progression. In addition to defense responses, phenolic compounds perform a wide array of antioxidant, structural, attractant, ultraviolet (UV) screen, and signal functions in plants [25]. These compounds are synthesized by the shikimate and phenylpropanoid pathways, and are characterized by their structures with at least one aromatic ring, with single or multiple hydroxyl (–OH) and methyl (–CH₃) groups linked, ranging from simple structures to highly polymerized compounds [25–27].

From the discovery of phenol in 1834 to the present day, more than 10,000 phenolic compounds are known, and many of them have been studied in relation to their functional properties [28]. The interest on phenolic compounds is mainly related to their antioxidant activity, preventing and reducing oxidate damages by free radicals to vital cellular constituents such as lipids, proteins and nucleic acids. Free radical scavenger potential of a phenolic compound depends on its reducing properties as hydrogen- or electron-donating agents, which in turn varies according to the number of hydroxyl groups and their position in relation to the carboxyl functional group [29, 30].

Phenolic compounds are categorized according to their structural differences, source of origin, and biological activity [28]. In general, they can be divided in two major classes, flavonoids and non-flavonoids. Flavonoids are the main group of phenolic compounds, characterized by a polyphenolic structure (C₆–

C3–C6) constituted of two aromatic rings (A and B) linked by a three-carbon bridge (frequently as a heterocyclic ring, named C). Flavonoids are subdivided into different subgroups, according to the substitution pattern (degree of unsaturation and oxidation) in C rings. Additionally, the sites of hydroxyl and methyl groups attached to flavonoid structure can result in glycoside modifications, thus flavonoids are found in both aglycone and glycoside forms. The main subgroups of phenolic compounds are anthocyanidins, flavan-3-ols, flavonols, flavones, flavanones, isoflavones, and chalcones [30–32].

Biosynthesis of phenolic compounds is regulated by several intrinsic and extrinsic factors, including plant hormones, developmental phase, water, temperature, minerals, salinity, UV radiation, and visible light, and can be stimulated by agricultural practices such as the application of plant growth regulators and the postharvest application of UV radiation [33–35].

Many flavonoids are often responsible alongside the carotenoids for color pigmentation of fruit and flowers in most angiosperm families [31, 36]. In red fruit and berries, major flavonoids are composed of anthocyanins, water-soluble pigments with pink, red, blue and purple hues [4]. The color provided by anthocyanin depends on its hydroxylation, methylation, and glycosylation; higher number of hydroxyl groups attached to B-ring increases blue coloration, while methylation results in reddish hues [37].

The use of fruit anthocyanins to enhance and provide color to food products has been increasing, since natural ingredients are associated by consumers with the health benefits and improvement in life quality, in contrast with critics to synthetic dyes and their insecurity for human health [38]. However, anthocyanins are highly susceptible to degradation, depending on temperature, pH and light, which leads to changes in their color to yellow or even colorless degradation under these conditions [37].

2.3.1 Methods for assessment of acerola phenolics: from spectrophotometry to smartphone-based sensor and high-performance liquid chromatography (HPLC)

2.3.1.1 Classical method

Folin-Ciocalteu colorimetric method is the most widespread analytical technique for estimation of total phenolic compounds (TPC) content in plant

extracts by the oxidation-reduction reaction of phenols with a mixture of phosphomolybdate and phosphotungstate (Folin-Ciocalteu reagent) to form a blue complex able to be quantified by spectrophotometry [39].

Since the first study on quantification of phenolic compounds in acerola, using the classical colorimetric Folin-Ciocalteu method [40], this fruit has shown to have an incredibly high content of these compounds. A comparative study of 18 non-traditional fruits from Brazil was carried out by Rufino et al. [41] and demonstrated that this fruit had the second highest content of polyphenols [1063 mg of gallic acid equivalents (GAE) per 100 g of fresh mass (fm)], slightly lower than that observed in camu-camu (*Myrciaria dubia*, Myrtaceae) (1,176 mg GAE 100 g⁻¹ fm), a little known Amazonian fruit also recognized as one of the richest natural sources of AsA worldwide.

Acerola (593.77 mg GAE 100 g⁻¹ fm) presented together with the panã (*Annona crassiflora*) (902.18 mg GAE 100 g⁻¹ fm), açaí (*Euterpe oleracea*) (708.22 mg GAE 100 g⁻¹ fm) and jabuticaba (*Myrciaria cauliflora*) (626.57 mg GAE 100 g⁻¹ fm) the highest TPC content in frozen pulps between 44 traditional and exotic Brazilian fruit species [42].

2.3.1.2 Innovations on Folin–Ciocalteu-based approaches for assessment of phenolic content in acerola

As alternatives to the use of spectrophotometer in determination of TPC content, which has high cost and little portability and that is limited to the laboratory routine, some innovative approaches have been proposed using acerolas. These new techniques mainly aim to make the assessment of plant-based phenolic content cheaper and more accessible, ceasing to be exclusive to the laboratory and accessible to industries.

Miranda et al. [43] designed a low-cost system for measuring the concentration of TPC and flavonoids in twelve fruit species, including acerola. Briefly, the system consists of an optical sensor, which requires a light emitting diode, an optical filter, a cuvette holder, a photodiode, an electronic board and a mechanical structure. Measurements were collected in transmittance and compared with those from a commercial spectrophotometer. The results did not indicate significant ($p > 0.05$) differences between the methods for determination of TPC (48.16 µg µL⁻¹ for the spectrophotometer and 60.27 µg µL⁻¹ for the sensor) and

flavonoids ($71.41 \mu\text{g } \mu\text{L}^{-1}$ for the spectrophotometer and $68.61 \mu\text{g } \mu\text{L}^{-1}$ for the sensor) in acerola, as well for the other fruit evaluated in the study.

Martins et al. [44] developed a simple and fast system based on colorimetry for quantification of phenolic compounds in acerola using acquisition of images by mobile devices followed by image conversion into the quantifiable RGB color system, i.e., a mathematical model that represents simultaneously red (R), green (G) and blue (B) colors as number values. Both spectrophotometry and smartphone-based method were in close agreement (145.0 and $146.0 \text{ mg GAE g}^{-1} \text{ dm}$, respectively). Additionally, the proposed method reduced in five times the reagent consumption and waste generation, being an efficient and greener approach for determination of the phenolic content.

2.3.1.3 HPLC: detailed phenolic composition of acerola

Recently, technological advances on food composition analysis have allowed an efficient and sensible separation, identification and quantification of phenolic compounds, whose biological and health properties are structure-dependent [45]. HPLC and mass spectrometry (MS) are undoubtedly recognized as the most advanced techniques for the elucidation and determination of phenolic compounds by the scientific community [46].

Phenolic compounds in acerola were separated by chromatography for the first time by Vendramini and Trugo [47]. The authors identified nine phenolic compounds in red-ripe acerolas including three anthocyanins (malvidin-3,5-diglucoside, cyanidin 3-glucoside and pelargonidin), four phenolic acids (*p*-coumaric acid, ferulic acid, caffeic acid and chlorogenic acid), and two flavonols (kaempferol and quercetin). Since this period, at least 76 phenolic compounds were identified using HPLC in acerolas, including 55 flavonoids (anthocyanins, flavan-3-ols, flavonols, flavones, flavanones and isoflavones) (Table 2.1) and 21 non-flavonoids (phenolic acids, stilbenes and lignans) (Table 2.2). Different classes of phenolic compounds previously reported in acerola are discussed in the next topics.

Table 2.1. Individual flavonoids (anthocyanins, flavan-3-ols, flavonols, flavones, flavanones, isoflavones and chalcones) reported in acerola fruit and by-products.

(continues)

Flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
Anthocyanins							
Cyanidin 3–rhamnoside	102601165	Skin and pulp	Ripe	HPLC–DAD	Ethanol	14.99–68.23 ^a	[15]
		Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	47–1152 ^b	[50]
Cyanidin 3–rutinoside	29231	Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	12–67 ^b	[50]
Cyanidin 3,5–diglucoside	441688	By-product	Ripe	HPLC–DAD	Ethanol	0.7 ^a	[58]
Delphinidin	68245	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Malvidin 3–glucoside	11249520	By-product	Ripe	HPLC–DAD	Ethanol	8.9 ^a	[58]
Pelargonidin	440832	Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	0.4–7.0 ^b	[50]
Pelargonidin 3–glucoside	443648	By-product	Ripe	HPLC–DAD	Ethanol	1.3 ^a	[58]
Pelargonidin 3–rhamnoside	44256696	Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	25–155 ^b	[50]
Peonidin–3–xylopyranoside	NL	Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	6–156 ^b	[50]
Petunidin 3–glucoside	443651	By-product	Ripe	HPLC–DAD	Ethanol	14.7 ^a	[58]
Phloretin	4788	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Tulipanin	5492231	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Flavan–3–ols							
<i>Monomeric catechins</i>							
(±)–Catechin	9064	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.64 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	0.8 ^b	[51]
		Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.68–2.51 ^a	[15]
		Skin and pulp	Ripe	HPLC–DAD	Methanol	871 ^a	[49]
		Skin and pulp	Intermediate	HPLC–DAD	Methanol	396 ^a	[49]
		Skin and pulp	Green	HPLC–DAD	Methanol	115 ^a	[49]
		Seed	–	UPLC–MS	Ethanol	1.57 ^b	[48]
		Seed	–	UPLC–MS	Acetone	3.12 ^b	[48]

(continues)

Flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
(±)–Catechin	9064	Seed	–	HPLC–UV	Water	1.09 ^a	[59]
		Seed	–	HPLC–UV	Methanol	1.13 ^a	[59]
		By-product	Ripe	HPLC–DAD	Ethanol	35.1 ^a	[58]
		Non–pomace	Green	UHPLC–MS/MS	Water	1.26–22.08 ^b	[54]
		Juice	Ripe	HPLC–DAD	NS	0.15 ^a	[52]
		Juice	Green	UHPLC–MS/MS	Water	0.42 ^a	[54]
(±)–Epicatechin	72276	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.63 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	0.7 ^b	[51]
		Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.003 ^a	[15]
		Skin and pulp	Ripe	HPLC–DAD	Methanol	704 ^a	[49]
		Skin and pulp	Intermediate	HPLC–DAD	Methanol	253 ^a	[49]
		Skin and pulp	Green	HPLC–DAD	Methanol	521 ^a	[49]
		Seed	–	UPLC–MS	Ethanol	159.5 ^b	[48]
		Seed	–	UPLC–MS	Acetone	33.1 ^b	[48]
		Seed	–	HPLC–UV	Water	1.17 ^a	[59]
		Seed	–	HPLC–UV	Methanol	9.19 ^a	[59]
(–)–Epicatechin gallate	107905	Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.25–0.72 ^a	[15]
(–)–Epigallocatechin gallate	65064	Skin and pulp	Green	HPLC–DAD	Ethanol	5.83 ^b	[60]
		Seed	–	HPLC–UV	Methanol	0.91 ^a	[59]
		By-product	Ripe	HPLC–DAD	Ethanol	3.8 ^a	[58]
<i>Proanthocyanidins</i>							
Procyanidin A2	124025	Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.52–0.93 ^a	[15]
		By-product	Ripe	HPLC–DAD	Ethanol	3.8 ^a	[58]
Procyanidin B1	11250133	Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.09–1.78 ^a	[15]
		By-product	Ripe	HPLC–DAD	Ethanol	20.9 ^a	[58]
Procyanidin B2	122738	Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.25–0.81 ^a	[15]

(continues)

Flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
Procyanidin B2	122738	By-product	Ripe	HPLC–DAD	Ethanol	39.1 ^a	[58]
Flavonols							
Isoquercitrin	5280804	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	33.2 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	43.2 ^b	[51]
		Skin and pulp	Ripe	HPLC–DAD	Ethanol	1.31–3.29 ^a	[15]
		Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	0.5–2.8 ^b	[50]
		By-product	Ripe	HPLC–DAD	Ethanol	15.8 ^a	[58]
Isorhamnetin	5281654	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.62 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	0.99 ^b	[51]
		Skin and pulp	Ripe	HPLC–DAD	Ethanol	2.08–4.70 ^a	[15]
		Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	1.1–1.3 ^b	[50]
		Skin and pulp	Green	HPLC–DAD	Ethanol	10.13–11.41 ^b	[60]
Isorhamnetin 3–galactoside	13245586	Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	2.7–3.0 ^b	[50]
Isorhamnetin 3–glucoside	5318645	Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	17–43 ^b	[50]
Kaempferol	5280863	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	2.39 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	1.61 ^b	[51]
		Skin and pulp	Ripe	HPLC–DAD	Methanol	162 ^a	[49]
		Skin and pulp	Intermediate	HPLC–DAD	Methanol	231 ^a	[49]
		Skin and pulp	Green	HPLC–DAD	Ethanol	6.18–6.30 ^b	[60]
		Skin and pulp	Green	HPLC–DAD	Methanol	125 ^a	[49]
		Seed	–	UPLC–MS	Ethanol	2.30 ^b	[48]
		Seed	–	UPLC–MS	Methanol	3.26 ^b	[48]
		Seed	–	UPLC–MS	Acetone	4.15 ^b	[48]
Kaempferol 3–robinobioside–7– alpha–L–arabinofuranoside	44258992	Juice	Ripe	HPLC–DAD	NS	0.37 ^a	[52]
		Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	1.5–5.5 ^b	[50]

(continues)

Flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
Kaempferol 3-glucoside	5282102	Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.34–0.83 ^a	[15]
		Skin and pulp	Green	HPLC-DAD	Ethanol	8.50–32.68 ^b	[60]
		By-product	Ripe	HPLC-DAD	Ethanol	12.0 ^a	[58]
Kaempferol 7-neohesperidoside	5483905	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	3.1–7.0 ^b	[50]
Kaempferol 3-arabinofuranoside	5318717	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	10.5–18.1 ^b	[50]
Myricetin	5281672	Juice	Ripe	HPLC-DAD	NS	0.09 ^a	[52]
Myricetin 3-glucuronide	44259442	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	0.1–14.3 ^b	[50]
Myricetin 3-glucoside	44259426	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	0.1–25.1 ^b	[50]
Myricetin 3-rhamnoside	5352000	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	74–196 ^b	[50]
Quercetin	5280343	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	12.0 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	12.1 ^b	[51]
		Skin and pulp	Ripe	HPLC-DAD	Methanol	118 ^a	[49]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	113 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Methanol	601 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Ethanol	6.63–8.13 ^b	[60]
		Seed	–	HPLC-UV	Water	0.007 ^a	[59]
		Seed	–	HPLC-UV	Methanol	0.03 ^a	[59]
		Juice	Ripe	HPLC-DAD	NS	1.36 ^a	[52]
		Skin and pulp	Ripe	HPLC-DAD	Methanol	213 ^a	[49]
Quercitrin	5280459	Skin and pulp	Intermediate	HPLC-DAD	Methanol	580 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Methanol	193 ^a	[49]
		Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	0.8–1.9 ^b	[50]
Quercitrin-2''-O-gallate	NL	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	0.8–1.9 ^b	[50]
Rutin	5280805	Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.06–0.48 ^a	[15]
		Skin and pulp	Ripe	HPLC-DAD	Methanol	1657 ^a	[49]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	1034 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Methanol	1018 ^a	[49]

(continues)

Flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
Rutin	5280805	Skin and pulp	Green	HPLC–DAD	Ethanol	2.35 ^b	[60]
		Seed	–	UPLC–MS	Ethanol	3.62 ^b	[48]
		Seed	–	UPLC–MS	Methanol	7.07 ^b	[48]
		Seed	–	UPLC–MS	Acetone	8.29 ^b	[48]
		Non–pomace	Green	UHPLC–MS/MS	Water	1.54–2.48 ^b	[54]
		By-product	Ripe	HPLC–DAD	Ethanol	1.2 ^a	[58]
		Juice	Green	UHPLC–MS/MS	Water	0.28 ^a	[54]
Flavones							
Acacetin	5280442	Seed	–	UPLC–MS	Ethanol, methanol or acetone	<LOQ	[48]
Apigenin	5280443	Skin and pulp	Ripe or fully ripe	HPLC–MS/MS	Methanol	<LOQ	[51]
		Seed	–	UPLC–MS	Ethanol, methanol or acetone	<LOQ	[48]
Chrysin	5281607	Seed	–	UPLC–MS	Ethanol, methanol or acetone	<LOQ	[48]
Luteolin	5280445	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.05 ^b	[51]
		Juice	Ripe	HPLC–DAD	NM	0.04 ^a	[52]
		Seed	–	UPLC–MS	Ethanol, methanol or acetone	<LOQ	[48]
Malonylapiin	5280803	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Pinobanksin	73202	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.15 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	0.32 ^b	[51]
Vitexin	5280441	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]

Flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	(conclusion)
							Reference
7,8,4'–Trihydroxyflavone	688853	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Flavanones							
Hesperetin	72281	By-product	Ripe	HPLC–DAD	Ethanol	32.6 ^a	[58]
Hesperidin	10621	Skin and pulp	Ripe	HPLC–DAD	Ethanol	1.02–3.26 ^a	[15]
Naringenin	932	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.11 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	0.28 ^b	[51]
		Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.22–1.73 ^a	[15]
		Seed	–	UPLC–MS	Ethanol	47.88 ^b	[48]
		Seed	–	UPLC–MS	Methanol	33.73 ^b	[48]
		Seed	–	UPLC–MS	Acetone	118.82 ^b	[48]
		By-product	Ripe	HPLC–DAD	Ethanol	16.7 ^a	[58]
Naringin	442428	Skin and pulp	Ripe or fully ripe	HPLC–MS/MS	Methanol	<LOQ	[51]
2,4',7–Trihydroxyisoflavanone	13953272	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Isoflavones							
Biochanin A	5280373	Seed	–	UPLC–MS	Ethanol, methanol or acetone	<LOQ	[48]
Daidzein	5281708	Seed	–	UPLC–MS	Ethanol, methanol or acetone	<LOQ	[48]
Demethyltexasin	5284649	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Formononetin	5280378	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Genistein	5280961	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Chalcones							
Naringenin chalcone	5280960	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]

^aExpressed in fresh mass basis; ^bExpressed in dry mass basis.

NL – not listed on PubChem; NS – not shown; LOQ – limit of quantification.

Table 2.2. Individual non-flavonoids (phenolic acids, stilbenes and lignans) reported in acerola fruit and by-products.

(continues)

Non-flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
Phenolic acids							
<i>Hydroxybenzoic acids</i>							
Ellagic acid	5281855	Skin and pulp	Ripe	HPLC–DAD	Methanol	253 ^a	[49]
		Skin and pulp	Intermediate	HPLC–DAD	Methanol	172 ^a	[49]
		Skin and pulp	Green	HPLC–DAD	Methanol	130 ^a	[49]
Gallic acid	370	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.4 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	0.38 ^b	[51]
		Skin and pulp	Ripe	HPLC–DAD	Methanol	536 ^a	[49]
		Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	1.3–2.9 ^b	[50]
		Skin and pulp	Intermediate	HPLC–DAD	Methanol	451 ^a	[49]
		Skin and pulp	Green	HPLC–DAD	Methanol	470 ^a	[49]
		Seed	–	HPLC–UV	Water	0.47 ^a	[59]
		Seed	–	HPLC–UV	Methanol	0.33 ^a	[59]
		Juice	Ripe	HPLC–DAD	NS	0.07 ^a	[52]
Protocatechuic acid	72	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.39 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	0.31 ^b	[51]
Salicylic acid	338	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.24 ^b	[51]
Syringic acid	10742	Seed	–	HPLC–UV	Water	4.61 ^a	[59]
		Seed	–	HPLC–UV	Methanol	3.72 ^a	[59]
Vanillin	1183	Skin and pulp	Ripe	HPLC	Methanol	0.24 ^a	[53]
<i>Hydroxycinnamic acids</i>							
Caffeic acid	689043	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.73 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	0.95 ^b	[51]
		Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.07–0.29 ^a	[15]

(continues)

Non-flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
Caffeic acid	689043	Skin and pulp	Ripe	HPLC–DAD	Methanol	682 ^a	[49]
		Skin and pulp	Intermediate	HPLC–DAD	Methanol	563 ^a	[49]
		Skin and pulp	Green	HPLC–DAD	Methanol	459 ^a	[49]
		Non–pomace	Green	UHPLC– MS/MS	Water	1.36–3.44 ^b	[54]
		By-product	Ripe	HPLC–DAD	Ethanol	0.9 ^a	[58]
		Juice	Green	UHPLC– MS/MS	Water	0.49 ^a	[54]
		Juice	Ripe	HPLC–DAD	NS	0.05–0.10 ^a	[52]
Caftaric acid	6440397	Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.34–0.45 ^a	[15]
		By-product	Ripe	HPLC–DAD	Ethanol	19.4 ^a	[58]
Chlorogenic acid	1794427	Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.17–0.38 ^a	[15]
		Seed	–	UPLC–MS	Methanol	0.28 ^b	[48]
		Seed	–	UPLC–MS	Acetone	4.25 ^b	[48]
		By-product	Ripe	HPLC–DAD	Ethanol	6.5 ^a	[58]
<i>m</i> –Coumaric acid	637541	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
<i>p</i> –Coumaric acid	637542	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.79 ^b	[51]
		Skin and pulp	Ripe	HPLC–MS/MS	Methanol	0.98 ^b	[51]
		Skin and pulp	Green	HPLC–DAD	Ethanol	28.87 ^b	[60]
		Seed	–	UPLC–MS	Ethanol	4.22 ^b	[48]
		Seed	–	UPLC–MS	Methanol	6.06 ^b	[48]
		Seed	–	UPLC–MS	Acetone	8.34 ^b	[48]
		Seed	–	HPLC–UV	Methanol	0.12 ^a	[59]
		Seed	–	HPLC–UV	Methanol	0.24 ^a	[59]
		Juice	Ripe	HPLC–DAD	NS	0.33–0.84 ^a	[52]
<i>p</i> –Coumaroylquinic acid	441280	Skin and pulp	Intermediate	HPLC–MS/MS	Methanol	3.9–8.1 ^b	[50]
Ferulic acid	445858	Skin and pulp	Ripe	HPLC–MS/MS	Methanol	1.27 ^b	[51]

(conclusion)

Non-flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
Ferulic acid	445858	Skin and pulp	Fully ripe	HPLC–MS/MS	Methanol	0.62 ^b	[51]
		Skin and pulp	Green	HPLC–DAD	Ethanol	0.34–1.63 ^b	[60]
		Seed	–	UPLC–MS	Ethanol	4.10 ^b	[48]
		Seed	–	UPLC–MS	Methanol	5.41 ^b	[48]
		Seed	–	UPLC–MS	Acetone	6.53 ^b	[48]
		Non–pomace	Green	UHPLC–MS/MS	Water	2.96–5.44 ^b	[54]
		Juice	Green	UHPLC–MS/MS	Water	0.61 ^a	[54]
		Juice	Ripe	HPLC–DAD	NS	0.04–0.45 ^a	[52]
Isochlorogenic acid	6436237	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
<i>n</i> -feruloylglycine	5280527	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Stilbenes							
<i>trans</i> –Resveratrol	445154	Skin and pulp	Ripe	HPLC–DAD	Ethanol	0.23–0.38 ^a	[15]
		Juice	Ripe	HPLC–DAD	NS	0.34 ^a	[52]
		By-product	Ripe	HPLC–DAD	Ethanol	8.1 ^a	[58]
<i>cis</i> –Resveratrol	1548910	By-product	Ripe	HPLC–DAD	Ethanol	2.5 ^a	[58]
Coumarin	323	Skin and pulp	Green	HPLC–DAD	Ethanol	1.69–4.06 ^b	[60]
Lignans							
Lariciresinol	332427	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Sesaminol	94672	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]
Secoisolariciresinol monoglucoside	101138945	Skin and pulp	Green and ripe	UPLC–MS/MS	Methanol	NS	[55]

^aExpressed in fresh mass basis; ^bExpressed in dry mass basis.

NL – not listed on PubChem; NS – not shown; LOQ – limit of quantification.

The sensitivity of HPLC in detection of phenolic compounds is generally based on their purification and pre-concentration from matrices of plant extracts (i.e., acerola fruit and by-products). Furthermore, HPLC combined with MS detectors is highly sensitive and show high power for identification achieve high specificity due to the mass selectivity of detection [36].

The column selection also influences the success of the processes of identification and quantification of phenolic compounds in plant extracts. In studies with acerola and by-products, as with other fruit crops, a normal phase C18 or reversed phase (RP-C₁₈) column is employed, with 10–25 cm of length, 2.1–4.6 mm i.d., and 3–5 μ m particle size [15, 48–53]. New types of columns, with 10 cm length, 2.1 mm i.d., and 1.7–1.8 μ m particle size have been used in advanced HPLC methods of phenolic compounds identification, such as UPLC (ultra performance liquid chromatography) [54, 55]. Identification of acerola phenolic compounds has been carried out with ambient column temperature (30–40 °C), although higher temperatures can be adopted to new columns and techniques [36]. The running time is another crucial factor that influences the HPLC determination of phenolics, ranging from 15 to 90 min in studies with acerola. The flow rate varied between 0.35 and 1 mL/min and the volume injected was 4–40 μ L.

Successful and accurate identification and quantification of acerola phenolic compounds with HPLC are confirmed by validation parameters, including calibration curves from external standards of the analyzed phenolics with excellent adjustment of linear regression, as well as low values for limit of detection (LOD) and limit of quantification (LOQ). Ferreira et al. [15] quantified 17 phenolic compounds in acerola fruit of seven varieties using HPLC–DAD, with LOD < 0.0027 mg/100 g, LOQ < 0.0102 mg/100 g, and $R^2 > 0.996$ for all evaluated compounds. In another study with acerola fruit using HPLC–DAD, low LOD (0.0039 mg/100 g) and LOQ (0.0128 mg/100 g) were found for nine phenolic compounds in fruit of three maturity stages [49].

In recent years, sophisticated systems of liquid chromatography combined with modern detectors have been employed for the identification of acerola phenolics, including UPLC–q–TOF–MS (ultra performance liquid chromatography coupled to a quadrupole-time-of-flight mass spectrometer) [54, 55]. This system consists in a powerful and reliable analytical approach to trace qualitatively and quantitatively the phenolic profile of plant extracts, outperforming the conventional

HPLC method, which may present limitations in the detection of compounds in very small amounts, besides disturbance of high background noise [56]. However, these systems are still very expensive and limited to a few labs around the world, which justifies the wide use of DAD coupled to the liquid chromatography in the identification of phenolics, as a low cost, sensitive, efficient, and flexible system [57].

2.3.2 Identification and quantification of flavonoids in acerola

2.3.2.1 Anthocyanins

The majority of polyphenols found in acerola are anthocyanins, responsible for the reddish/purplish fruit color, similarly to other red fruit. The anthocyanin profile of acerola includes cyanidins, delphinidins, malvidins, pelargonidins, peonidins, and petunidins (Table 2.1). These anthocyanin subclasses are the six most widespread in nature [61]. Cyanidin is the main anthocyanin found in acerolas, corroborating with Olivas-Aguirre et al. [62] as the most widely distributed anthocyanin in red and blue fruit.

Anthocyanins are a large class of water-soluble pigments with a wide range of color diversity, present in vacuoles of different plant tissues, including the fruit [63]. Fruit color is not only the main indicator of sensory quality, but also correlated with nutritional quality [64]. Anthocyanins isolated from acerola show strong ability as free radical scavengers [65] and exhibit several health-promoting effects.

Anthocyanins extracted from fruit are promising for use as food colorant. However, they are unstable and highly susceptible for degradation, depending on conditions such as pH, light, oxygen, and temperature. In this sense, techniques can be adopted for anthocyanin stabilization. In acerola, the stability of anthocyanins seems to be severely compromised by the very high AsA content in the fruit, which condensates on carbon 4 of anthocyanins, resulting in losses of both compounds [66].

The use of inorganic host materials as additives to increase stability of acerola anthocyanins was suggested by Ribeiro et al. [67]. These authors demonstrated that the incorporation of montmorillonite (Mnt) clay into clarified acerola juice at 4% (dry basis) was enough for intercalation of major anthocyanins into Mnt interlayers, resulting in variations from pale red to darker and redder shades after 60 days, and thus preserving the red color of the juice.

Innovative and advanced approaches for stabilization of anthocyanin have been proposed, including co-pigmentation, acylation with various organic acids, complexation metal ions, and microencapsulation, but their application in acerola is still incipient.

2.3.2.2 *Flavan-3-ols: monomeric catechins and proanthocyanidins*

(+)-Catechin and (-)-epicatechin were the major flavan-3-ols previously reported in acerola, besides the isomers (-)-epicatechin gallate and (-)-epigallocatechin gallate were also observed in fruit and by-product (Table 2.1). Catechin content in acerola is comparable to that found in other fruit such as grape [68], apple [29, 69], pear [70], and cherry [71, 72], known as some of major sources of catechin in the world.

Catechins are colorless and astringent, responsible for the fruit bitter taste. Their extraction in different plant tissues is a hard task, due to polymerization with other molecules and high susceptibility to degradation by oxygen, high temperature, light and alkaline conditions. In this way, encapsulation and protected coating are strategies to stabilize catechin molecules [73].

Several beneficial properties has been attributed to catechin and derivatives, including anticancer, anti-obesity, antidiabetic, anticardiovascular, anti-infectious, hepatoprotective, and neuroprotective effects [74]. Furthermore, the antioxidant activity of catechins occurs through the scavenge of free radicals, chelation of redox active transition-metal ions, inhibition of redox active transcription factors, inhibition of pro-oxidant enzymes, and induction of antioxidant enzymes [75]. Studies with catechin from apple after ingestion have shown that this phenolic compound remains stable during gastric digestion, besides being rapidly absorbed from the small intestine [76].

Concerning the presence of proanthocyanidins (or condensed tannins) in acerola fruit and by-products, three were previously reported, namely procyanidin A2, procyanidin B1 and procyanidin B2 (Table 2.1). These compounds, together, accounted for 1.7–6.0% of total phenolic compounds analyzed by RP–HPLC/DAD in ripe acerolas of different cultivars [15].

Proanthocyanidins are synthesized by the polymerization or oligomerization of flavan-3-ols units (catechins and/or epicatechins). Proanthocyanidins are formed in the flavonoid pathway, sharing the same upstream pathway with anthocyanins,

including the function of the anthocyanidin synthase as key role in the biosynthesis of both [77].

Proanthocyanidins are mainly produced in fruit at earlier maturity stages and are responsible for their bitterness [78]. They are synthesized as end products of the flavonoid pathway and are involved in the browning of plant tissues, including fruit, as substrates of polyphenol oxidases, causing higher browning than other phenolics [79, 80].

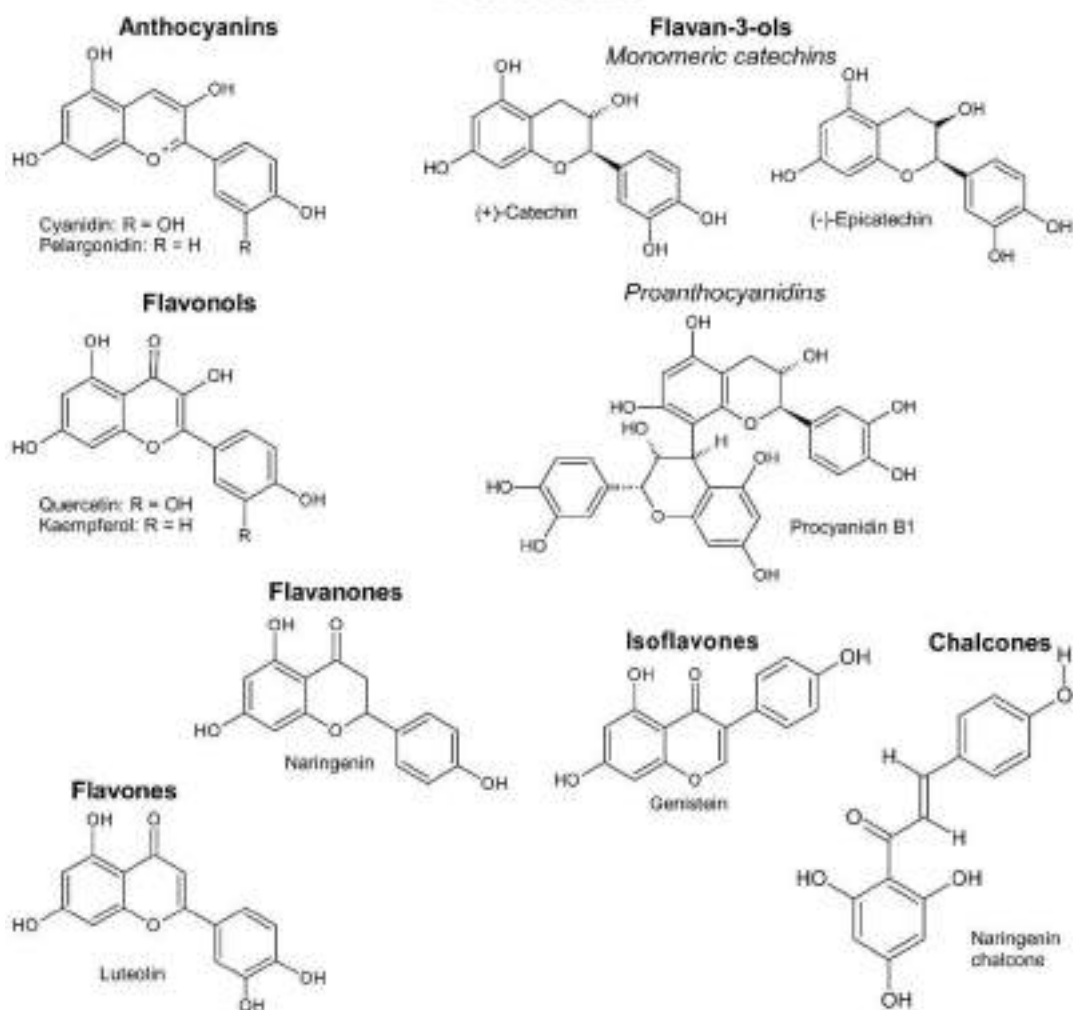
2.3.2.3 *Flavonols*

Flavonols were the phenolic class with the highest diversity of compounds in acerola, with 17 flavonols previously found, including the aglycones kaempferol, myricetin, quercetin, quercitrin, isoquercitrin, isorhamnetin, and rutin, as well as some of their glycosylated forms (Table 2.1). Glucose was the most frequent sugar type linked to flavonols in acerola, but other mono- and di-saccharides were also found, such as galactose, arabinofuranose, robinobiose, neohesperidose, and rhamnose. Other compounds attached to flavonols in acerola were glucosiduronic acid (in myricetin-3-O-glucuronide) and gallic acid (in quercitrin-2''-O-gallate).

Flavonols is usually the main class of phenolic compounds in acerolas at green maturity stage, when the anthocyanins are absent [60]. In that case, kaempferol, isorhamnetin, and quercetin have been reported as the main flavonols in unripe green fruit [49, 51, 60].

Flavonols are flavonoids with a ketone group and a hydroxyl at 3-position on the C ring, where glycosylation generally occur [31]. Quercetin and kaempferol are the major classes of flavonols, differing from each other by one extra OH group at the C3 of the B ring in quercetin (Fig. 2.3) [81]. Both were the flavonols reported with the greatest variety of glycosylations in acerola, with five distinct forms besides aglycone for quercetin, considering isoquercitrin, quercitrin, and rutin are glycoside derivatives of quercetin, and four distinct glycosylated forms for kaempferol (Table 2.1). At least 347 and 279 different glycosidic combinations are possible for kaempferol and quercetin, respectively [82].

Flavonoids



Non-flavonoids

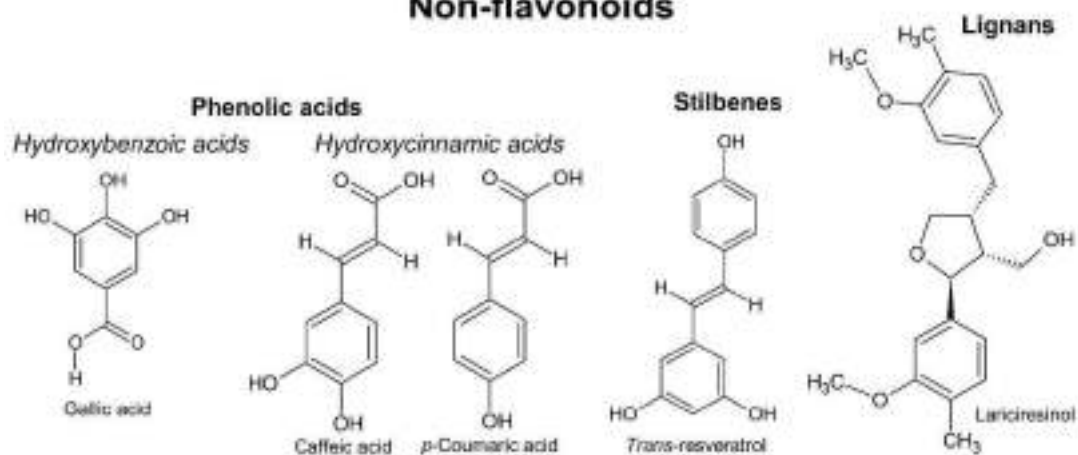


Figure 2.3. Chemical structures of representative phenolic compounds in acerola fruit and by-products.

Flavonol glycosides and aglycones are involved in several functions in plants, including UV photoprotection, reproduction, and internal regulation of cell physiology, besides acting as a free radical scavenger and contributor to plant immune system [83].

2.3.2.4 Flavones and flavanones

Eight flavones (acacetin, apigenin, chrysin, luteolin, malonylapiin, pinobanksin, vitexin and 7,8,4'-trihydroxyflavone) and five flavanones (hesperetin, hesperidin, naringenin, naringin and 2,4',7-trihydroxyisoflavanone) were found in acerolas (Table 2.1). These classes have less participation in the phenolic composition of acerolas than other flavonoid classes [15, 51].

Flavones and flavanones are generally colorless or pale yellow. Flavones are structurally similar to flavonols, with a double bond between positions 2 and 3 and oxidized at position 4 of the C ring, differing only in the absence of hydroxy group at C3 in flavones. The flavanones, in turn, have a similar structure to the flavones, but with the absence of double bond between C2 and C3 (Fig. 2.3) [27, 80, 84].

Naringenin was the major flavanone reported in acerola (Table 2.1). Naringenin is the first product resulted from the synthesis of flavonoids, from which many other flavonoids are formed [78].

2.3.2.5 Isoflavones and chalcones

The literature search indicated that, so far, at least five isoflavones and one chalcone have been identified in acerola fruit, but without quantification of these phenolic compounds. The isoflavones biochanin A and daidzein were reported in seed of acerola agro-industrial residues [48], while formononetin, genistein, 6,7,4'-trihydroxyisoflavone and the chalcone 2',4,4',6'-tetrahydroxychalcone (naringenin chalcone) were found in skin and pulp of green and red-ripe acerolas [55].

2.3.3 Identification and quantification of non-flavonoids in acerola

2.3.3.1 Phenolic acids

Phenolic acids are classified in two major substituted acid derivatives, hydroxybenzoic and hydroxycinnamic acids. The main differences between both classes of phenolic acids are related to the methoxylation and hydroxylation

positions of their aromatic rings and the number of carbons: C6–C1 basic skeleton for hydroxybenzoic acids, and C6–C3 basic skeleton for hydroxycinnamic acids [71].

A total of six hydroxybenzoic (ellagic, gallic, protocatechuic, salicylic and syringic acids, and vanillin) and nine hydroxycinnamic acids (caffeic, caftaric, chlorogenic, m-coumaric, p-coumaric, p-coumaroylquinic, ferulic and isochlorogenic acids, and n-feruloylglycine) were found in studies with acerola fruit and by-products (Table 2.2).

2.3.3.2 Stilbenes and lignans

Stilbenes described in acerola fruit and by-products include resveratrol and coumarins (Table 2.2). Resveratrol was reported for the first time in acerola in its *trans* form by Nowak et al. [52], in cold-pressed juices prepared from whole fruit without addition of water and sugar, in a level of 0.34 mg 100 mL⁻¹. Afterwards, *trans*-resveratrol was found in the skin and pulp of ripe fruit (0.23–0.38 mg 100 g⁻¹ fm) [15] and in by-products of fruit pulp processing (8.1 mg 100 g⁻¹ fm) [58], where *cis* form of resveratrol was also observed (2.5 mg 100 g⁻¹ fm).

Stilbenes are characterized by two aromatic rings joined by an ethylene bridge. Resveratrol has one of the simplest structures among the stilbenes, and present two forms: *trans* form is not sterically hindered, while *cis* form is sterically hindered and therefore less stable [68, 80]. Resveratrol is an antimicrobial and antifungal phytoalexin produced in the plants as a response to injury or infection [26]. It is the most studied stilbene and one of the most popular phenolic compounds, especially for its high concentration in wines, whose moderated and continuous ingestion plays a key role on the prevention of chronic heart diseases [57].

Even if the skin of red grapes (and consequently red wines) are the most known sources of resveratrol in human diet, its presence was also stated in several red fruits other than acerola, such as açaí (0.38 mg 100 g⁻¹ fm), blackberry (6.48 mg 100 g⁻¹ fm), blueberry (1.58 mg 100 g⁻¹ fm) and raspberry (1.12–3.85 mg 100 g⁻¹ fm) [4].

A total of three lignans (lariciresinol, sesaminol and secoisolariciresinol monoglucoside) were identified in the skin and pulp of both green unripe and red ripe acerolas, but without quantification [55]. Lignans are less common and less

studied in fruit and vegetables when compared to flavonoids, but are involved in important pathways of cell signaling, which arouses the interest in future investigations about its usefulness [26, 27].

2.3.4 Bioavailability and bioaccessibility of phenolic compounds in acerola

In recent years, the importance of research related to the bioavailability and bioaccessibility of phenolic compounds was raised, once the biological activities and health-promoting properties of these compounds are not only based on the absolute quantity of intake, but also their behavior in the gastrointestinal system [76]. The ambiguity in the concepts and the undifferentiated use require clear definitions of both terms [85]. Bioavailability is defined as the fraction of a phenolic compound (or any phytochemical or nutrient) that is absorbed, i. e. that reaches the bloodstream, and is available for use in metabolic processes. Bioaccessibility refers to the amount of phenolic compounds released from the food matrix during digestion, becoming absorbable (available for intestinal absorption) [86]. Bioavailability is part of the bioaccessibility, thus the bioaccessible fraction is always equal to or higher than the bioavailable fraction [85].

Studies on bioavailability and bioaccessibility of phenolic compounds of acerola are scarce. For the best of our knowledge, there are no reports in the literature related to the assessment on phenolic content of acerola fruit after gastrointestinal digestion. Bioaccessibility in acerola was previous reported only in frozen pulp, which was compared with other Brazilian fruit pulps. Stafussa et al. [87] found that acerola pulp exhibited remarkably high content of phenolic compounds (13,890.90 mg GAE 100 g⁻¹ dm) and high antioxidant activity by DPPH (727.86 µM Trolox g⁻¹ dm) and DPPH (958.20 µM Trolox g⁻¹ dm) methods, outperforming other phenolic-rich fruit such as açai and jaboticaba. Furthermore, a considerable reduction of phenolic content and antioxidant activity occurred for frozen pulp of all species after in vitro digestion in gastric and intestinal stages; bioaccessibility of phenolic compounds in acerola after both phases was 13.74%, while 8.35% of antioxidant activity was bioaccessible after gastrointestinal digestion [87].

Assis et al. [88] assessed seven flavonoids, three phenolic acids and two stilbenes in acerola purees after oral, gastric, and intestinal digestion and found great variations; cis-resveratrol (6.79%) and chlorogenic acid (7.18%) presented the

lowest bioaccessibility, while rutin (52.24%) and catechin (52.19%) were the most bioaccessible. Additionally, fermentation with probiotic cultures (*Lactobacillus* spp.) increased the bioaccessibility of all phenolic compounds, except cis-resveratrol and kaempferol 3-glucoside.

In acerola juices prepared with frozen pulps diluted in water (1:3, w/v), Fonteles et al. [89] reported a bioaccessibility of 13.3% for phenolic compounds, which was increased with high-temperature short time (22.8%) or ultra-high temperature (22.3%) processing. Acerola by-products (skins and seeds) extracted with water were assessed for phenolic compounds, and presented a bioaccessible fraction of 10.2% [90]. Additionally, the authors found a reduction in bioaccessibility to 4.8% when samples were ultrasonically extracted.

2.3.5 Factors affecting phenolic composition of acerola

2.3.5.1 Genotype/cultivar

It is very clear that the genotype of acerola plant greatly influences the fruit postharvest quality and chemical composition [16–18, 91]. Likewise, the phenolic composition of acerola fruit is also affected by the choice of cultivar. In the last decade, first studies were accomplished by research groups in different regions of Brazil to investigate genetic influence on the phenolic compounds in edible portion of acerola fruit by the classical Folin-Ciocalteu method [92–94].

‘Flor Branca’, one of the major acerola cultivars produced in the Brazilian Northeast Region due to high production regularity, showed less phenolic content when compared to other acerola varieties [92, 94]. The cultivar ‘Okinawa’ was compared to other varieties in at least three studies, and has been noted for its high content of AsA, but not of phenolic compounds [92, 93, 95].

Studies by Mariano-Nasser et al. [93] and Ferreira et al. [15] assessed the same four acerola cultivars (Apodi, Cereja, Roxinha and Frutacor) and conducted similar results; ‘Cereja’ acerola stood out in terms of phenolic compounds, in addition to its high AsA content, and is a great choice for processing and production of frozen pulp, once its high acidity and low sugar content is not recommended in fruit for fresh consumption.

Recently, the use of HPLC revealed both quantitative and qualitative variations on acerola phenolic composition, depending on the genotype. In seven Brazilian acerola varieties, the sum of phenolic compounds quantified individually

ranged between 237.02 mg kg⁻¹ fm (cv. Jaburu) and 845.89 mg kg⁻¹ fm (cv. Roxinha), which represents a difference of almost four times between the varieties with the highest and lowest phenolic concentration [15]. The study also revealed that variation in types of phenolic compounds is genotype-dependent, like the 16 phenolic compounds found in ‘Apodi’ acerolas, in contrast to 11 found in ‘BRS Cabocla’. Furthermore, only five of the 17 studied phenolic compounds were reported in all genotypes.

In the same way, the anthocyanin content is also influenced by the genotype, which reflects in diversity of skin color in acerola cultivars, from dark orange/light-red to dark-purple fruit. Cyanidin content in ‘Flor Branca’ ripe acerolas was 520.76 mg 100 g⁻¹ dm, 262% higher than that found in ‘Jaburu’ in the same study [94]. Besides that, pelargonidin content was 97.04 mg 100 g⁻¹ dm in ‘Flor Branca’, but this anthocyanin was absent in ‘Jaburu’ acerolas.

2.3.5.2 Fruit maturity stage

Throughout acerola maturity stages, several physical, biochemical, and physiological changes are accomplished in the fruit, visually represented by color shift from light green to final reddish hues, which serve as an indicator of harvest time. Thus, a large increase is observed in the anthocyanin content during acerola ripening, in the same way as observed in most red fruit crops [96]. In acerolas harvested at four maturity stages, cyanidin content was 0.49–1.15 mg 100 g⁻¹ dm in small-green fruit, increasing in big-green (9.47–21.63 mg 100 g⁻¹ dm) and intermediate (44.87–111.84 mg 100 g⁻¹ dm) stages and reaching maximum levels in red-ripe fruit (143.65–520.76 mg 100 g⁻¹ dm) [94].

Nascimento et al. [49] assessed phenolic acids and flavonols in green, intermediate and red acerolas by HPLC–DAD, and reported rutin as major phenolic compound in green (101.8 mg 100 g⁻¹ fm) and ripe (16.57 mg 100 g⁻¹ fm) fruit, in contrast to quercetin (11.27 mg 100 g⁻¹ fm) as the main polyphenol in fruit at intermediate stage.

Most of anthocyanins and flavones, as well as chlorogenic and isochlorogenic acids, one flavanone, one lignan, and one chalcone predominantly accumulated in the red acerola fruit rather than in green unripe ones [55]. Meanwhile, ferulic acid, n-coumaric and feruloylglycine were found in higher amounts in unripe fruit than in ripe ones.

Betta et al. [51] determined phenolic composition of acerolas in two red stages, named ripe and fully ripe. Most of the phenolic acids, flavan-3-ols, flavonols, and flavanones assessed individually showed highest concentrations in the ripe stage rather than in fully ripe. A decrease in flavonol content throughout fruit ripening was also related in blueberries, strawberries and cranberries (*Vaccinium macrocarpon* Ait.) [97]. This behavior may be a consequence of the use of phenolics from other classes for biosynthesis of anthocyanins in late ripening stages, like the conversion of flav-2-en-3-ol 3-O-glucoside (a flavonol glycoside) to cyanidin 3-rutinoside as a probable phenomenon in the seed-coat of black soybean (*Phaseolus vulgaris* L.), a cyanidin-rich crop [98].

2.3.5.3 Extraction methods

Extraction is the first procedure to obtain bioactive compounds from fruit or other parts of plants. Different approaches have been applied to separate phenolic compounds from acerola fruit and by-products [99]. Considering the diversity on structure of phenolic compounds, the extraction method influences directly on the recovery rate of phenolic compounds from fruit samples [5, 100]. The use of different organic solvents such as acetone, ethanol, methanol, and their aqueous solutions affect the extraction efficiency, based on the solubility, polarity, and affinity to the solute. Furthermore, the application of physical forces and thermal treatment may optimize the phenolic extraction [4].

Conventional extraction methods such as maceration and Soxhlet methods have been applied in the extraction of phenolic compounds in fruit crops, for their low cost, high accessibility in most laboratories and satisfactory recovery. However, the high volume of solvents and manual procedures required in conventional methods, as well as the concernment about solvent toxicity and environmental pollution, led to the development of emergent extraction approaches [101, 102].

Emergent unconventional extraction techniques reported in acerola include subcritical water extraction (SWE), enzyme-assisted extraction (EAE) and ultrasound-assisted extraction (UAE), as described below.

SWE is an eco-friendly technique that uses only water in a liquid state at a temperature between 100 and 374 °C, under high pressure. In acerola by-product (seeds and pomace), SWE had a higher extraction efficiency and AOX in fresh and

dry samples when compared to Soxhlet method. Additionally, an increase in temperature of SWE from 70 to 130 °C improved the extraction yield, phenolic content, and antioxidant activity [99].

EAE involves the use of cell wall degrading enzymes to weaken cell wall and ensure the extraction of cellular content. In acerola residues, the combination of skin and EAE method using the protease/peptidase enzyme showed the best result of total phenolics when compared to conventional method and seed [103].

UAE method applies ultrasound energy by implosion of cavitation bubbles, resulting in damages to cell wall with increased contact between phenolic compounds and solvents. This process reduces the processing time, energy and solvent consumption [100]. Carvalho Gualberto et al. [48] assessed the combination of extraction methods (UAE and conventional shaking) and solvents (ethanol, methanol and acetone), and found that UAE and acetone were the best conditions for extraction of phenolic compounds in acerola residues. In acerola wastes evaluated by Silva et al. [104], the UAE yielded better results than those obtained with the conventional solid-liquid extraction.

2.4 BIOLOGICAL ACTIVITIES AND HEALTH BENEFITS OF PHENOLIC COMPOUNDS IN ACEROLA FRUIT AND BY-PRODUCTS

The first study on biological activity of acerola was published in 2005 by Hanamura et al. [105]. These authors isolated three phenolic compounds (cyanidin 3-rhamnoside, pelargonidin 3-rhamnoside and quercetin-3-rhamnoside) from acerola fruit and reported effects of these compounds on in vitro O_2^- scavenging activity, as well as an inhibitory effect on diabetes-related molecules (α -glucosidase and advanced glycation end products).

Since then, several health-promoting properties have been attributed to phenolic compounds extracted from acerola. Most of the works published about phenolic compounds in acerola in the 2000s and early 2010s were focused on quantification of antioxidant activity in fruit, juice and frozen pulp by the widely used methods ABTS and DPPH [106–108]. In the last ten years, residues generated as by-products from acerola processing industry, including seeds and residual skin and pulp, began to be investigated as a source of antioxidants for food and nutraceutical applications [109, 110].

2.4.1 Antioxidant activity

In vitro antioxidant activity is the most evaluated biological property attributed to phenolics of acerola fruit and by-products, due to remarkable advances in analytical methods and tools for its quantification during the last few decades. It is known that acerola is rich in various bioactive compounds with antioxidant properties, although phenolic compounds and AsA have the strongest antioxidant capacity [108].

Antioxidants act in different cellular processes, including direct scavenging of ROS, activation of antioxidant enzymes, metal-chelating activity, increase in levels of α -tocopherol and uric acid, inhibition of NADPH oxidases, mitigation of oxidative stress by NO, and increase in the antioxidant properties of low-molecular-weight antioxidants [32].

Antioxidant potential is variable between different phenolic compounds, depending on substitutions in aromatic rings and arrangements in molecules related to hydrogenation, hydroxylation, methylation, malonylation, sulfation, and glycosylation [31]. The presence of two hydroxyl groups in the ring tends to improve the antioxidant potential of a phenolic compound, since hydrogens and electrons are donated to stabilize free-radicals. C2=C3 double bonds associated with 4-carbonyl groups also increase antioxidant activity by providing planarity, electron expansion, and displacement between adjacent rings. Conversely, the presence of 3-OH in a phenolic compound tends to suppress its antioxidant action. Furthermore, O-glycosylation seems to reduce the free radical scavenger property of a flavonoid when compared to its corresponding aglycone [30, 111].

In vitro antioxidant activity of natural extracts can be determined by a range of assays with different mechanisms of action, whose results may vary between them. The lack of consensus on the most convenient and applicable method reflects the need for multiple assays to consistently determine the antioxidant capacity of a plant material. In acerola fruit and by-products, most used methods are based on the capacity of plant extract to neutralize the organic free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Table 2.3). These colorimetric assays are widely disseminated in scientific research worldwide due to their low cost, easy operation and good reproducibility when compared to other methods, despite depending on a spectrophotometer [112].

Table 2.3. Antioxidant activity measured by different assays (DPPH, ABTS, FRAP and ORAC) in acerola fruit and by-products.
(continues)

Method	Contents	Fruit fraction	Maturity stage	Extraction solvent	References
DPPH	1,910.87-2,154.93 μM Trolox g^{-1} dm	Skin and pulp	Green	Ethanol	[60]
	38.33 μg fm g^{-1} DPPH (IC_{50})	Skin and pulp	Green	Methanol	[49]
	95.0-251.0 mM Trolox kg^{-1} fm	Skin and pulp	Green	Methanol	[113]
	54.7-142.0 mM Trolox kg^{-1} fm	Skin and pulp	Intermediate	Methanol	[113]
	39.57 μg fm g^{-1} DPPH (IC_{50})	Skin and pulp	Intermediate	Methanol	[49]
	1120.4 mg Trolox g^{-1} dm	Skin and pulp	Ripe	Methanol/acetone	[51]
	138.1-200.0 mM Trolox kg^{-1} fm	Skin and pulp	Ripe	Ethanol	[15]
	3,276.67-7,738.03 g fm g^{-1} DPPH (IC_{50})	Skin and pulp	Ripe	Methanol/acetone	[95]
	40.4-101.0 mM Trolox kg^{-1} fm	Skin and pulp	Ripe	Methanol	[113]
	46.20 μg fm g^{-1} DPPH (IC_{50})	Skin and pulp	Ripe	Methanol	[49]
	40.0 μg fm g^{-1} DPPH (IC_{50})	Skin and pulp	Ripe	Methanol	[53]
	963.3 mg Trolox g^{-1} dm	Skin and pulp	Fully ripe	Methanol/acetone	[51]
	2,226.24-2,305.27 μM Trolox 100 g^{-1} dm	Seed	-	Ethanol	[48]
	2,221.39-2,305.83 μM Trolox 100 g^{-1} dm	Seed	-	Methanol	[48]
	2,214.16-2,242.86 μM Trolox 100 g^{-1} dm	Seed	-	Acetone	[48]
	67.4 μM Trolox mL^{-1}	Juice	Green	Water	[54]
	5149.0 mg Trolox L^{-1}	Juice	Ripe	Not mentioned	[52]
	88-299 μM Trolox g^{-1}	Non-pomace	Green	Water	[54]
	6.87 μg mL^{-1} (IC_{50})	Non-pomace (pressure)	Green	Ethanol	[22]
	38.17 μg mL^{-1} (IC_{50})	Bagasse (seed and peel)	Green	Ethanol	[22]
	4.24 μg mL^{-1} (IC_{50})	Juice powder	Green	Ethanol	[22]
ABTS	7,475.86-8,613.54 μM Trolox g^{-1} dm	Skin and pulp	Green	Ethanol	[60]
	3,628-14,004 μM Trolox 100 g^{-1} fm	Skin and pulp	Green	Methanol/acetone	[94]
	4,104-4,617 μM Trolox 100 g^{-1} fm	Skin and pulp	Intermediate	Methanol/acetone	[94]

(conclusion)

Method	Contents	Fruit fraction	Maturity stage	Extraction solvent	References
ABTS	3,293-4,236 μM Trolox 100 g^{-1} fm	Skin and pulp	Ripe	Methanol/acetone	[94]
	2.72-5.84 μM Trolox g^{-1} fm	Skin and pulp	Ripe	Methanol/acetone	[95]
	135.7-208.3 mM Trolox kg^{-1} fm	Skin and pulp	Ripe	Ethanol	[15]
	5,556.22-12,055.18 μM Trolox 100 g^{-1} dm	Seed	-	Ethanol	[48]
	7,943.29-16,752.68 μM Trolox 100 g^{-1} dm	Seed	-	Methanol	[48]
	14,404.44-25,493.45 μM Trolox 100 g^{-1} dm	Seed	-	Acetone	[48]
	21.4 mM Trolox	Juice	Ripe	Not mentioned	[52]
	204-380 μM Trolox g^{-1}	Non-pomace	Green	Water	[54]
FRAP	824.23-1,447.97 μM Trolox g^{-1} dm	Skin and pulp	Green	Ethanol	[60]
	29.3-53.5 mM Fe^{2+} 100 g^{-1} fm	Skin and pulp	Ripe	Ethanol	[15]
	501.8 μM Trolox g^{-1} dm	Skin and pulp	Ripe	Methanol/acetone	[51]
	2.2 mM Fe^{2+} 100 g^{-1} fm	Skin and pulp	Ripe	Methanol	[53]
	338.3 μM Trolox g^{-1} dm	Skin and pulp	Fully ripe	Methanol/acetone	[51]
	23,789.66-86,045.58 μM FS 100 g^{-1} dm	Seed	-	Ethanol	[48]
	17,105.57-64,941.90 μM FS 100 g^{-1} dm	Seed	-	Methanol	[48]
	33,480.00-57,171.28 μM FS 100 g^{-1} dm	Seed	-	Acetone	[48]
ORAC	1,950.10-2,454.42 μM Trolox g^{-1} dm	Skin and pulp	Green	Ethanol	[60]
	43.5-79.0 μM Trolox kg^{-1} fm	Skin and pulp	Green	Methanol	[113]
	36.5-62.0 μM Trolox kg^{-1} fm	Skin and pulp	Intermediate	Methanol	[113]
	36.2-53.0 μM Trolox kg^{-1} fm	Skin and pulp	Ripe	Methanol	[113]
	2.13 $\mu\text{g mL}^{-1}$ (IC_{50})	Non-pomace (pressure)	Green	Ethanol	[22]
	7.86 $\mu\text{g mL}^{-1}$ (IC_{50})	Bagasse (seed and peel)	Green	Ethanol	[22]
	2.44 $\mu\text{g mL}^{-1}$ (IC_{50})	Juice powder	Green	Ethanol	[22]

FS – ferrous sulfate

A very high antioxidant activity was observed in green acerola fruit (skin and pulp) assessed by the DPPH assay, in both ethanolic (1,910.87–2,154.93 $\mu\text{M Trolox g}^{-1} \text{ dm}$) [60] and methanolic (95.0–251.0 mM Trolox $\text{kg}^{-1} \text{ fm}$) [113] extracts. Advancement in ripening results in a small reduction in antioxidant activity of acerola, as observed in ripe (1120.4 mg Trolox $\text{g}^{-1} \text{ dm}$) and fully ripe (963.3 mg Trolox $\text{g}^{-1} \text{ dm}$) fruit [51]. Poletto et al. [22] evaluated the composition of by-products generated in the acerola processing of acerola and reported the following DPPH free radical scavenging activity (IC_{50}): 38.17 $\mu\text{g mL}^{-1}$ for bagasse (seed and peel), 6.87 $\mu\text{g mL}^{-1}$ for non-pomace (from the juice clarification step), and 4.24 $\mu\text{g mL}^{-1}$ for juice powder. Additionally, DPPH assay was also adopted for measurement of antioxidant activity of acerola juice prepared with green (67.4 $\mu\text{M Trolox mL}^{-1}$) [54] and ripe (5149.0 mg Trolox L^{-1}) [52] fruit (Table 2.3).

Results of antioxidant activity in fruit skin and pulp using ABTS assay corroborated with the DPPH method, reflecting the reduction on antioxidant activity during acerola ripening, from 3,628–14,004 $\mu\text{M Trolox 100 g}^{-1} \text{ fm}$ in green fruit, to 4,104–4,617 and 3,293–4,236 $\mu\text{M Trolox 100 g}^{-1} \text{ fm}$ in intermediate and ripe stages, respectively [94]. In agro-industrial residues of acerola composed of seeds, the antioxidant activity showed oscillations depending on the solvent and extraction method, ranging from 5,556.22 $\mu\text{M Trolox 100 g}^{-1} \text{ dm}$ (using methanol and ultrasonic bath) and 25,493.45 $\mu\text{M Trolox 100 g}^{-1} \text{ dm}$ (using acetone and shaker) [48]. In addition, the antioxidant activity of a water extract of acerola juice prepared with ripe fruit was also reported in the literature (21.4 mM Trolox) [52] (Table 2.3).

The Ferric Reducing Antioxidant Power (FRAP) is other colorimetric method that has also been considered for determining the antioxidant capacity of acerola [15, 51, 53, 60]. FRAP is a single electron transfer method based on the measurement of the reduction in the colorless complex of ferric iron and tripyridyltriazine (TPTZ) (Fe^{3+} -TPTZ) to the intensely blue ferrous complex (Fe^{2+} -TPTZ) by the plant extract containing antioxidants [114].

Ethanolic and methanolic extracts of ripe acerola evaluated by the FRAP assay presented antioxidant activity of 29.3–53.5 mM $\text{Fe}^{2+} 100 \text{ g}^{-1} \text{ fm}$ [15] and 2.2 mM $\text{Fe}^{2+} 100 \text{ g}^{-1} \text{ fm}$ [53], respectively. Antioxidant capacity of acerola seeds showed a variation between 17,105.57 and 86,045.58 $\mu\text{M ferrous sulfate 100 g}^{-1}$

dm by the FRAP assay, depending on the extraction method and solvent [48] (Table 2.3).

The Oxygen Radical Absorbance Capacity (ORAC) assay was also adopted in previous reports for determination of antioxidant capacity in acerola [22, 60, 113]. ORAC is based on the ability of the radical chain reaction by antioxidants through monitoring the inhibition of peroxy radical-induced oxidation [114]. ORAC method showed a slight reduction in antioxidant activity of fruit (skin and pulp) from green (43.5–79.0 $\mu\text{M Trolox kg}^{-1} \text{ fm}$) to intermediate (36.5–62.0 $\mu\text{M Trolox kg}^{-1} \text{ fm}$) and ripe (36.2–53.0 $\mu\text{M Trolox kg}^{-1} \text{ fm}$) stages. In addition, Poletto et al. [22] used ORAC assay for determining antioxidant capacity (IC_{50}) of by-products generated in the acerola processing, and reported 2.13 $\mu\text{g mL}^{-1}$ for non-pomace from the juice clarification step, 7.86 $\mu\text{g mL}^{-1}$ for bagasse, and 2.44 $\mu\text{g mL}^{-1}$ for juice powder (Table 2.3).

2.4.2 Antibacterial activity

Acerola bagasse flour was tested against the microorganisms *Listeria monocytogenes* ATCC 19117, *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 15442, and *Salmonella cholerasuis* ATCC 6539 [115].

Staphylococcus aureus was inhibited by contact with acerola puree extract [minimum inhibitory concentration (MIC) = 6.25 mg mL^{-1}], which was attributed to the high content of anthocyanins, especially pelargonidin, and rhamnosides of cyanidin and pelargonidin [87]. Phenolic compounds extracted from different portions of acerola fruit were tested against *S. aureus* by Delva and Goodrich-Schneider [113]. The antimicrobial activity of flavonoids from the edible fraction (skin and pulp) was clear in green fruit and moderate in red fruit, while the non-edible fraction (seeds) had a clear activity against *S. aureus*. Additionally, phenolic acids from seeds also showed moderate antimicrobial activity.

In several plant species, the antimicrobial activity has been attributed to their secondary metabolites, including phenolic compounds and alkaloids [116].

2.4.3 Antihyperglycemic, antihyperlipidemic, anti-inflammatory and hepatoprotective activities

A methanolic extract of bagasse flour from acerola agro-industrial residue containing phenolic compounds (gallic acid, catechin, epicatechin gallate,

epicatechin, siringic acid, p-cumaric acid and quercetin) was able to inhibit in vitro digestive enzymes α -amylase and α -glucosidase, and can help in the treatment of obesity, associated comorbidities and type 2 diabetes [117].

In a study by Dias et al. [118], the effect of acerola juice intake was investigated in mice with cafeteria diet-induced obesity. The results showed that acerola juice prevents weight gain (based on body weight and adiposity) and dyslipidemia (based on triglycerides), and restores inflammation to a normal range, which was attributed by the authors to the presence of phenolic compounds in the juice.

Phenolic-rich lyophilized extract of acerola bagasse was tested against the toxic action of carbon tetrachloride (CCl_4) in Wistar rats by Marques et al. [59]. According to the results, there was a decrease in the activity of aspartate aminotransferase, alanine aminotransferase and gamma glutamyl transferase, and an increase in superoxide dismutase, total antioxidant capacity and albumin content in relation to control treatment, confirming the hepatoprotective action of acerola extract.

2.5 APPLICATIONS OF PHENOLIC COMPOUNDS FROM ACEROLA IN FOOD AND PHARMACEUTICAL INDUSTRIES

In tropical regions such as Brazil, several native and exotic unknown or underexploited fruit species present high potential for use in the agroindustry, including acerola [41]. Acerola is a fruit with intense metabolism, characterized by very high respiration rate and ethylene-mediated molecular, biochemical and physiological changes, resulting in a high perishability and fragility after harvest [119]. Thus, the fruit processing is key to reduce acerola postharvest losses.

Fresh consumption of acerola is actually restricted to some varieties selected for high fruit sweetness and low acidity, as well as to regions close to the production area. In order to overcome limitations due to short fruit postharvest life, acerolas have been processed in several products (Fig. 2.4), including frozen pulp, juice, marmalade, frozen concentrate, jam, and liquor [20].

The production of frozen fruit puree is the main destination for ripe acerolas (Fig. 2.4). Previous studies confirmed the high amounts of phenolic compounds in acerola frozen purees, which presented the highest antioxidant activity ($\text{DPPH} = 7433.37 \mu\text{mol Trolox}/100 \text{ g fm}$ and $\text{ABTS} = 8511.84 \mu\text{mol Trolox}/100 \text{ g}$

fm) among the 44 fruit pulps evaluated [42]. The processing of frozen fruit is promising in acerola industries, which enables its global distribution to consumers interested in phenolic-rich products. In recent years, the consumption of acerola frozen pulp acerola has increased in Japan, United States, and Europe [20].

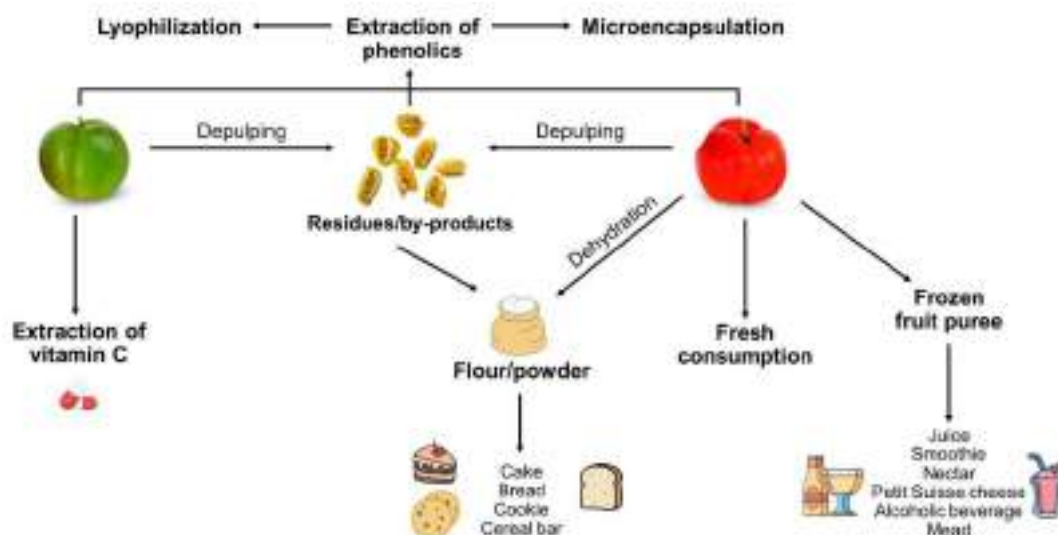


Figure 2.4. Illustrative representation of potential technological processing of acerola fruit and its residue.

In addition to being an alternative to reduce fruit postharvest losses, the acerola frozen purees are versatile for the preparation of phenolic-rich products, including juices [52], smoothies [120], nectars [121], Petit Suisse cheese [122], alcoholic beverages [123], and meads [124]. Acerola is widely consumed worldwide as juice, prepared with acerola fruit [54] or in blends with other tropical fruit [125, 126]. A high diversity of phenolic compounds has been reported in acerola juices, including phenolic acids, flavan-3-ols, flavonols, and flavones, as well as resveratrol (stilbene) [52].

In recent years, some approaches have been proposed to stabilize phenolic compounds and other antioxidant compounds such as ascorbic acid and carotenoids in acerola pulps and juices, which are highly susceptible to degradation when exposed to light, moisture, heat, and oxygen. Microencapsulation is a technique that has shown positive effects in conservation phenolic compounds in acerola, using maltodextrin and gum Arabic as encapsulating agents, and dried by spray and freeze-drying [126, 127]. This technology successfully preserved bioactive compounds and antioxidant activity in acerola pulp for a period of at least 180 days

[128], which demonstrates its high potential for application in functional and nutraceutical foods.

The industrial extraction of vitamin C from green unripe fruit represents an important portion of the global acerola market. With contents that can exceed 4,000 mg/100 g, green acerolas have high potential for the production of extracts, supplements, and concentrated powders of vitamin C for use in food and pharmaceutical purposes. In addition to the high ascorbic acid content, green acerolas contain several phenolic compounds, such as flavonols, flavan-3-ols, isoflavones, phenolic acids, stilbenes, and lignans [49, 55, 60]. Together, these bioactive compounds make acerola an excellent alternative for dietary supplementation and development of new functional foods. For this, processes such as microencapsulation and lyophilization can be applied to extracts of unripe acerola fruit, in order to increase the stability of these compounds, which are suitable as an antioxidant ingredient in food emulsions [8].

Acerola-based products manufacturing results in the discarding of fruit skin and seeds, constituting a residue. These fractions (also named by-products) represent between 10 and 60% of total fruit weight [91] and concentrate high levels of phenolic compounds [54, 99], suggesting their potential use in the food and pharmaceutical industries. Recent researches have focused on potential technological processes that affect phenolic composition of acerola residues, adding value to these by-products. The industrial application of acerola residuals is an important alternative to reduce both environmental impacts and production costs [129].

The processing of acerola by-products into flour and powder results in a product with several antioxidant compounds, destined for nutritional enrichment of cakes, breads, cookies, and cereal bars [115, 130]. Some characteristics found in the flour produced from acerola residues, such as high content of dietary fibers, emulsion stability, and water and oil absorption capacity, make it ideal for incorporation into meat and bakery products [131].

The extraction of phenolic compounds from acerola by-products for use as active compounds in edible coatings is emerging in postharvest researches focusing on fruit conservation. A chitosan solution containing phenolic compounds extracted from acerola by-products was applied on guavas, being able to delay fruit ripening, preserving firmness and maintaining green color [132].

Extracts of acerola flour demonstrated high potential for several biotechnological applications, especially in the cosmetic industry, due to the presence of triterpene saponins with good surface activity and emulsifying capacity [133]. Furthermore, extracts of acerola fruit reduced the cellular melanin level and inhibited tyrosinase activity, suggesting a potential as a component in skin-whitening cosmetics [134].

2.6 CONCLUSIONS AND FUTURE PERSPECTIVES

Scientific evidences suggest that the acerola is a promising superfruit with great potential in the food and pharmaceutical industries. At least 76 phenolic compounds were identified using high performance liquid chromatography in acerolas, including 55 flavonoids (anthocyanins, flavan-3-ols, flavonols, flavones, flavanones, isoflavones and chalcones) and 21 non-flavonoids (phenolic acids, stilbenes and lignans).

Phenolic compounds in acerola show several biological properties, including antioxidant, antibacterial, antihyperglycemic, antihyperlipidemic, anti-inflammatory, and hepatoprotective activities. However, studies are further required to assess the seasonal and genotypic influence on the phenolics of acerola and their bioaccessibility.

Acerola is an anthocyanin-rich fruit with high potential for pigment extraction, but stabilization of anthocyanins in juice and pulp should be further elucidated and improved.

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ARTICLE III

Bayesian and classical approaches for the estimation of genetic parameters and coefficients of repeatability of acerola quality traits*

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Bayesian and classical approaches for the estimation of genetic parameters and coefficients of repeatability of acerola quality traits

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ABSTRACT

Although acerola (*Malpighia emarginata* DC.) is a tropical fruit of high interest due to its high ascorbic acid content and attractive sensory attributes, fruit production is characterized by high genetic variability. Additionally, the use of new biometric tools for acerola breeding is scarce. This study aimed to estimate genetic parameters and the coefficient of repeatability, as well as determine the optimal number of fruits for quality trait analyses in different acerola genotypes, using different approaches. Twenty-three (Experiment I) and thirty-five (Experiment II) genotypes were evaluated in a randomized block design with four replicates and three plants per plot. Twenty fruits per plant were harvested and evaluated for the following quality traits: diameter, mass, skin color (lightness, chroma and hue), firmness, soluble solids (SS), titratable acidity (TA), SS/TA ratio and ascorbic acid content. The genetic parameters and the coefficient of repeatability were estimated for each experiment using classical and Bayesian methods. Both approaches achieved similar results on estimating variance components, genetic parameters and the coefficient of repeatability. Genetic parameters showed favorable conditions for acerola selection. The coefficient of repeatability was high for all acerola quality traits. A total of 17 fruits are required for the effective selection of acerola genotypes with an accuracy of 95%.

Keywords: *Malpighia emarginata* DC.; analysis of variance; genetic variability; fruit quality; repeated measures.

3.1 INTRODUCTION

Acerola (*Malpighia emarginata* DC.) is a tropical fruit well-known for its high ascorbic acid (vitamin C) content, attractive red color and high antioxidant activity (Delva & Schneider, 2013; Prakash & Baskaran, 2018). Acerola is usually consumed as fresh fruit or processed products (Ferreira et al., 2022; Gualberto et al., 2021).

Currently, Brazil is the global largest producer, consumer and exporter of acerola, which is cultivated in all states, but it is mainly concentrated in the Northeast region, which has tropical climatic conditions that favor the growth and development of acerola trees (Ferreira et al., 2021). Acerola commercial cultivation in Brazil started in the 1980s and is still characterized by high genetic variability that results in irregular fruit production, low productivity and poor fruit quality as a result of propagation by seeds (Ritzinger, Kobayashi, & Oliveira, 2017).

With the rising global appeal for fruit consumption, there is a need for genetic breeding programs to select genotypes with high yield and fruit quality to meet the demands of consumers, growers and exporters. In the breeding programs of fruit crops, the determination of physicochemical quality traits and their related genetic parameters is indispensable to improve the efficiency of the selection of superior genotypes and to efficiently preserve germplasm resources (Liu, Qi, Song, Li, & Li, 2018; Zaouay & Mars, 2014). Furthermore, the use of biometric tools optimizes genetic selection and allows the estimation of experimental accuracy.

In this context, the coefficient of repeatability has been extensively adopted in genetic breeding programs of several fruit species (Alcoforado, Pedrozo, Mayer, & Lima-Primo, 2019; Andrade Júnior et al., 2020; Catarina et al., 2020; Diel et al., 2020; Jesus, Lima, Souza, & Girdardi, 2021; Malikouski et al., 2021). The coefficient of repeatability indicates whether multiple measurements of a quality trait in a genotype are the same over time or space, based on the contribution of the genotype to the total variance (Cruz, Regazzi, & Carneiro, 2012), which allows the estimation of an optimal number of fruits to ensure high accuracy in quality trait analysis.

In acerola, a previous study by Lopes, Bruckner, Cruz, Lopes and Freitas (2001) estimated the coefficient of repeatability in some quality traits of sixteen genotypes and found values between 0.03 and 0.94 using four classical methods of determination (analysis of variance, principal component analysis based on the

correlation and covariance matrices and structural analysis). However, there are no studies estimating genetic parameters and the coefficient of repeatability in acerola using Bayesian inference.

Bayesian inference is a data analysis approach based on Bayes' theorem that has been shown to be more informative and flexible in obtaining the components of variance to estimate genetic parameters than classical approaches because it allows the assessment of small samples and the inclusion of prior information, increasing selection accuracy (Evangelista et al., 2022; Valadares et al., 2022).

This work aimed to estimate genetic parameters and the coefficient of repeatability, as well as determine the optimal number of fruits for quality trait analyses in different acerola genotypes, using different approaches.

3.2 MATERIAL AND METHODS

3.2.1 Plant material and experimental conditions

Two experiments were carried out in an experimental orchard in Petrolina, Pernambuco, Brazil, situated at 9°09'S latitude and 40°22' W longitude at an altitude of 365 m. The local climate is BSh (semi-arid), according to Köppen's classification (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013), and the soil is classified as a dystrophic Yellow Argisol (Santos et al., 2018). The orchard was established in 2012, and cultural practices including micro-sprinkler irrigation, fertilization, pruning, and control of weeds, pests and diseases were carried out according to technical recommendations (Ritzinger, Kobayashi, & Oliveira, 2003).

Both experiments were conducted under a randomized complete block design, with four replications each. The experimental unit was composed of three plants, which were grown at a spacing of 4.0 × 3.0 m. Experiments I and II were composed of 23 and 35 genotypes, respectively, which were cultivated in the Active Germplasm Bank at the Brazilian Agricultural Research Corporation (Tropical Semi-Arid Embrapa), Petrolina, PE, Brazil.

A total of 20 acerolas at the red-ripe maturity stage were collected per plant in September 2018 (Experiment I) and September 2019 (Experiment II). The climatic data during the fruit set period, i.e., 20–25 days from anthesis to harvest, depending on the genotype (Maranhão Ribeiro et al., 2018; Santos et al., 2019), were monitored at the experimental orchard weather station. Meteorological data for September 2018 and September 2019 were as follows:

minimum/mean/maximum temperature 21.1/27.6/34.8°C and 21.3/27.4/34.0°C, rainfall 0 and 0.04 mm, relative humidity 56.7 and 55.3%, global solar radiation 26.0 and 24.4 MJ m⁻² day⁻¹, and reference evapotranspiration of 6.9 and 6.8 mm day⁻¹, respectively.

After harvest, the fruit was washed, sanitized, dried and selected based on uniformity of color, size, shape, and absence of damage and fungal infection. Ten fruit quality traits were analyzed, as described below.

3.2.2 Acerola quality traits

Fruit diameter (mm) was determined with 0.01 mm precision using a digital caliper model CD-6 CS (Mitutoyo Corp., Japan). The weight (g) was evaluated with an accuracy of 0.1 g using a semi-analytic balance model VI 2400 (Acculab, USA).

Skin color was analyzed with a colorimeter model CR-400 (Konica Minolta, Japan), according to the three-dimensional CIEL*C*h space, which records the following measurements: lightness (*L**), varying from black (0) to white (100); chroma (*C**), which indicates the color intensity; and hue angle (*°h*), which indicates the color, where 0/360° represents red, and 90°, 180°, and 270° represent yellow, green and blue, respectively (Pathare, Opara, & Al-Said, 2013).

Pulp firmness (N) was measured with a texture analyzer (model TA.XT Plus, Stable MicroSystem, UK) equipped with a P/75 compression plate. Firmness was determined using the compression test, which recorded fruit resistance against 10% deformation in its volume.

Soluble solid (SS) content (%) was measured with an accuracy of 0.2% using a digital refractometer model PAL-1 (Atago, Japan). Titratable acidity (TA) (% of malic acid) was determined by titrating 5 mL of juice with 0.1 M NaOH until reaching a pH of 8.1. The SS/TA ratio was calculated for each sample by dividing the SS value by its respective TA value.

The ascorbic acid content (AsA, in mg 100 g⁻¹) was determined using Tillman's method according to AOAC method no. 942.15 (AOAC, 2016). One milliliter of juice was diluted in 100 mL of 0.5% oxalic acid, which was titrated with 0.02% 2,6-dichlorophenol indophenol (DFI).

3.2.3 Frequentist and Bayesian analysis

In each experiment, genetic parameters were estimated using the frequentist method, i.e., analysis of variance (ANOVA) and Bayesian inference. The model for the frequentist approach was $Y_{ij} = \mu + g_i + a_j + \varepsilon_{ij}$, where Y_{ij} is the observation of the i -th genotype in the j -th environment; μ is the overall mean; g_i is the random effect of the i -th genotype under the influence of the permanent environment; a_j is the fixed effect of the temporary environment in the j -th measurement; and ε_{ij} is the experimental error established by the temporary environmental effect of the j -th measurement in the i -th genotype (Cruz et al., 2012).

Bayesian inference was applied using Monte Carlo via Markov chains (MCMC) through the Gibbs sampling algorithm. Bayesian inference analysis was carried out using the *MCMCglmm* package (Hadfield, 2010) of R (R Foundation for Statistical Computing, Austria). A total of 1,500,000 iterations were generated per trait. A burn-in of 100,000 and a thin sampling interval of 1000 iterations, where the variance components were obtained (*a posteriori* distribution), were used. Different degrees of freedom (ν) (0.002, 0.02, 2, 4 and 7) were adopted to test informative *priors* (Resende & Alves, 2020). Chain convergence was tested using the Geweke test without autocorrelation ($p > 0.05$) in the *boa* package (Smith, 2007). After confirming these criteria for each quality trait, genetic parameters and the coefficient of repeatability were estimated.

3.2.4 Estimation of genetic parameters and coefficient of repeatability

The following genetic parameters were estimated: genetic coefficient of variation ($CV_g = \sqrt{\sigma_g^2} / \mu \times 100$); residual (experimental) coefficient of variation ($CV_e = \sqrt{\sigma_e^2} / \mu \times 100$); variation index ($I_v = CV_g / CV_e$); phenotypic variance (σ_p^2); genetic variance (σ_g^2); environmental (residual) variance (σ_e^2); and heritability ($h^2 = \sigma_g^2 / \sigma_p^2 \times 100$). The phenotypic and genotypic correlations were evaluated using a t-test at a 5% probability level.

The coefficient of repeatability (ρ) was estimated with four classical methods, according to Cruz et al. (2012): a) analysis of variance (ANOVA) ($\rho = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$); b) principal component analysis based on a correlation matrix (PCA-Cor); c) principal component analysis based on a covariance matrix (PCA-Cov) (Abeywardena, 1972); and d) structural analysis based on a correlation matrix (SA-

Cor) (Mansour, Nordheim, & Rutledge, 1981). The coefficient of repeatability from Bayesian inference was calculated using variance components and the same formula as the ANOVA method.

After the estimation of the coefficient of repeatability in all methods, for all quality traits, the minimum required number of fruit (η_0) for prediction of the real value of the individuals was calculated based on predefined R^2 values of 0.80, 0.85, 0.90, and 0.95, as follows:

$$\eta_0 = \frac{R^2 (1 - \rho)}{(1 - R^2) \rho}$$

3.3 RESULTS

Genetic parameters of acerola genotypes determined by the frequentist method, represented by the genetic coefficient of variation, environmental coefficient of variation, variation index, phenotypic variance, genetic variance, environmental variance and heritability are shown in Table 3.1.

Table 3.1. Estimates of genetic parameters of 10 postharvest physicochemical fruit traits from acerola genotypes using the frequentist method.

Parameter	FD	FM	L	C	h	PF	SS	TA	SS/TA	AsA
CV _g	8.04	25.47	5.90	10.08	18.36	31.59	8.83	14.31	17.54	19.05
CV _e	2.13	4.60	2.52	3.77	5.57	6.20	3.58	3.75	4.73	5.78
CV _g /CV _e	3.77	5.53	2.34	2.67	3.29	5.10	2.47	3.82	3.71	3.30
σ_p^2	3.78	2.79	5.82	19.26	26.19	12.99	0.78	0.046	1.42	105894
σ_g^2	3.53	2.70	4.92	16.89	23.98	12.51	0.67	0.043	1.32	96968
σ_e^2	0.25	0.09	0.90	2.36	2.21	0.48	0.11	0.003	0.10	8926
h ²	93.42	96.84	84.58	87.72	91.56	96.30	85.90	93.59	93.22	91.57
Experiment II (35 genotypes)										
CV _g	11.66	35.28	7.62	12.92	13.62	33.79	18.88	19.96	23.35	26.84
CV _e	2.19	4.21	2.54	3.09	4.78	5.32	3.08	3.97	4.26	4.98
CV _g /CV _e	5.33	8.38	3.00	4.18	2.85	6.35	6.13	5.03	5.48	5.39
σ_p^2	6.51	3.46	10.06	33.80	16.93	11.99	2.85	0.080	2.42	181331
σ_g^2	6.29	3.41	9.06	31.97	15.08	11.70	2.77	0.077	2.34	175304
σ_e^2	0.22	0.05	1.01	1.83	1.85	0.29	0.07	0.003	0.08	6027
h ²	96.60	98.60	89.99	94.59	89.06	97.58	97.40	96.20	96.78	96.68

FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; AsA: ascorbic acid. CV_g: genetic coefficient of genetic variation; CV_e: residual (experimental) coefficient of variation; σ_p^2 : phenotypic variance; σ_g^2 : genetic variance; σ_e^2 : environmental (residual) variance; h²: heritability.

In Experiment I, the CV_g ranged between 5.90 and 31.59%, while those for Experiment II were between 7.62 and 35.28%. In both experiments, lightness was

the trait with the lowest CV_g , while pulp firmness and fruit mass had the highest CV_g in Experiments I and II, respectively. The CV_g was higher in Experiment II for all quality traits, except for the pulp hue. In 80% of quality traits (considering both experiments together), CV_g was higher than 10%, considering the desired variability for genotype selection.

The CV_e ranged between 2.13 and 6.20% in Experiment I and between 2.19 and 5.32% in Experiment II. All CV_e values were considered low (<10%). Fruit mass and pulp firmness were the traits with the lowest and highest CV_e , respectively, in both experiments.

The heritability was very high (>80%) for all quality parameters. Fruit lightness had the lowest h^2 in Experiment I (84.58%) and hue in Experiment II (89.06%). The highest h^2 values were observed for fruit mass in both experiments, at 96.84% and 98.60% in Experiments I and II, respectively.

Genetic parameters were also estimated using Bayesian inference and are shown in Table 3.2.

Table 3.2. Estimates of genetic parameters of 10 postharvest physicochemical fruit traits from acerola genotypes using the Bayesian method.

Parameter	FD	FM	L	C	h	PF	SS	TA	SS/TA	AsA
Experiment I (23 genotypes)										
CV_g	8.32	26.22	6.02	10.48	19.16	33.14	9.04	14.52	18.06	19.43
CV_e	2.16	4.70	2.56	3.88	5.77	6.26	3.65	3.76	4.80	5.84
CV_g/CV_e	3.86	5.58	2.35	2.70	3.32	5.29	2.48	3.86	3.76	3.33
σ_p^2	4.03	2.95	6.04	20.75	28.50	14.25	0.82	0.05	1.50	109951
σ_g^2	3.78	2.86	5.12	18.24	26.13	13.76	0.70	0.044	1.40	100847
σ_e^2	0.25	0.09	0.92	2.51	2.37	0.49	0.11	0.003	0.10	9104
h^2	93.71	96.89	84.70	87.92	91.70	96.56	86.02	93.71	93.40	91.72
Experiment II (35 genotypes)										
CV_g	11.84	36.50	7.92	13.17	13.76	34.79	19.35	20.84	24.26	27.37
CV_e	2.26	4.38	2.65	3.15	4.92	5.45	3.10	4.03	4.39	5.15
CV_g/CV_e	5.23	8.34	2.98	4.18	2.80	6.38	6.25	5.17	5.53	5.32
σ_p^2	6.72	3.70	10.88	35.15	17.35	12.70	2.99	0.09	2.61	188765
σ_g^2	6.48	3.65	9.78	33.25	15.38	12.40	2.91	0.084	2.53	182316
σ_e^2	0.24	0.05	1.10	1.90	1.96	0.30	0.07	0.003	0.08	6449
h^2	96.47	98.58	89.90	94.59	88.68	97.61	97.50	96.40	96.83	96.58

FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; AsA: ascorbic acid. CV_g : genetic coefficient of genetic variation; CV_e : residual (experimental) coefficient of variation; σ_p^2 : phenotypic variance; σ_g^2 : genetic variance; σ_e^2 : environmental (residual) variance; h^2 : heritability.

The estimates of the variance components (σ_g^2 and σ_p^2) were higher using the Bayesian method, with a slight increase in CV_g and CV_e when compared to the frequentist method. CV_g ranges of acerola genotypes were 6.02–33.14% (Experiment I) and 7.92–36.50% (Experiment II) using the Bayesian approach.

In both experiments, h^2 was slightly higher when estimated by Bayesian inference for most quality traits. In Experiment I, the lowest and highest h^2 were 84.70 (lightness) and 96.89% (fruit mass), respectively. In Experiment II, h^2 varied between 88.68 (lightness) and 98.58% (hue).

The phenotypic and genotypic correlations for the 10 quality traits in acerola genotypes are shown in Table 3.3. A total of 9 and 11 significant phenotypic correlations were found in Experiments I and II, respectively, with coefficient ranges (in modulus) of 0.41–0.96 and 0.34–0.90. Of these, two were strong correlations ($0.90 < |r| < 1.00$), nine were strong correlations ($0.60 < |r| \leq 0.90$), and nine were medium correlations ($0.30 < |r| \leq 0.60$). When considering genotypic correlations, nine significant correlations were found in Experiment I and eleven in Experiment II. Most significant genotypic correlations were classified as strong (50%) or medium (40%).

For both experiments, fruit mass and diameter had strong and positive phenotypic (0.96 and 0.90) and genotypic (0.97 and 0.91) correlation coefficients. All correlations between color traits (L^* , C^* and $^{\circ}h$) were significant and positive, and most of them were strong. Other significant phenotypic and genotypic correlations in both experiments included $FM \times (PF \text{ and } SS)$ and $L \times AsA$. As expected, the SS/TA ratio was positively correlated with TA and negatively correlated with SS . In Experiment II, AsA was negatively correlated with SS/TA ratio, considering phenotypic (-0.47) and genotypic (-0.48) correlations.

Table 3.3. Estimates of phenotypic (upper diagonal) and genotypic (lower diagonal) correlations for 10 postharvest physicochemical fruit traits from acerola genotypes.

	FD	FM	L	C	h	PF	SS	TA	SS/TA	AsA
Experiment I (23 genotypes)										
FD		0.96**	-0.19	-0.27	-0.22	0.33	-0.36	-0.12	-0.08	-0.25
FM	0.97**		-0.15	-0.34	-0.09	0.43*	-0.41*	-0.03	-0.17	-0.28
L	-0.22	-0.18		0.77**	0.78**	0.13	-0.18	-0.02	-0.12	-0.52*
C	-0.32	-0.38	0.75**		0.58**	-0.06	0.05	-0.03	0.01	-0.12
h	-0.25	-0.10	0.77**	0.57**		0.33	-0.23	0.14	-0.25	-0.24
PF	0.36	0.44*	0.10	-0.09	0.32		-0.36	-0.16	-0.04	-0.03
SS	-0.38	-0.44*	-0.14	0.12	-0.22	-0.38		-0.15	0.61**	0.34
TA	-0.15	-0.04	-0.04	-0.04	0.14	-0.18	-0.19		-0.87**	0.37
SS/TA	-0.06	-0.17	-0.10	0.05	-0.24	-0.03	0.62**	-0.88**		-0.10
AsA	-0.27	-0.31	-0.62**	-0.16	-0.28	-0.05	0.38	0.40	-0.11	
Experiment II (35 genotypes)										
FD		0.90**	-0.11	-0.26	0.05	0.34*	-0.25	-0.16	-0.08	-0.23
FM	0.91**		-0.04	-0.24	0.18	0.36*	-0.26	-0.09	-0.15	-0.09
L	-0.12	-0.04		0.71**	0.72**	-0.31	-0.14	-0.18	0.07	-0.14
C	-0.27	-0.25	0.73**		0.68**	-0.41	0.03	-0.14	0.16	-0.05
h	0.06	0.20	0.74**	0.70**		-0.11	-0.12	-0.18	0.10	-0.11
PF	0.35*	0.36*	-0.34*	-0.43	-0.13		-0.08	0.24	-0.31	0.23
SS	-0.25	-0.26	-0.14	0.04	-0.11	-0.07		0.39*	0.47**	0.24
TA	-0.18	-0.08	-0.18	-0.15	-0.20	0.26	0.39*		-0.60**	0.72**
SS/TA	-0.08	-0.16	0.08	0.18	0.12	-0.32	0.48	-0.60**		-0.47**
AsA	-0.23	-0.09	-0.14	-0.05	-0.12	0.24	0.24	0.74**	-0.48**	

FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; AsA: ascorbic acid.

* and **: significant correlations at 5 and 1% by the t-test, respectively.

Variation between quality traits was observed for the coefficient of repeatability, whose values ranged from 0.58 for lightness in Experiment I to 0.95 for fruit mass in Experiment II. Estimates of the coefficient of repeatability in our study are considered high since all quality traits except lightness in Experiment I showed a coefficient of repeatability greater than or equal to 0.60 (Table 3.4).

Classical methods for estimating the coefficients of repeatability showed very similar values. Bayesian estimation resulted in similar coefficients of repeatability for most quality traits. In a few quality traits, the Bayesian approach revealed values slightly lower than the other methods for this parameter, such as for SS in the first experiment (0.56 in Bayesian inference $\times$ 0.66 in PCA-Cor method).

Table 3.4. Estimates of the coefficient of repeatability (ρ) and its coefficient of determination (R^2) for 10 postharvest physicochemical fruit traits from acerola genotypes.

Quality trait	ANOVA		PCA-Cor		PCA-Cov		AS-Cor		Bayesian	
	ρ	R^2	ρ	R^2	ρ	R^2	ρ	R^2	ρ	R^2
Experiment I (23 genotypes)										
FD	0.78	93.42	0.80	93.96	0.79	93.86	0.79	93.83	0.78	93.29
FM	0.88	96.84	0.90	97.24	0.90	97.22	0.90	97.22	0.88	96.79
L	0.58	84.58	0.62	86.90	0.58	84.80	0.61	86.29	0.53	81.94
C	0.64	87.72	0.68	89.44	0.67	88.97	0.67	89.09	0.61	86.27
h	0.73	91.56	0.75	92.47	0.75	92.28	0.75	92.29	0.72	90.95
PF	0.87	96.30	0.87	96.46	0.87	96.36	0.87	96.42	0.87	96.43
SS	0.60	85.90	0.66	88.45	0.61	86.11	0.65	87.90	0.56	83.74
TA	0.78	93.59	0.83	94.98	0.81	94.62	0.82	94.86	0.78	93.28
SS/TA	0.77	93.22	0.81	94.43	0.79	93.94	0.81	94.30	0.77	92.94
AsA	0.73	91.57	0.75	92.47	0.77	92.90	0.75	92.19	0.72	90.97
Experiment II (35 genotypes)										
FD	0.88	96.60	0.88	96.82	0.88	96.81	0.88	96.79	0.87	96.34
FM	0.95	98.60	0.95	98.66	0.95	98.63	0.95	98.66	0.94	98.56
L	0.69	89.99	0.72	91.29	0.70	90.13	0.72	90.99	0.66	88.77
C	0.81	94.59	0.82	94.93	0.82	94.64	0.82	94.84	0.80	94.29
h	0.67	89.06	0.70	90.25	0.69	89.92	0.68	89.65	0.63	87.23
PF	0.91	97.58	0.92	97.84	0.92	97.94	0.92	97.82	0.91	97.55
SS	0.90	97.40	0.91	97.61	0.91	97.61	0.91	97.59	0.90	97.44
TA	0.86	96.20	0.87	96.34	0.86	96.21	0.87	96.30	0.87	96.26
SS/TA	0.88	96.78	0.89	96.89	0.88	96.85	0.89	96.85	0.88	96.73
AsA	0.88	96.68	0.88	96.82	0.88	96.74	0.88	96.79	0.87	96.07

ANOVA: analysis of variance; PCA-Cor: principal component analysis based on correlation matrix; PCA-Cov: principal component analysis based on covariance matrix; SA-Cor: structural analysis based on correlation matrix; FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; AsA: ascorbic acid.

Based on classical methods for estimating the coefficient of repeatability, the minimum required number of measurements for prediction of the real value of the individuals was 13.86, 11.46, 13.62 and 12.07 fruit in the first experiment, and 9.34, 8.21, 8.52 and 8.78 in the second experiment, considering ANOVA, PCA-Cor, PCA-Cov and SA-Cor methods, respectively, with an R^2 of 95%. For the same accuracy, the number of measurements estimated by Bayesian inference was 16.75 and 11.12 fruit in Experiments I and II, respectively. The diameter, mass, pulp firmness, titratable acidity and SS/TA ratio determined by the PCA-Cor and PCA-Cov approaches required the smallest number of fruit (<6) to predict the real value with a 95% accuracy for both experiments independent of the adopted approach (Figure 3.1).

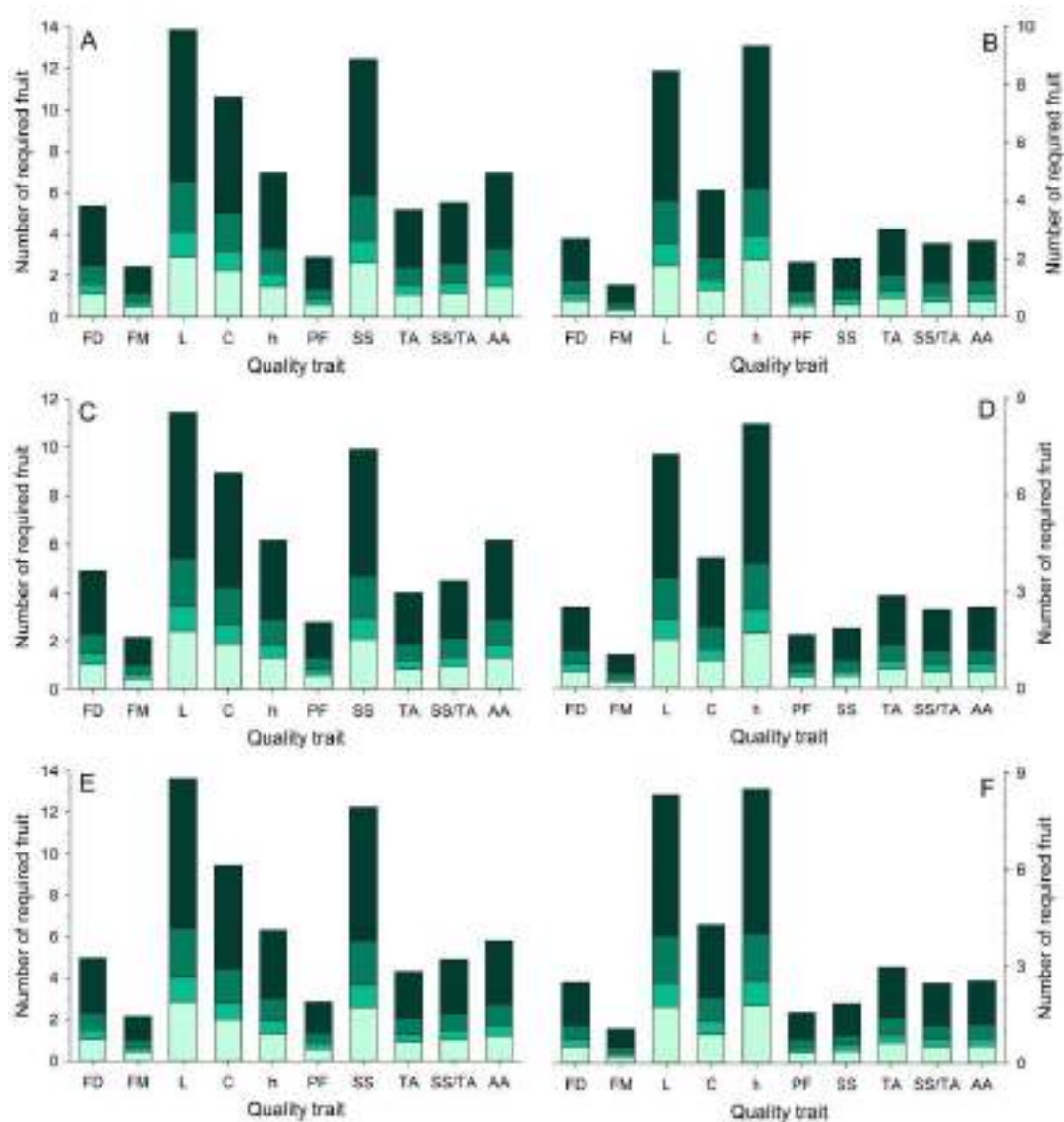


Figure 3.1. Number of fruit required for different R^2 values (80, 85, 90 and 95%) estimated by analysis of variance (A and B), principal component analysis based on correlation matrix (C and D), principal component analysis based on covariance matrix (E and F), structural analysis based on correlation matrix (G and H), and Bayesian inference (I and J) for 10 postharvest physicochemical traits of fruit from 53 acerola genotypes. Figures on the left represent Experiment I (23 genotypes), and those on the right represent Experiment II (35 genotypes).

(continues)

FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; AsA: ascorbic acid.

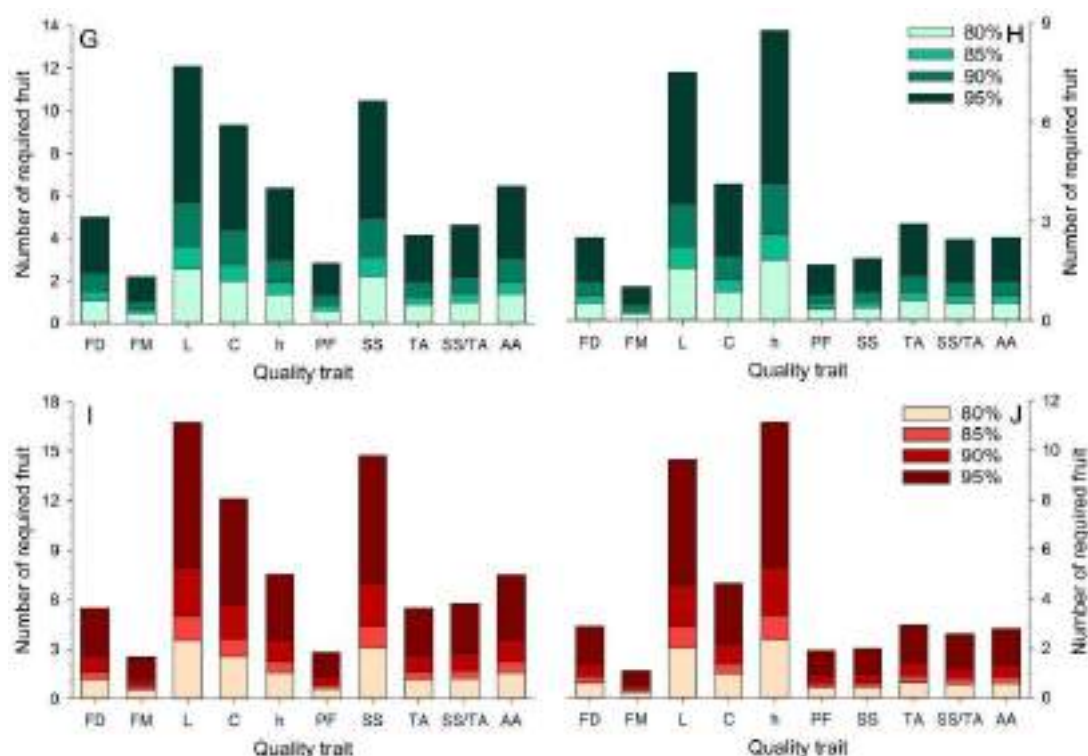


Figure 3.1. Number of fruit required for different R² values (80, 85, 90 and 95%) estimated by analysis of variance (A and B), principal component analysis based on correlation matrix (C and D), principal component analysis based on covariance matrix (E and F), structural analysis based on correlation matrix (G and H), and Bayesian inference (I and J) for 10 postharvest physicochemical traits of fruit from 53 acerola genotypes. Figures on the left represent Experiment I (23 genotypes), and those on the right represent Experiment II (35 genotypes).

(conclusion)

FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; AsA: ascorbic acid.

3.4 DISCUSSION

The commercial cultivation of acerola in Brazil started in the late 1980s and was mostly focused on the production of ascorbic acid (vitamin C) for the food and pharmaceutical industries. At that time, acerola trees were mainly propagated by seeds (sexual method), which resulted in a high genetic variability in Brazilian acerola orchards (Ritzinger et al., 2017) that has been observed until the present day and reported in studies that have evaluated the fruit quality of different acerola genotypes cultivated in different regions (Farinelli et al., 2021; Ferreira et al., 2021; Ferreira et al., 2022; Magalhães et al., 2018). In this context, the use of biometric tools is key for genotype characterization to optimize selection.

The assessment of genotypes in fruit breeding programs requires time, labor and expenses. Therefore, the study of experimental parameters, such as the coefficient of correlation and repeatability, can optimize genotype selection by estimating an ideal number of measurements required to ensure high quality characterization accuracy (Alcoforado et al., 2019; Jesus et al., 2021).

In this study, the high values of genetic coefficients of variance (CV_g) (> 10%) and high heritability (h^2) (> 60%) for most quality traits indicate favorable conditions for selection as a result of high genetic variability (Jesus et al., 2021). The environmental coefficient of variation (CV_e) was lower than 10% for the 10 studied quality traits. When assessing quality parameters of eight acerola genotypes, Mariano-Nasser et al. (2017) found low CV_e values (3.0–7.9%) for skin lightness, soluble solids, titratable acidity and ascorbic acid and medium CV_e values (13.8–19.9%) for skin chroma and hue. Furthermore, the relative CV (CV_g/CV_e) was higher than 1 for all quality traits, which is recommended for genotype selection (Azevedo et al., 2021; Vasconcelos et al., 2020).

Fruit mass and pulp firmness presented the highest CV_g among all quality traits assessed in both experiments, confirming exploitable and desirable genetic variability. Larger and firmer fruit are strongly desirable in acerola breeding programs to improve pulp yield and facilitate processing, as well as to reduce fruit losses due to higher resistance to postharvest handling, storage and shipping (Freitas et al., 2022). However, lightness showed the lowest CV_g in both experiments. Lightness is part of the CIEL*C*h color space system that can range from dark (0) to light (100), which varies less than other color parameters, such as chroma and hue angle (Lazaro et al., 2019).

Some significant correlations in acerola quality traits include those between both physical traits (diameter and mass) and between pairs of skin color parameters (lightness, chroma and hue), confirming previous results observed in 103 acerola trees (phenotypes) evaluated by Farinelli et al. (2021) and in 24 genotypes evaluated by Magalhães et al. (2018). The genetic coefficients of correlation for acerola were higher than phenotypic ones, which confirms the high genetic variability in the studied genotypes.

The coefficient of repeatability was estimated for each quality trait in each experiment using four classical methods and Bayesian inference. In all methods, the variance components (genetic and environmental variances) were the basis for

calculating the coefficient of repeatability. A higher repeatability coefficient (close to 1) means that multiple measurements of a quality trait in one evaluated genotype are consistent in different times or spaces (Cruz et al., 2012), and thus, not many fruit are required to assess this trait with high accuracy in genetic breeding programs. A high coefficient of repeatability (>0.60) was observed for the 10 quality traits, similar to Lopes et al. (2001) with 16 acerola genotypes evaluated for similar traits, including fruit diameter (0.88), mass (0.92), titratable acidity (0.81) and ascorbic acid (0.84).

The estimates of genetic parameters found in this study indicate similar results for different approaches (Bayesian $\times$ classical methods), which agrees with studies on kale (Brito et al., 2019) and physic nut (*Jatropha curcas* L.) (Evangelista et al., 2022). Some advantages of Bayesian inference in relation to frequentist determination of variance components (and consequently coefficients of repeatability) include the feasibility of using previous data information (*priori*), the lower sensitivity to outliers and lower data assumptions, such as experimental balance and high sample size (Singh, L-Yassin, & Omer, 2015), resulting in more accurate estimates of variance, as reported in guava (Silva et al., 2020) and soybean (Volpato et al., 2019).

Despite several studies on the genotypic variability of acerola quality, only a few studies have involved the evaluation of repeated measures in acerola breeding. Furthermore, Bayesian inference was applied for the first time as an alternative method for estimating genetic parameters in acerola.

3.5 CONCLUSION

Frequentist and Bayesian approaches achieved similar results in estimating variance components and genetic parameters of acerola quality traits. The coefficient of repeatability was high for all acerola quality traits and varied little when estimated using five different methods. A total of 17 fruits are required for the effective selection of acerola genotypes with an accuracy of 95%.

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ARTICLE IV

Genetic diversity in acerola (*Malpighia emarginata* DC.) quality traits revealed by artificial neural networks*

***Situation:** Not submitted

Genetic diversity in acerola (*Malpighia emarginata* DC.) quality traits revealed by artificial neural networks

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ABSTRACT

Acerola is a tropical fruit well-known for its high ascorbic acid (AsA) content. Studies on genetic diversity are important to identify superior acerola genotypes with desired attributes for breeding programs. The aim of this study was to evaluate the genetic diversity of acerola genotypes, based on fruit quality traits at different maturity stages. Fruits of 35 genotypes were harvested at green and red maturity stages and evaluated for diameter, mass, color, firmness, soluble solids (SS), titratable acidity (TA), SS/TA ratio and AsA. Genetic diversity was assessed by unweighted pair cluster method with mean hierarchical clustering based on the Mahalanobis distance and through artificial intelligence using Kohonen self-organizing maps. Genetic diversity was observed among genotypes for all quality traits and maturity stages. Okinawa was the most divergent in green fruit due to its highest mass and firmness, as well as a high AsA content, and therefore is recommended for industrial extraction of AsA. BRS Rubra was the most divergent in red maturity stages and had the highest SS and SS/TA ratio, being recommended for fresh consumption and processing. The SS/TA ratio was the trait that most contributed to genetic diversity of acerola, in both green (24.84%) and red (46.36%) maturity stages.

Keywords: Barbados cherry; vitamin C; fruit quality; active germplasm bank; plant breeding; quantitative traits; postharvest quality; kohonen self-organizing maps; superfruits; biometrics.

4.1 INTRODUCTION

Acerola (*Malpighia emarginata* DC., Malpighiaceae), also known as Barbados cherry, West Indian cherry, and Guarani cherry, is a tropical fruit native to West Indies and tropical America that has high ascorbic acid (vitamin C) contents, which can exceed 100 times that found in orange and lemon [1–3]. In addition, many other bioactive compounds with high antioxidant activity are found in acerola, which is recognized as a “super fruit” due to several health benefits [4–6].

Brazil is the largest producer, consumer, and exporter of acerola worldwide, with production distributed in all states, especially in the Northeastern region, known as the São Francisco Valley, which accounts for 25% of the country acerola production. In that region, each orchard can be harvested until eight times per year due to the short crop cycle and favorable edaphoclimatic conditions [7].

Acerola fruit quality is determined by several factors, including soil and climate conditions, cultural practices, genotypes, and maturity stage [7–10]. Fruits for fresh consumption are harvested at the red-ripe maturity stage with high diameter, firmness and soluble solids content, as well as low acidity, while fruits for ascorbic acid extraction are harvested at early green maturity stages due to higher content [11].

Historically, Brazilian acerola trees have been sexually propagated by seeds, which resulted in heterogeneous orchards with irregular production and low fruit quality [12]. Moreover, acerola commercial cultivation in Brazil started in the last decades and has been accomplished with a few cultivars [13]. Therefore, acerola breeding requires to be optimized using statistical and biometric tools that will help determining the genetic diversity based on the assessment of fruit quality traits at different maturity stages.

In a plant breeding program, exploring genetic diversity offers breeders insights into the genetic variability among assessed populations. This information aids in identifying the most contrasting parent plants, streamlining crossbreeding efforts for optimal results, and consequently, enhances genetic gains and efficiency in the development of new cultivars [14].

Indeed, genetic diversity determined based on fruit quality traits has been investigated in several fruit crops, including strawberry [15], mango [16], passion fruit [17], plum [18], papaya [19], and apple [20]. Hence, the aim of this study was

to evaluate the genetic diversity of acerola genotypes, based on fruit quality traits at different maturity stages.

4.2 MATERIAL AND METHODS

4.2.1 Plant material and experimental conditions

The experiment was carried out with 35 acerola (*Malpighia emarginata* DC.) genotypes belonging to the germplasm collection of the Brazilian Agricultural Research Corporation (Tropical Semi-arid Embrapa), Petrolina, Pernambuco, Brazil (9° 09' S, 40° 22' W, altitude of 365 m). The fruit were produced in 2019 and 2020. The orchard has a BSh Semi-arid climate, according to the Köppen's classification [21] and a dystrophic Yellow Argisol soil [22].

The orchard was established in 2012 with vegetatively propagated plants. The trees were spaced at 4 m between plants and 3.5 m between rows. Crop management practices were performed as recommended by Ritzinger et al. [23]. Irrigation was daily performed based on the crop evapotranspiration.

The experiment followed a randomized block design with three replications and three plants per replication. A total of ten fruit per replication were harvested at two maturity stages (fully expended-green and red-ripe). After harvest, the fruit were transported to the Postharvest Laboratory and were washed, sanitized, dried at room temperature, and analyzed for the following quality traits.

4.2.2 Characterization of fruit quality traits

Diameter (mm) was determined with a digital caliper (model CD-6 CS, Mitutoyo, Japan). Mass (g) was evaluated with a semi-analytical balance (model AS 2000, Marte, Brazil). Color was determined using a colorimeter (model CR-400, Konica Minolta, Japan) and recorded as hue angle (°h), which represents the color that ranges from 0/360° (red), 90° (yellow), 180° (green), and 270° (blue).

Pulp firmness (N) was assessed with a texture analyzer (model TA.XT Plus, Stable Micro Systems, UK) equipped with stainless compression plate. The pulp firmness values represent the maximum force required to compress 10% of each fruit diameter. Soluble solids (SS) content (%) was evaluated with a digital refractometer that contains automatic temperature compensation (model PAL-1, Atago, Japan). Titratable acidity (TA) was quantified by titration of 1 mL of fruit juice, diluted in 50 mL of distilled water, with 0.1 N NaOH using an automatic

titrator (model Titrino Plus, Metrohm, Brazil). TA results were expressed as percentage of malic acid. The SS/TA ratio was calculated by dividing the SS value by the TA value in each sample. Ascorbic acid (AsA) content (mg/100 g) was determined by titration of fruit juice with 2,6-dichlorophenol indophenol, following the AOAC method no. 967.21 [24].

4.2.3 Statistical analysis

4.2.3.1 Statistical model

The analysis of variance was performed for all acerola quality traits in each maturity stage, according to the model $Y_{ij} = m + G_i + B_j + e_{ij}$, where Y_{ij} represents the observation of i_{th} genotype in j_{th} block; m represents the overall mean; G_i represents the effect of i_{th} genotype; B_j represents the effect of j_{th} block; and e_{ij} represents the experimental error associated with observation Y (residue).

4.2.3.2 Assessment of genetic diversity

The Mahalanobis distance (D^2) was estimated based on the means of fruit quality traits [25], as follows:

$$D_{ii'}^2 = \delta' \psi^{-1} \delta$$

where: $D_{ii'}^2$ is the Mahalanobis distance between genotypes i and i' ; ψ^{-1} is the matrix of variances and residual covariances; $\delta' = [d_1, d_2, \dots, d_v]$ being $d_j = Y_{ij} - \bar{Y}_{i\cdot}$; and $\bar{Y}_{i\cdot}$ is the mean of the i_{th} genotype in relation to the j_{th} trait.

Acerola genotypes were then clustered by the unweighted pair group method with arithmetic mean (UPGMA), based on the distance matrix, and were represented on a dendrogram. Clustering consistency was verified by the cophenetic correlation coefficient. The number of groups was defined by maximizing the Caliński-Harabasz pseudo-F index, calculated as follows [26]:

$$\text{Pseudo-F} = \left(\frac{A}{W}\right) \cdot \left(\frac{n-k}{k-1}\right)$$

where: A and W are the among- and within-cluster variances, respectively; n is the number of genotypes; and k is the number of existing clusters.

The relative contribution of quality traits on acerola genetic diversity was estimated by the method described by Singh [27]. In addition, genetic diversity was

assessed using computational intelligence, through artificial neural network (ANN). Kohonen's self-organizing maps (SOMs) were applied. Different network configurations were tested by varying the number of rows (2–5) and columns (2–5) [28]. The best architecture was selected with 500 epochs for each configuration. For acerola quality traits, the genetic diversity was best represented by the combination of five rows and five columns (25 neurons), with rectangular topology. Euclidean distance was adopted to configure the ANNs.

All statistical analyses were performed using GENES software, integrated with the R software [29].

4.3 RESULTS AND DISCUSSION

4.3.1 Characterization of fruit quality traits

Significant effects ($p \leq 0.05$) of acerola genotypes were found for all fruit quality traits, in both fully expanded-green and red-ripe maturity stages (Table 4.1). Considering that all genotypes were cultivated under the same environmental conditions, these findings indicate high levels genetic variability in the studied population, which is desirable to allow the discrimination of superior and divergent genotypes to be used in breeding programs [30].

Fruit diameter varied between 13.3 and 21.9 mm (fully expended-green), and 16.3 and 25.9 mm (red-ripe). Fruit mass showed ranges between 2.0–5.8 g (fully expended-green), and 2.6–9.1 g (red-ripe) (Figure 4.1). These values are slightly higher than those observed in diameter (14.3–27.8 mm) and mass (1.5–8.8 g) of red-ripe fruit of 103 acerola phenotypes evaluated by Farinelli et al. [31].

Red acerolas used by the processing industry to produce frozen pulp are required to have minimum mass of 4 g and diameter of 15 mm to guarantee high yield and quality of the processed product [32]. Green acerolas used to obtain vitamin C are also desired to have higher mass to increase yield and efficiency of ascorbic acid extraction, as well as for easier and faster harvest [33].

Table 4.1. Summary of the analysis of variance among 35 acerola (*Malpighia emarginata* DC.) genotypes for eight quality traits, assessed in fully expanded-green and red-ripe maturity stages.

Source of variation	Quality traits							
	Diameter	Mass	Firmness	Color	Soluble solids	Titrateable acidity	SS/TA ratio	Ascorbic acid
Fully expanded-green maturity stage								
Block (df = 2)	1.58 ^{ns}	0.09 ^{ns}	294.00 ^{ns}	20.55 [*]	8.77 ^{***}	0.36 ^{***}	0.02 ^{ns}	681,600 [*]
Genotypes (df = 34)	349.17 ^{***}	84.94 ^{***}	46692.00 ^{***}	1917.47 ^{***}	25.02 ^{***}	5.52 ^{***}	95.03 ^{***}	27,114,096 ^{***}
Residue (df = 68)	48.05	8.32	4536.00	178.75	14.54	0.83	7.99	5,758,150
CV (%)	4.59	10.25	15.12	1.41	5.91	6.88	6.82	10.17
Red-ripe maturity stage								
Block (df = 2)	6.60 [*]	1.00 ^{ns}	8.00 [*]	4.96 ^{ns}	1.95 ^{ns}	0.01 ^{ns}	0.21 ^{ns}	19,603 ^{ns}
Genotypes (df = 34)	424.67 ^{***}	204.74 ^{***}	720.76 ^{***}	1687.45 ^{***}	186.47 ^{***}	6.42 ^{***}	338.74 ^{***}	14,670,868 ^{***}
Residue (df = 68)	65.58	12.77	79.34	303.04	22.03	0.52	18.39	2,627,663
CV (%)	4.57	8.37	10.08	7.48	5.89	6.26	7.18	12.58

Values presented are mean squares of analysis of variance. *, * and *** represent F-values significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

df: Degrees of freedom; CV: Coefficient of variation. ns: non-significant.

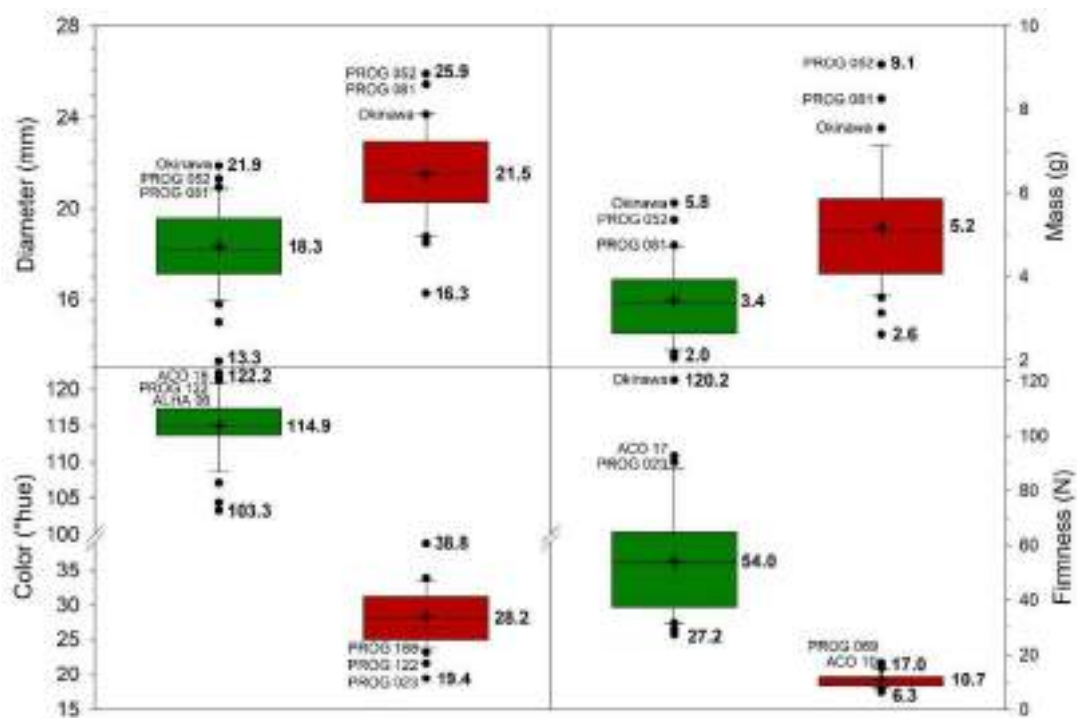


Figure 4.1. Distribution of maximum, mean and minimum values of 35 acerola (*Malpighia emarginata* DC.) genotypes for fruit diameter, mass, color and firmness, assessed in fully expanded-green and red-ripe maturity stages.

The box plot displays the minimum, mean, and maximum values for the quality traits. The plus symbol (+) indicates the mean value. The genotypes shown represent the extreme values for each quality trait.

Color is an important and reliable quality trait used to determine fruit maturity and harvest time [4]. Hue angle values close to 0° indicate reddish color and higher values, in ascending order, represent orange, yellowish or greenish colors. Unripe green acerolas showed a hue range of 103.3–122.2°, which decreased in the subsequent maturity stage. In ripe fruit, the minimum hue value was 19.4° and the maximum was 38.8° (Figure 4.1). These findings represent the typical color changes occurring in the acerola fruit, which ranges from bright glossy green to darkish-red or purple color, depending on the genotype [9].

In green acerolas, the pulp firmness varied between 27.2 and 120.2 N (green), while red fruits show a variation between 6.3 and 17.0 N (Figure 4.1). Fruit softening is one of the most intense ripening-related changes in acerola, which takes place due to cell wall disassemble by the action of several enzymes, such as pectinesterases, polygalacturonases, polysaccharide lyases and galacturonosyltransferases [34].

Acerolas at the fully expanded-green maturity stage had a variation between 6.7 and 9.1% for soluble solids (SS) content. The SS content showed an increase in red-ripe fruit (7.3–12.0%), compared to less advanced maturity stage (Figure 4.2).

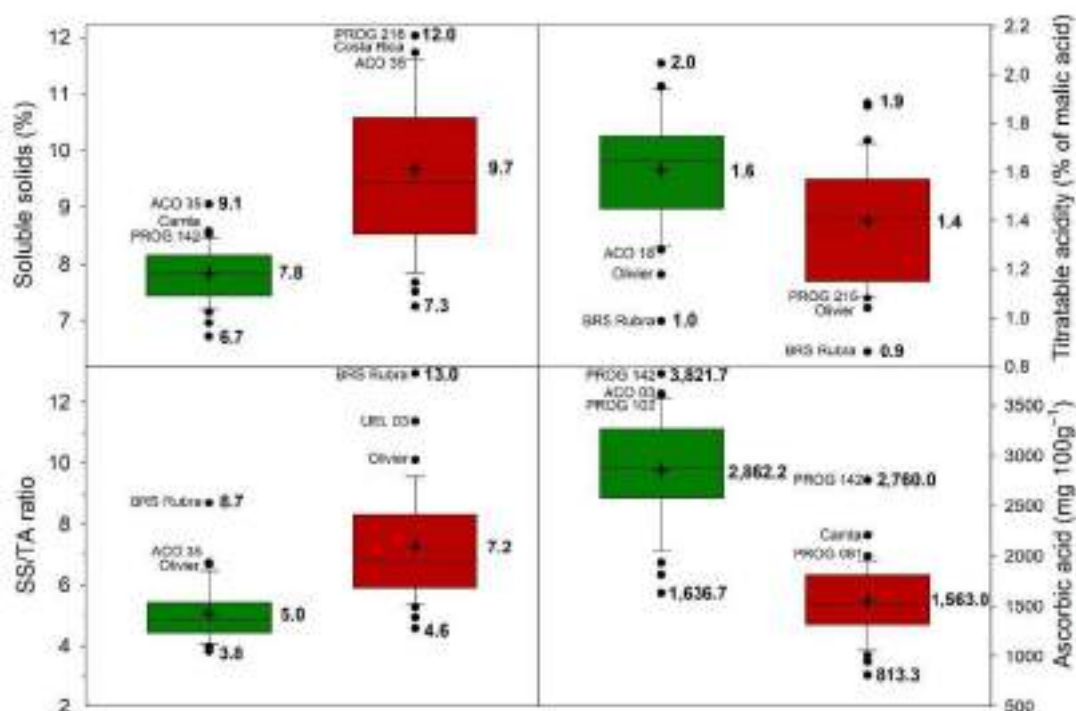


Figure 4.2. Distribution of maximum, mean and minimum values of 35 acerola (*Malpighia emarginata* DC.) genotypes for fruit soluble solids (SS), titratable acidity (TA), SS/TA ratio and ascorbic acid, assessed in fully expanded-green and red-ripe maturity stages.

The box plot displays the minimum, mean, and maximum values for the quality traits. The plus symbol (+) indicates the mean value. The genotypes shown represent the extreme values for each quality trait.

SS content is an important quality trait in acerolas destined to the fresh market because it determines fruit sweetness and consumer acceptance. The values observed in red-ripe acerolas are within the range of 3.3–11.8% reported by Moura et al. [35] in 45 acerola genotypes. Minimum SS contents of 7.0% and 7.5% are required by European and Japanese markets, respectively [36].

Titrate acidity (TA) showed ranges of 1.0–2.0% (fully expanded-green), and 0.9–1.9% of malic acid (red-ripe) (Figure 4.2). Acerola is a very acidic fruit due to the presence of several organic acids, including malic (0.46–1.10%) and succinic (0.05–0.09%) acids, as reported in red-ripe fruit of seven cultivars under

semi-arid conditions [7]. However, for fresh consumption, red-ripe acerolas must have low acidity to improve consumer quality and acceptance [11].

The SS/TA ratio serves as a better indicator of fruit flavor than each of these traits alone. It can also be utilized as a quality index for determining harvest maturity for the fresh market [4,37]. In our study, acerola genotypes presented SS/TA ratios ranging between 3.8 and 8.7 (fully expanded-green), and between 4.6 and 13.0 (red-ripe) (Figure 4.2). Similar SS/TA ratios were reported by Moura et al. [35] in 45 acerola genotypes (4.32–11.94). In another study involving four acerola cultivars (Sertaneja, Okinawa, Costa Rica, and Flor Branca) produced under irrigation in the São Francisco Valley, SS/TA ratios between 4.22 and 7.45 were reported, which are lower than the ratios observed in other fruit species such as mango (23.44–98.54) and guava (18.87–25.52) [33].

Green acerolas showed a range of ascorbic acid content between 1,636.7 mg and 3,821.7 mg 100 g⁻¹ in fully expanded-green fruits. As expected, the ascorbic acid content decreased in red-ripe acerolas, ranging from 813.3 to 2,760.0 mg 100 g⁻¹, respectively (Figure 4.2). The market for green acerolas, used for ascorbic acid extraction, is expanding due to the increasing worldwide demand for products with health benefits, which has also stimulated production in acerola growing countries, such as in Brazil [6]. In our study, most genotypes showed at least 1,000 mg 100 g⁻¹ of ascorbic acid at the red-ripe maturity stage, which is a desirable content to the processing industry. However, very high ascorbic acid content will increase acidity, reducing consumer acceptance of acerola fruit.

4.3.2 Genetic diversity of acerola genotypes

Acerola genotypes were clustered by the unweighted pair group method with arithmetic mean (UPGMA), based on the Mahalanobis distances between them. According to the cluster analysis, four groups of acerola genotypes were formed in both fully-expanded green and red-ripe maturity stages (Figure 4.3), following the Caliński-Harabasz pseudo-F criteria. Cophenetic correlation coefficients were 0.861 (fully-expanded green) and 0.746 (red-ripe), indicating an adequate fit by the clustering method ($r \geq 0.700$).

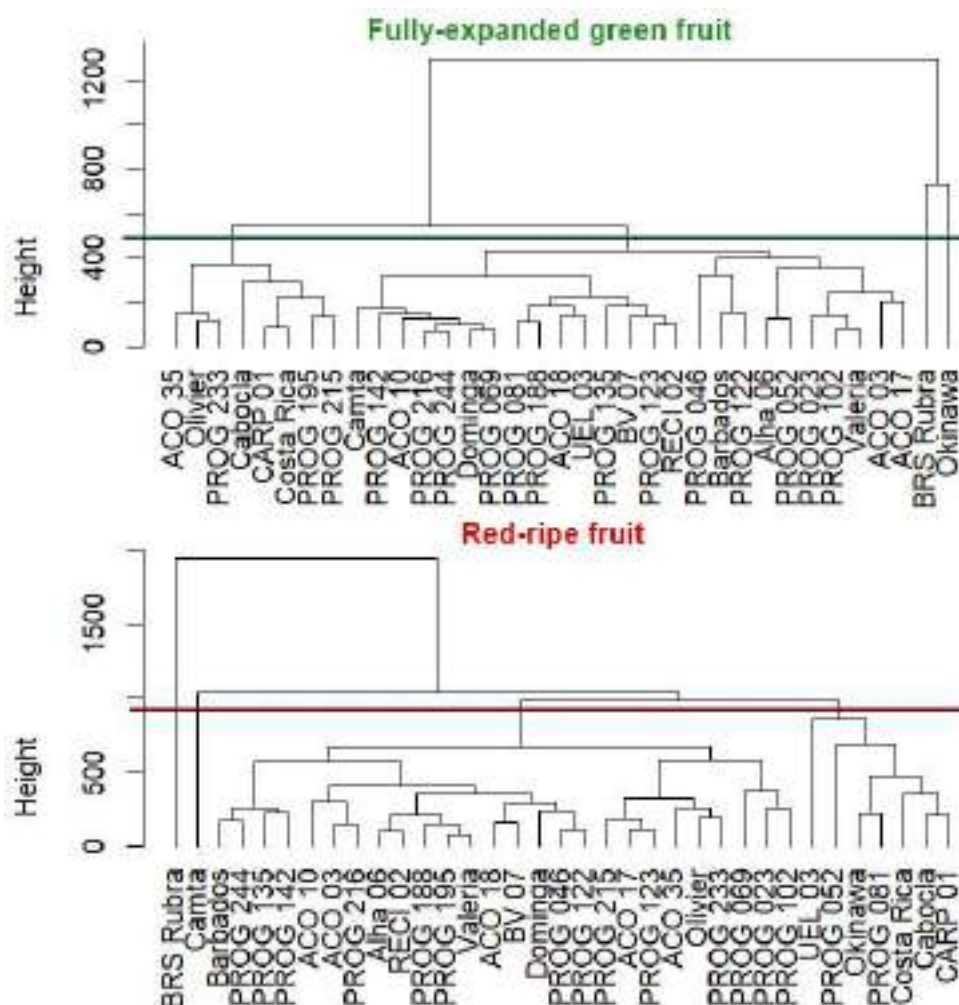


Figure 4.3. Dendrograms of 35 acerola (*Malpighia emarginata* DC.) genotypes in two maturity stages, generated by the unweighted pair group method with arithmetic mean (UPGMA) method based on the Mahalanobis' distance, using the fruit quality traits.

Acerola genotypes were clustered by the unweighted pair group method with arithmetic mean (UPGMA), based on the Mahalanobis distances between them. According to the cluster analysis, four groups of acerola genotypes were formed in both fully-expanded green and red-ripe maturity stages (Figure 4.3), following the Caliński-Harabasz pseudo-F criteria. Cophenetic correlation coefficients were 0.861 (fully-expanded green) and 0.746 (red-ripe), indicating an adequate fit by the clustering method ($r \geq 0.700$).

For the fully-expanded green maturity stage, Cluster I was composed of eight genotypes (ACO 35, Olivier, PROG 233, Cabocla, CARP 01, Costa Rica, PROG 195 and PROG 215). Most of genotypes were included in the Cluster II ($n = 25$). The clusters III and IV were formed by only one genotype in each, BRS Rubra and Okinawa. In the red-ripe fruit, the genotypes BRS Rubra and Camta

formed the clusters I and II separately. Most of genotypes composed the cluster III ($n = 26$), while the cluster IV englobed seven genotypes, namely UEL 03, PROG 052, Okinawa, PROG 081, Costa Rica, Cabocla and CARP 01.

SS/TA ratio was the trait that most contributed to genetic diversity in both maturity stages (24.84% and 46.36% for green and red, respectively) (Table 4.2). In a previous study with 11 peach cultivars, the SS/TA ratio was also one of the major contributors to genetic diversity of fruit physicochemical quality traits [38].

Table 4.2. Relative contribution (%) of each fruit quality trait to the genetic diversity among 35 acerola (*Malpighia emarginata* DC.) genotypes at different maturity stages, estimated by the Singh [27] method.

Fruit quality trait	Fully expanded-green stage	Red-ripe stage
Diameter	4.08	2.36
Mass	20.28	22.80
Firmness	17.79	11.76
Color	17.52	8.73
Soluble solids	3.92	1.97
Titrateable acidity	4.14	0.00
SS/TA ratio	24.84	46.36
Ascorbic acid	7.43	6.02

When observing the dendrograms, BRS Rubra was the most divergent genotype in both maturity stages, which stood out for the highest SS/TA ratio between all acerola genotypes, in both green (8.7) and red (13.0) fruits. Additionally, BRS Rubra had the lowest titrateable acidity, regardless of maturity stage (1.0 and 0.9% of malic acid in green and red fruits, respectively).

BRS Rubra is an acerola cultivar launched in 2004 by the Brazilian Agricultural Research Corporation with good fruit quality for both the processing industry and consumption of fresh fruit. Since then, BRS Rubra has stood out in breeding programs due to its high SS/TA ratio, ideal for achieving the perception of sweetness desired by consumers [39]. Recently, cytogenetic studies revealed that the BRS Rubra cultivar is triploid ($3n = 30$) [40–41] – unlike most acerola cultivars such as Cabocla and Costa Rica (diploids, $2n = 20$) – which restricts its use as a parent.

Fruit mass was the 2nd most important contributor to genetic diversity of green (20.28%) and red (22.80%) acerolas. In addition, firmness had a contribution higher than 10% in both maturity stages (17.79% and 11.76% for green and red

stages, respectively) (Table 4.2). For both maturity stages, Okinawa genotype stood out in terms of fruit mass, with the highest mean in green fruits (5.8 g). Furthermore, Okinawa acerolas had the highest firmness in green fruits (120.2 N), which contributed for its diversity in relation to other genotypes in this maturity stage (Figure 4.3). In addition to these attributes, Okinawa fruits have a high content of ascorbic acid and a slower change from the green to ripe stage, which facilitates the harvesting of fruits at earlier maturity stages [42]. Therefore, this genotype is highly recommended for the industrial extraction of ascorbic acid in fruits for the manufacture of products such as food enrichers and pharmaceuticals.

4.3.2.1 Artificial neural networks

Through the self-organizing maps by 25 neurons established with the five rows and five columns, the 35 acerola genotypes were classified into 5 classes, regardless the maturity stage (Table 4.3).

Table 4.3. Grouping of acerola genotypes into classes formed using artificial neural networks by self-organizing maps.

Class	Fully expanded-green stage	Red-ripe stage
1	Okinawa	BRS Rubra
2	BRS Rubra, ACO 17, Valéria	Camta, Barbados, PROG 135
3	PROG 102, PROG 052, Barbados, RECI 02, PROG 122, PROG 142, PROG 216, PROG 244, Dominga, BV 07, PROG 195, PROG 188	PROG 102, CARP 01, PROG 122, ACO 03, PROG 023, ACO 35, ACO 10, ACO 17, RECI 02, PROG 046, PROG 123, Olivier, Alha 06, ACO 18
4	Camta, PROG 135, Alha 06, ACO 18, ACO 10, PROG 233, Cabocla, UEL 03, ACO 03	UEL 03, PROG 216, Dominga, BV 07, PROG 142, PROG 188, PROG 081, PROG 069, PROG 195, PROG 215, PROG 244, PROG 233, Valéria
5	ACO 35, Olivier, CARP 01, PROG 023, Costa Rica, PROG 123, PROG 215, PROG 081, PROG 046, PROG 069	Okinawa, Cabocla, Costa Rica, PROG 052

Class 1 consisted of only one genotype in each maturity stage, Okinawa in green fruit and BRS Rubra in red fruit, demonstrating the genetic diversity of these accessions in relation to the remainder (Table 4.3). Additionally, this class corroborated the representation of the dendrogram created by the UPGMA method (Figure 4.3).

Class 2 held three genotypes each, in both maturity stages. Class 3 consisted of 12 (green) and 14 (red) genotypes and classified the largest number of genotypes. In green fruit, classes 4 and 5 clustered nine and ten genotypes, respectively, while in red fruit, these classes were formed of thirteen and four genotypes, respectively.

Self-organizing maps obtained through ANNs are an emerging approach for assessing the genetic diversity in plant breeding studies [43–46]. Self-organizing maps are widely applied in several areas, present easy implementation, and are adequate for data mining through clustering representation [47]. In the acerola active germplasm bank assessed in this study, the self-organizing maps had a greater discriminating power between the genotypes compared to the UPGMA method, which corroborates with previous research [44–45]. This difference can be attributed to the weight of means and variances of quality traits, which are taken into consideration in the clustering process, unlike ANNs whose cluster formation is not influenced by experimental errors [46].

4.4 CONCLUSION

The collection analyses of 35 acerola genotypes assessed in this study showed a wide diversity for fruit quality traits in the evaluated maturity stages, underscoring the potential for selecting superior genotypes for breeding programs. Notably, the SS/TA ratio was the trait that most contributed to genetic diversity of acerola, in both green (24.84%) and red (46.36%) maturity stages.

In both approaches, Okinawa was the most divergent in green fruit due to highest mass and firmness, along with elevated ascorbic acid content, and therefore is recommended for industrial extraction of ascorbic acid. Conversely, BRS Rubra was the most stood out in the red-ripe stage and had the highest SS and SS/TA ratio, being recommended for fresh consumption and processing.

These findings highlight the importance of genetic diversity studies in identifying genotypes with desirable traits to enhance acerola breeding programs. The significant genetic variability provides a valuable resource for future breeding efforts, aiming to enhance acerola fruit quality and expand its market potential.

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ARTICLE V

Multivariate statistics unveil seasonal and genotypic variations and relations between antioxidant compounds in acerola*

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Multivariate statistics unveil seasonal and genotypic variations and relations between antioxidant compounds in acerola

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ABSTRACT

This study assessed the combined effects of season and cultivars on the fruit quality traits, bioactive compounds, and antioxidant activity of acerola (*Malpighia emarginata*), and investigated the contributions of bioactive compounds on antioxidant activity. Red-ripe fruits of four acerola cultivars—three pre-selected (BRS Rubra, Cabocla, and Costa Rica) and one commercial (Junko)—were harvested during two seasons, spring and summer. We evaluated seasonal and genotypic variations in quality traits (color, soluble solids content, titratable acidity, and SSC/TA ratio), bioactive compounds (ascorbic acid, phenolic compounds, anthocyanins and yellow flavonoids), and antioxidant activity. Multivariate statistics of principal component analysis and path analysis were used to assess the relationships and the direct and indirect effects of bioactive compounds on the fruit antioxidant activity. Both cultivars and seasons influenced fruit quality traits, whereas most of bioactive compounds and antioxidant activity were primarily driven by genotypic variations. This suggests a greater influence of cultivar on functional compounds compared to seasonal conditions, potentially allowing breeding programs to develop acerola cultivars rich in these compounds. Phenolic compounds and ascorbic acid had the major contribution on the antioxidant activity of acerola. Thus, breeding focused on the functional value of the fruits should prioritize the assessment of these compounds.

Keywords: *Malpighia emarginata*, phenolic compounds, ascorbic acid, anthocyanins, flavonoids, path analysis.

5.1 INTRODUCTION

Several scientific research have assumed the fruit consumption is correlated with a high intake of antioxidant compounds, which plays a key role in the prevention of several chronic diseases (Angelino et al., 2019). This happens because the antioxidant molecules present in fruits act to contain the damage caused by reactive oxygen species (ROS). The ROS are highly reactive molecules produced from oxygen metabolism, which easily reacts with other molecules in a cell (Sinenko et al., 2021). At low or moderate concentrations, ROS present positive functions in cell biology, especially as second messenger signaling molecules. However, an elevated formation of ROS results in molecular damages, known as oxidative stresses, which can result in cell death (Meitha et al., 2020).

Diseases such as cancer, respiratory, cardiovascular, neurodegenerative, and digestive diseases, as well as the aging process, are a result of the overproduction of ROS, which cause damage to important biomolecules such as lipids, proteins, and DNA (Liu et al., 2018). Thus, a balanced diet, abundant in antioxidant compounds sourced from fruits, such as the Mediterranean diet, can help reduce organism vulnerability to oxidative stress and potentially mitigate the decline in antioxidant defenses that often accompanies illness (Mazzoni et al., 2016).

Berries are the most consumed fruits in the Mediterranean diet, as rich sources of nutrients and phytochemicals, including minerals, vitamins, dietary fibers, and phenolic compounds (Skrovankova et al., 2015; Battino et al., 2019). Several berries are used as functional food ingredients and dietary supplements (Golovinskaia & Wang, 2021). In botanical terms, a berry is a fleshy fruit with seeds, produced from a single flower with one ovary. However, this term is popularly attributed to small edible, juicy, and colorful fruits, such as grapes, strawberries, raspberries, blueberries, blackberries, goji berries, and cranberries (Veberic et al., 2015). Most of berries are known for their high anthocyanin content. Anthocyanins are the major group of phenolic compounds responsible for the vibrant red, blue, and purple colors of fruits, vegetables, and flowers, including berries. Besides acting as colorants, these compounds also offer potential health benefits due to their strong antioxidant activity (Xue et al., 2023).

In recent years, the cultivation of berries has expanded in several areas, aiming to attend the global demand by consumers (Clark & Finn, 2014; Chaves et

al., 2018 López et al., 2021). The use of native berries has also increased since several investigations constated the presence of elevated levels of bioactive compounds and their potential effects on human health. As an intercontinental country, Brazil presents a rich biodiversity of berry fruits, with several native berry fruits produced by plants of the Arecaceae, Myrtaceae, and Malpighiaceae botanical families (Neri-Numa et al., 2018).

Malpighiaceae is a family comprising 74 genera and more than 1,300 species, most of them being lianas, shrubs and trees occurring in the American tropics, especially Brazil (Mannochio-Russo et al., 2022). Several studies confirmed that most genera in Malpighiaceae family present important biological activities, including antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, anti-depressant, and cytotoxic properties (Abbas et al., 2022). The most economically important species within the Malpighiaceae family is *Malpighia emarginata*, often called acerola.

The acerola fruit is a fleshy, thin-skinned, red-purple berry with tri-lobed seeds, measuring up to 2.9 cm in diameter, and weighing between 1.5 and 15.0 g. While a few varieties produce sweet fruits, most are acidic, with a mean acidity ranging from 0.8 to 1.7% and soluble solids from 6.4 to 11.0% (Mohammad, 2011; Vilvert et al., 2024a). Acerola is recognized since the late-1940s as the richest natural source vitamin C in the world, with up to 4,000 mg 100 g⁻¹ (Huang et al., 2022), comparable only to the Amazonian fruit camu-camu (*Myrciaria dubia*, Myrtaceae) (García-Chacón et al., 2023).

In addition to ascorbic acid, acerola berries are very rich in phenolic compounds, with elevated antioxidant activity and surpassing other tropical fruits like mango and guava (Batista et al., 2018). A recent study summarized at least 76 phenolics of different classes already found in the acerola fruits and by-products (Vilvert et al., 2024b). Most of acerola phenolics are flavonoids, with proven beneficial effects on human health, including anti-inflammatory, anti-oxidative, and neuroprotective effects (Miskinis et al., 2023). In acerolas, the highest concentration of flavonoids includes anthocyanins (Carneiro Ferreira et al., 2021), water-soluble pigments responsible for red and purple hues on fruits, with a high antioxidant capacity. These natural pigments stand out as a potential raw material to be used as natural food colorants (Cerino et al., 2023). Considering natural antioxidants are more attractive to consumers than synthetic ones (Gutiérrez-del-

Río et al., 2021), acerola fruits are a potential source of natural antioxidants for the development of functional foods.

The relationships between bioactive compounds and antioxidant are of great interest for plant breeding programs focused on the functional value of the fruits. Several multivariate statistical tools are available to accurately and clearly investigate the relationships established between different variables. In addition to widespread principal component analysis (PCA), the path analysis has been applied successfully to clarify interrelations between bioactive compounds and antioxidant activity in several horticultural crops, including blackberry (Li et al., 2018), strawberry (Zebrowska et al., 2021), guava (Kherwar & Usha, 2016), pepper (Diel et al., 2020), and cornelian cherry (*Cornus mas* L.) (Hassanpour et al., 2011).

Although acerolas are constituted of abundant and diversified bioactive compounds with beneficial health properties, the fruit quality and accumulation of functional compounds is dependent on the cultivar, growing conditions, maturity stage, and postharvest treatments (Rabelo et al., 2020; Ribeiro & de Freitas, 2020; Guedes et al., 2022; Miskinis et al., 2023; Macedo et al., 2023; Vilvert et al., 2024a). With a growing global demand on functional foods, this study aimed to assess the seasonal variation on the fruit phytochemical composition of different acerola cultivars, as well as to investigate the contribution of bioactive compounds to the antioxidant activity using multivariate statistical approaches.

5.2 MATERIAL AND METHODS

5.2.1 Chemical reagents

Sodium hydroxide, oxalic acid, hydrochloric acid, ethanol, methanol, acetone, gallic acid, Folin–Ciocalteu’s reagent, anhydrous sodium carbonate, 2,6-dichloro-indophenol (DFI), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid (ABTS), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St. Louis, USA).

5.2.2 Plant material

Acerolas berries were collected from the acerola germplasm bank (AGB) at the Tropical Semi-arid Embrapa, in Petrolina, PE, Brazil, situated at 09°09' S latitude and 40°22' W longitude, at an altitude of 365 m. The region has a Semi-

arid climate (BSh) according to Koppen's classification (Alvares et al., 2013). The meteorological data during experiment conduction were collected from the automatic weather station located in the study area and are shown in Figure 5.1.

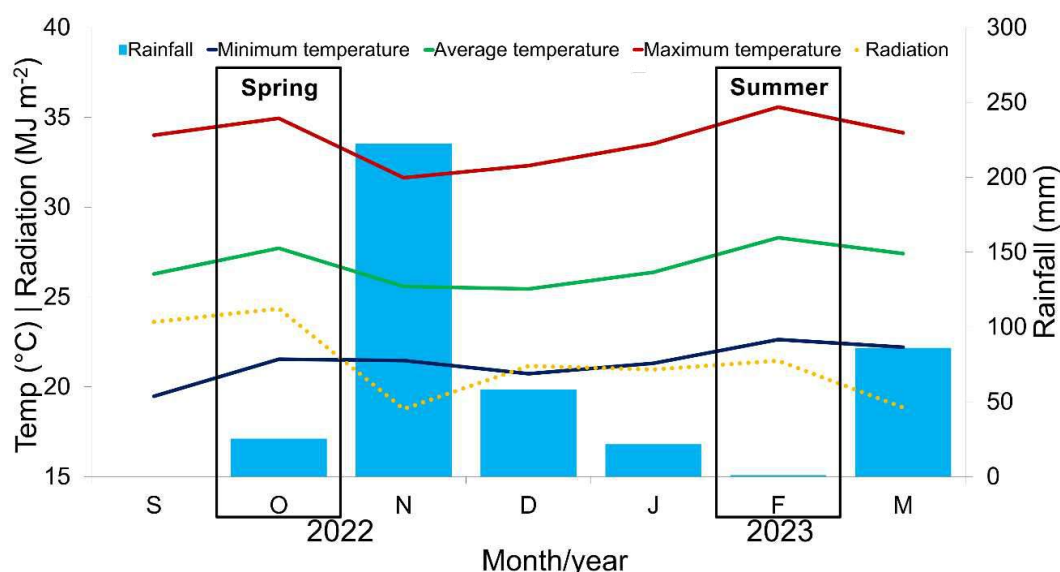


Figure 5.1. Minimum, mean and maximum temperature, global radiation and total monthly rainfall in the experimental area, between September 2022 and March 2023. Source: Agrometeorological Station of Bebedouro, Petrolina, Pernambuco, Brazil (09°09' S, 40°22' W) (Embrapa, 2024).

The orchard was established in 2012, and the acerola trees were spaced 4 m between plants and 3.5 m between rows. Irrigation, fertilization, and phytosanitary treatments were carried out according to technical recommendations for the crop (Ritzinger et al., 2003).

5.2.2.1 Fruit harvest

Four acerola cultivars (BRS Rubra, Cabocla, Costa Rica, and Junko) (Fig. 5.2) were harvested in two seasons, spring (Oct 2022) and summer (Feb 2023).



Figure 5.2. Acerola cultivars assessed in this study. The fruits in the figure were harvested in the spring season.

The fruits were collected at the red-ripe maturity stage, characterized by a skin fully covered with red/purple color. The cultivars BRS Rubra, Cabocla, and Costa Rica were chosen based on the selection criteria of multiple desired traits for fresh consumption (Ferreira et al., 2022), while Junko is a control treatment, as the main acerola cultivar commercialized (Malegori et al., 2017). After harvest, the fruits were immediately transported to the Postharvest Laboratory and selected for homogeneity in color and size and absence of mechanical damage, diseases, and insect damage.

Later, the fruits were washed and divided into three replications, each weighing 500–1,000 grams. They were then processed and stored at -20°C . The analyzes were carried out as described below.

5.2.3 Characterization of acerola quality traits

Skin color was measured at the fruit equatorial region with a colorimeter model CR-400 (Konica Minolta, Tokyo, Japan). Color measurements were recorded as hue angle, where $0^{\circ}/360^{\circ}$ represents a red color, while angles of 90° , 180° , and 270° represent yellow, green, and blue colors, respectively (Pathare et al., 2013).

Soluble solids content (SSC, in %) and titratable acidity (TA, in % of malic acid) were determined in fruit juice samples with a pocket Brix-acidity meter (model PAL-BX ACID3, Atago, Tokyo, Japan). The SSC was determined on undiluted acerola juice, while TA was measured after diluting the juice in a 1/50 ratio with distilled water. For each sample, SSC/TA ratio was calculated by dividing the SSC by its respective TA.

5.2.4 Determination of bioactive compounds in acerolas

5.2.4.1 Ascorbic acid

Ascorbic acid content was determined by titration with Tillman's solution (DFI - 2,6-dichlorophenol indophenol) at 0.02% (Strohecker & Henning, 1967). One gram of acerola pulp was diluted in 100 mL of 0.5% oxalic acid. Later, 1 mL of this solution was diluted in 50 mL of distilled water and titrated with Tillman's solution until permanent light pink color development. Results were expressed as mg of ascorbic acid per 100 g of fresh mass (fm).

5.2.4.2 Phenolic compounds

5.2.4.2.1 Preparation of the phenolic extract

For the analysis of phenolic compounds, a phenolic extract was prepared as described by Larrauri et al. (1997), by weighing 2 g of acerola pulp, which were left to extract in 20 mL of 50% methanol (v/v) for 1 h. The samples were then centrifuged at $10,000 \times g$ for 15 min at ambient temperature. The supernatant (liquid extract) was filtered and transferred to a 50 mL volumetric flask. A second extraction on the residue was done for 1 h using 20 mL of 70% acetone (v/v). The process followed centrifugation and the filtration of the supernatant, which was added to the 50 mL volumetric flask with previous liquid extract. Finally, the flask volume was completed to 50 mL using distilled water.

5.2.4.2.2 Determination of total phenolic compounds (TPC) content

The TPC content was determined spectrophotometrically in the acerola phenolic extracts, using the Folin–Ciocalteu method (Singleton & Rossi, 1965). The extracts were mixed with 1 mL of Folin–Ciocalteu reagent (1:3, v/v), 2 mL of 20% sodium carbonate (w/v) solution and 2 mL of distilled water and were left to rest under absence of light. After 30 min, absorbances at 700 nm were read in the spectrophotometer. A calibration curve was obtained using gallic acid (0–50 μg) and the results were expressed as mg gallic acid equivalent (GAE) per 100 g fm.

5.2.4.3 Anthocyanins and yellow flavonoids

Anthocyanins and yellow flavonoids were determined by the method of Fuleki & Francis (1968). One gram of acerola pulp was extracted with 10 mL of a 95% ethanol solution acidified with 1.5 N hydrochloric acid in a ratio of 85:15 (v/v), and vortexed for 2 min. The mixture was completed to 50 mL with the extracting solution. Protected from the light, the extract was stored overnight at 4 °C, and then filtered on Whatman No. 1 paper.

For determination of anthocyanins and yellow flavonoids, the absorbance of the samples was measured at 535 and 374 nm, and the results were expressed as mg of cyanidin and quercetin per 100 g fm, respectively.

5.2.5 Assessment of antioxidant activity in acerolas

The antioxidant activity of acerolas was evaluated using the ABTS (2,2-azinobis-(3-ethyl benzothiazoline-6- sulfonic acid)) (Re et al., 1999) and the DPPH (2,2-diphenyl-1-picrylhydrazyl) (Brand-Williams et al., 1995) radical scavenging methods.

5.2.5.1 ABTS method

In the ABTS assay, the radical was generated by reacting the ABTS stock solution (7 mmol L^{-1}) with potassium persulfate (140 mmol L^{-1}) and storing the mixture in the absence of light for 16 hours at room temperature. Before analysis, this mixture was diluted in ethanol until an absorbance of 0.7 ± 0.005 at 734 nm was achieved.

Three different dilutions for each phenolic extract (section 5.2.4.2.1) were prepared in triplicate. In the dark, 30 μL of each dilution were mixed to 3 mL of the ABTS radical solution using a Vortex mixer. Absorbance was measured at 734 nm after 6 minutes. Results were expressed as μM of Trolox per gram of fm, using a standard Trolox curve.

5.2.5.2 DPPH method

In the DPPH assay, three different dilutions of each phenolic extract were prepared in triplicate. In the absence of light, 100 μL of each extract dilution was added to 3.9 mL of the DPPH radical solution (0.06 mM). The mixture was vortexed and left to rest for 45 minutes, considered the time of stabilization of the DPPH radical. The control consisted of 100 μL of a mixture of 50% methanol, 70% acetone and water, in a 4:4:2 ratio. Absorbance readings were taken at 515 nm.

The antioxidant activity by the DPPH method was expressed as EC_{50} , i.e. the concentration of extract required to reduce the DPPH radical by 50%. Thus, the higher the DPPH value (in g of fruit per g of DDPH), the lower the antioxidant activity.

5.2.6 Statistical analysis

5.2.6.1 Experimental design

The experiment was set up in a completely randomized design, in a full factorial arrangement 4×2 . The plots consisted of combinations between four cultivars (BRS Rubra, Cabocla, Costa Rica and Junko) and two harvest seasons (springer and summer).

The statistical model is expressed as follow:

$$Y_{ijk} = \mu + C_i + S_j + (C \times S)_{ij} + \varepsilon_{ijk}$$

where:

Y_{ijk} is the response variable for the k -th replicate of the i -th cultivar and the j -th season.

μ is the overall mean.

C_i is the effect of the i -th cultivar ($i = 1, 2, 3, 4$).

S_j is the effect of the j -th season ($j = 1, 2$).

$(C \times S)_{ij}$ is the interaction effect between the i -th cultivar and the j -th season.

ε_{ijk} is the random error term associated with the k -th replicate of the i -th cultivar and the j -th season.

5.2.6.2 Univariate statistics

The data were analyzed using a two-way analysis of variance (ANOVA) to determine the main effects of cultivars and harvest seasons, as well as their interaction effects, on the fruit quality traits, bioactive compounds and antioxidant activity of acerola.

The ANOVA was carried out in five steps: 1) examination of extreme outliers in the dataset; 2) verification of normality assumption by analyzing the model residuals, using QQ plot and Shapiro-Wilk test; 3) checkup of homogeneity of variances by residuals vs fits plot and Levene's test; 4) application of F-test to verify the isolated and combined effects of cultivars and seasons on variables; 5) application of multiple pairwise comparisons between treatment means using Tukey's test ($p \leq 0.05$). The ANOVA-related analyses were carried out using the R packages *ggpubr*, *tidyverse*, and *rstatix*.

5.2.6.3 Multivariate statistics

Before the execution of multivariate statistical analysis, the original dataset was standardized, considering the variables were measured on different scales. The data were transformed to have a mean of 0 and a standard deviation of 1, using the formula:

$$z = \frac{x - \mu}{\sigma}$$

where: z is the standardized value, x is the observed value of the variable in each sample, μ is the mean of the variable, and σ is the standard deviation of the variable.

The principal component analysis (PCA) was applied to dataset to indicate the linear relationships between the variables. For this, the traits were reduced to few principal components that account for most of the data variance, based on the correlation matrix among the traits. The number of principal components (PCs) was defined according to the significance of the eigenvalues (≥ 1). The PCA was applied using the software STATISTICA 12 (StatSoft Inc., Tulsa, USA).

The contribution of the bioactive compounds on the antioxidant activity of acerolas was assessed through a multivariate path analysis. Firstly, a multicollinearity test was applied to the variables, where variance inflation factors (VIF) lower than 10 indicated low multicollinearity. Additionally, the condition number (CN) was calculated as the ratio between the larger and the smaller eigenvalue of the matrix, being $CN < 100$ (weak collinearity, highly recommended), $100 \leq CN < 1000$ (medium), and $CN \geq 1000$ (strong, not recommended) (Montgomery & Peck, 1981).

In path analysis, the bioactive compounds were considered independent variables, while the antioxidant activity – measured by ABTS and DPPH methods and analyzed separately – was the dependent variable. The multicollinearity test and the path analysis were applied using the using the R packages *foreign*, *car* and *semPlot*.

5.3 RESULTS

5.3.1 Seasonal and genotypic variation on acerola quality traits

A two-way ANOVA was applied to assess the genotypic and seasonal effect on acerola quality traits. Both cultivars and harvest seasons caused significant variations in the color, soluble solids content (SSC), titratable acidity (TA), and SSC/TA ratio of acerola fruits ($p \leq 0.05$). Furthermore, the interaction between cultivars and season significantly contributed to the variation of all the quality parameters ($p \leq 0.05$) (Table 5.1).

Table 5.1. Summary of analysis of variance for genotypic and seasonal effect on acerola quality traits, bioactive compounds and antioxidant activity.

Trait	Cultivar (C)	Season (S)	C × S	CV (%)
	(df = 3)	(df = 1)	(df = 3)	
Sum of squares				
Color	465.8***	225.6***	56.6**	6.93
Soluble solids	99.23***	5.54**	4.44*	7.00
Titratable acidity	0.69***	0.21***	0.06*	8.97
SSC/TA ratio	359.9***	67.48***	29.02**	11.08
Ascorbic acid	5063308***	35334 ^{ns}	576626*	14.82
Total phenolic compounds	1618659***	70004*	120506*	13.20
Anthocyanins	115.13*	85.69*	7.81 ^{ns}	18.07
Yellow flavonoids	47.10*	0.25 ^{ns}	0.75 ^{ns}	21.33
Antioxidant by ABTS	81591511***	642292 ^{ns}	12094812***	9.88
Antioxidant by DPPH	1531792***	6024 ^{ns}	704005***	14.22

*, ** and *** represent sources of variation significant by the F-test, with $p \leq 0.05$, 0.01 and 0.001, respectively. ^{ns}: non-significant ($p > 0.05$). df: degrees of freedom. CV: coefficient of variation.

Acerola color was presented as hue angle, whose values closer to 0° indicate a redder color. The spring harvest presented redder fruits than in summer for all cultivars, except for Cabocla. The fruits of the Junko cultivar exhibited a redder color during the spring harvest. In the summer harvest, Costa Rica displayed a less reddish color than other cultivars (Fig. 5.3A).

BRS Rubra and Cabocla acerolas showed higher soluble solids content in the spring compared to summer, whereas the other cultivars were not affected by the season. A substantial genotypic effect was noted on the acerola SS content, with Junko exhibiting the lowest SS content in both seasons, approximately 37% lower in the spring and 31% lower in the summer harvests, respectively (Fig. 5.3B).

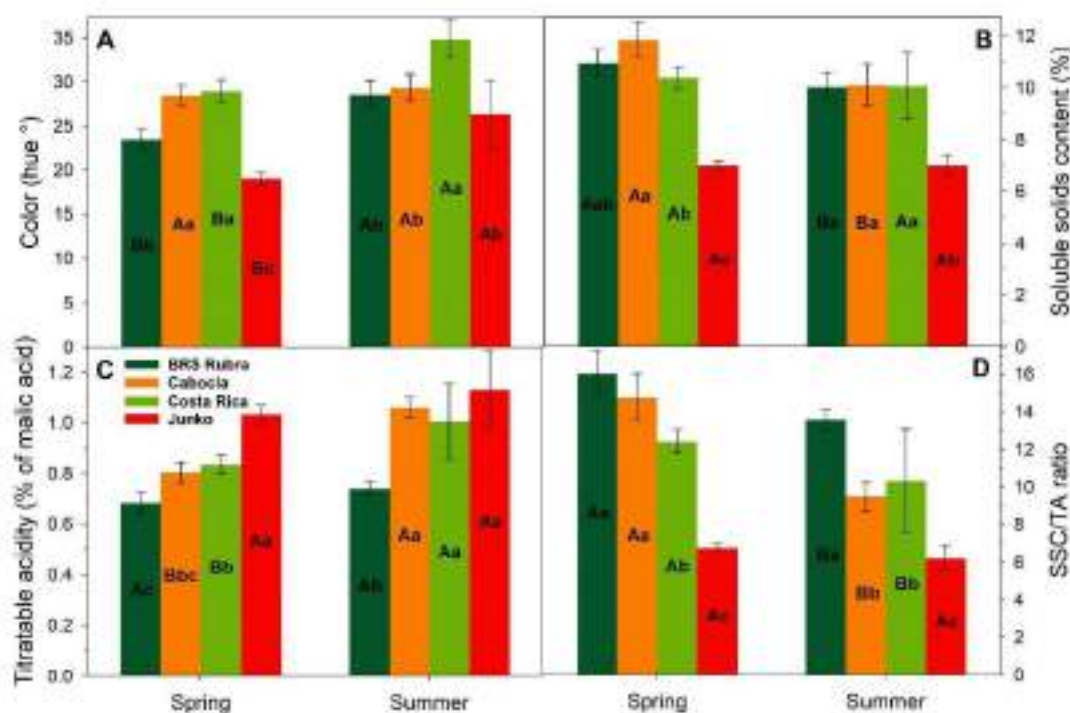


Figure 5.3. Genotypic and seasonal effect on color (A), soluble solids content (SSC) (B), titratable acidity (TA) (C) and SSC/TA ratio (D) of acerolas.

Different lowercase letters indicate statistically significant difference between cultivars by Tukey test ($p \leq 0.05$). Different uppercase letters indicate statistically significant difference between harvest seasons by Tukey test ($p \leq 0.05$).

TA was higher in summer than in spring for the Cabocla and Costa Rica cultivars. Regardless of the harvest season, BRS Rubra acerolas had the lowest TA between the cultivars (Fig. 5.3C). Consequently, the acerola SSC/TA ratio was higher in the cultivar BRS Rubra in both cycles, although there was no difference with Cabocla in the spring harvest. Furthermore, fruits harvested in the spring had a higher SSC/TA than in summer for all cultivars, except for Junko (Fig. 5.3D).

5.3.2 Seasonal and genotypic variation on acerola bioactive compounds and antioxidant activity

Both cultivars and harvest seasons influenced on the total phenolic compounds (TPC) and anthocyanins of acerolas, while only a genotypic effect was observed for the contents of ascorbic acid and yellow flavonoids ($p \leq 0.05$). Variations in antioxidant activity, investigated using ABTS and DPPH assays, were significantly caused by cultivars ($p \leq 0.05$), but not by harvest seasons ($p > 0.05$). Additionally, the interaction between cultivars and seasons was significant for

ascorbic acid and TPC, as well as for antioxidant activity by both methods ($p \leq 0.05$) (Table 5.1).

Junko cultivar had the higher ascorbic acid content in both seasons, along with Cabocla in the spring season. Furthermore, Cabocla was the only cultivar affected by season, with a higher ascorbic acid content in the spring (Fig. 5.4A).

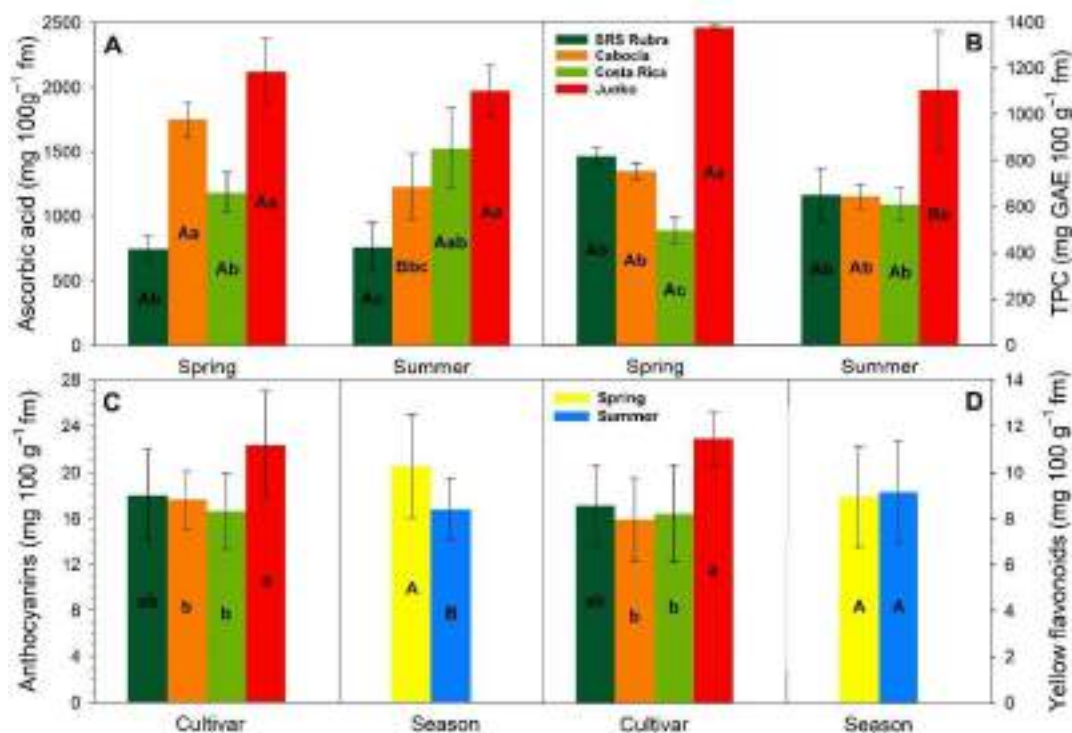


Figure 5.4. Genotypic and seasonal effect on the contents of ascorbic acid (A), total phenolic compounds (TPC) (B), anthocyanins (C) and yellow flavonoids (D) of acerola.

Different lowercase letters indicate statistically significant difference between cultivars by Tukey test ($p \leq 0.05$). Different uppercase letters indicate statistically significant difference between harvest seasons by Tukey test ($p \leq 0.05$).

The TPC content in acerolas was higher in Junko in both harvests, with content approximately 50% higher in spring and 42% higher in summer than in other cultivars. Seasonal variability influenced only the Junko cultivar, whose fruits showed higher TPC content in spring (Fig. 5.4B).

Regardless of the harvest season, Junko acerolas had a higher content of anthocyanins (22.4 mg 100 g⁻¹) and yellow flavonoids (11.4 mg 100 g⁻¹) than Cabocla and Costa Rica. For all cultivars, spring harvest provided a higher anthocyanin content (20.5 mg 100 g⁻¹) in the fruits than summer harvest (16.8 mg 100 g⁻¹) (Fig. 5.4C), while yellow flavonoids were not affected by seasonal variations, with an average of 9.1 mg 100 g⁻¹ (Fig. 5.4D).

A high genotypic effect was observed in the antioxidant activity of acerolas, assessed by ABTS and DPPH assays. In both harvest seasons, Jundo cultivar had a higher antioxidant activity, expressed by high ABTS and low DPPH values (Fig. 5.5). This is a clear response for the higher content of bioactive compounds in Jundo acerolas, compared to other cultivars (Fig. 5.4).

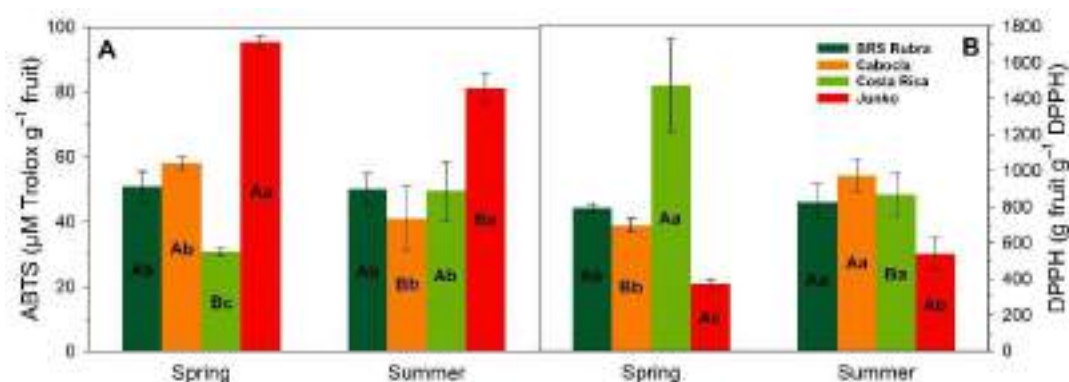


Figure 5.5. Genotypic and seasonal effect on the antioxidant activity of acerola, assessed by ABTS (A) and DPPH (B) methods.

Different lowercase letters indicate statistically significant difference between cultivars by Tukey test ($p \leq 0.05$). Different uppercase letters indicate statistically significant difference between harvest seasons by Tukey test ($p \leq 0.05$).

5.3.3 Relationships between acerola variables and responses of cultivars and seasons by PCA

A principal component analysis (PCA) was applied to summarize the data information in a graphical approach gathering two components that explain most of variance, with a minimum loss of information. According to the results, the first principal component (PC1) and PC2 accounted for 69.49% and 17.13% of the data variance, respectively, explaining together for more than 86.6% of total variation (Fig. 5.6; Table 1A, 2A).

All the assessed bioactive compounds together with antioxidant activity by ABTS method were on the left side of the PCA plot. By overlapping these parameters and analyzing the samples, it was evident that Jundo acerolas presented the highest content of ascorbic acid, phenolic compounds, anthocyanins and yellow flavonoids, as well as high antioxidant activity, which were mainly regulated by the cultivar, with less influence from the harvest season (Fig. 5.6).

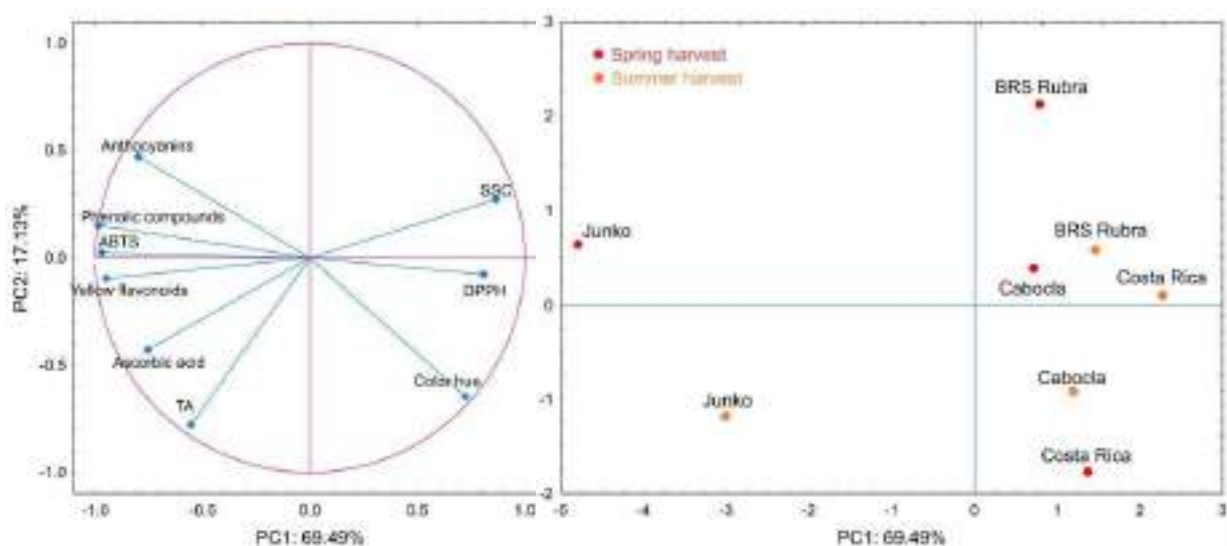


Figure 5.6. Principal component analysis for quality traits of acerola genotypes, harvested in different harvest seasons.

The first quadrant of the PCA plot is highly correlated with SSC. BRS Rubra acerolas of both seasons, as well as Costa Rica acerolas harvested in spring, overlapped these parameters, indicating a greater perception of sweetness by consumers due to the high SSC/TA ratio. Contrastingly, these samples presented a lower antioxidant activity than the other cultivars, expressed by the high DPPH value (Fig. 5.5).

5.3.4 Relationship between acerola bioactive compounds and antioxidant activity by path analysis

Correlation analysis confirmed the degree of relationship between the bioactive compounds and the antioxidant activity (determined by both ABTS and DPPH assays) in acerola (Fig. 5.6). Additionally, variance inflation factors (VIF) lower than 10 for ascorbic acid (1.73), phenolic compounds (2.66), anthocyanins (1.60) and yellow flavonoids (1.65), as well as a condition number lower than 100 (57.89), confirm a low multicollinearity between the variables. Thus, we examined in detail the direct and indirect effects of ascorbic acid, phenolic compounds, anthocyanins and yellow flavonoids on the acerola antioxidant activity, using a path analysis.

The contents of ascorbic acid (0.744), phenolic compounds (0.928), anthocyanins (0.614) and yellow flavonoids (0.616) were positively and significantly correlated with the antioxidant activity assessed by the ABTS method

(Table 5.2). In contrast, the DPPH method was negatively and significantly correlated with ascorbic acid (−0.564), phenolic compounds (−0.820), anthocyanins (−0.439) and yellow flavonoids (−0.480), considering the higher the value of DPPH assay, the lower the antioxidant activity (Table 5.3). Thus, the path analysis was applied to sectionize the correlation coefficient of different bioactive compounds into direct and indirect effects on acerola antioxidant activity, measured by both methods.

Table 5.2. Path analysis indicating direct and indirect effects of different bioactive compounds on antioxidant activity (ABTS method) of acerola (*Malpighia emarginata* DC.) cultivars.

Effect on antioxidant activity	Bioactive compounds			
	Ascorbic acid	Phenolic compounds	Anthocyanins	Yellow flavonoids
Direct effect	0.246	0.710	0.058	0.041
Indirect effect via AsA		0.157	0.112	0.110
Indirect effect via PC	0.454		0.428	0.442
Indirect effect via ANT	0.026	0.035		0.023
Indirect effect via YF	0.018	0.026	0.016	
Correlation with antioxidant activity	0.744***	0.928***	0.614**	0.616**

Residual = 0.318. *, ** and *** represent sources of variation significant by the F-test, with $p \leq 0.05$, 0.01 and 0.001, respectively.

Table 5.3. Path analysis indicating direct and indirect effects of different bioactive compounds on antioxidant activity (DPPH method) of acerola (*Malpighia emarginata* DC.) cultivars.

Effect on antioxidant activity	Bioactive compounds			
	Ascorbic acid	Phenolic compounds	Anthocyanins	Yellow flavonoids
Direct effect	−0.096	−0.820	0.084	0.041
Indirect effect via AsA		−0.061	−0.044	−0.043
Indirect effect via PC	−0.525		−0.495	−0.511
Indirect effect via ANT	0.038	0.051		0.033
Indirect effect via YF	0.018	0.026	0.016	
Correlation with antioxidant activity	−0.564**	−0.805***	−0.439*	−0.480*

Residual = 0.585. *, ** and *** represent sources of variation significant by the F-test, with $p \leq 0.05$, 0.01 and 0.001, respectively.

The phenolic compounds content showed the highest direct effect on antioxidant activity of acerolas (ABTS 0.710, DPPH −0.820), followed by ascorbic

acid. Ascorbic acid, yellow flavonoids and anthocyanins contents had indirect effects through phenolic compounds on antioxidants by ABTS (0.454, 0.442, 0.428) and DPPH assays (−0.525, −0.511, −0.495) (Tables 5.2 and 5.3).

5.4 DISCUSSION

The quality of acerola berries is defined by a balance among their appearance, flavor, texture, aroma, sweetness, and nutritional value, influencing consumer' preference and readiness to purchase. In the last years, the identification and quantification of health-promoting bioactive compounds in acerola has been gained attention, especially ascorbic acid and phenolic compounds.

Considering acerola is a small, tart, and perishable fruit, breeding programs have dedicated efforts to launching acerola cultivars that produce larger, sweeter, firmer, and less acidic fruits. Studies have shown a diverse range of basic quality parameters among different acerola cultivars, including diameter, color, texture, soluble solids, and acidity, which holds significant implications for the selection of superior cultivars intended for the development of new cultivars and as parental candidates in breeding programs (Vilvert et al., 2024a). Moreover, genotypic variations have been noted in concentrations of key acerola bioactive compounds such as ascorbic acid, phenolic compounds, anthocyanins, and flavonoids (Souza et al., 2014; Carneiro Ferreira et al., 2021; Farinelli et al., 2021; Franca et al., 2021). This diversity underscores the potential for targeted breeding efforts aimed at enhancing the nutritional and functional attributes of acerola fruits through the selection and manipulation of cultivars with desirable phytochemical profiles.

Different acerola cultivars display a wide range of fruit skin color, from orange to red and dark purple (Ferreira et al., 2022). In our study, red color was more intense in fruits harvested in the spring, which was represented by low hue values. Considering temperature and water availability (by irrigation) were very similar between both harvest seasons, as pointed out in the Figure 5.1, the greatest solar radiation observed in the spring may have been the key factor for the more intense red color in acerolas. The synthesis of anthocyanin is dependent on genetic, environmental, and cultural factors, mainly temperature and solar radiation, which have been targets of investigations in different fruit species, such as grapes. The exposure of fruits to ultraviolet radiation stimulates the expression of genes involved in the synthesis and accumulation of anthocyanins (Matus, 2016).

In addition to fruit color, traits such as SS, TA and SS/TA ratio have a crucial role in determining acerola fruit quality. In our study, all these parameters were depended on the cultivars and harvest seasons, consistent with previous reports (Nasser et al., 2018; Ferreira et al., 2022). Furthermore, their variance caused by the cultivars appeared to be higher than those by the seasons. BRS Rubra acerolas stood out in terms of SS and SS/TA ratio, as well as low TA, which is important for the fruit sweet perception. This corroborates with Mamede et al. (2009), which reported the lowest acidic flavor and highest consumer acceptability of BRS Rubra, in comparison to other acerola cultivars. A continuous monitoring of fruit flavor parameters across the years can optimize the harvesting time to reach a better fruit quality to attend consumers' expectations (Zhang et al., 2022).

Bioactive compounds compose fruits and by-products in different concentrations and combinations. These differences are mainly attributed to variations in climatic conditions (temperature, solar radiation, and precipitation), geographic location, cultivar, and maturity stage, as well as extraction parameters (solvent, method, time, and temperature) (Trigo et al., 2020). The present study fills a gap pointed out by Vilvert et al. (2024b), specifically concerning the assessment of the combined effects of seasonal and genotypic variations on the bioactive compounds of acerola.

Ascorbic acid and diverse groups of phenolic compounds (including flavonoids and anthocyanins) play a key role on the scavenging of reactive oxygen species (ROS) (Franca et al., 2021; Zhang et al., 2022). These compounds, either individually or combined, are the main responsible for the antioxidant properties and associated health benefits provided by berries (Skrovankova et al., 2015). Our results show a high genotypic effect on the ascorbic acid content, while most of cultivars were not affected by harvest seasons. Junko cultivar stood out in terms of ascorbic acid content in both seasons, in accordance with a previous study (Ribeiro & Freitas, 2020).

Ascorbic acid (or vitamin C) is an essential water-soluble vitamin that act as a strong antioxidant, overcoming oxidative stresses and neutralizing free radicals and reactive oxygen species (Budiarto et al., 2024). Additionally, this vitamin is well-known for enhance body immunity against viral infections, including influenza and COVID-19 (Shahbaz et al., 2022). Another important function of ascorbic acid is acting as food preserver and nutrient quality indicator in processing

and storage; retention of ascorbic acid generally results in minimum changes and losses of other nutrients (Skrovankova et al., 2015).

Typically, acerola fruits reach an ascorbic acid peak of up to 4,000 mg 100 g⁻¹, then undergoes a drastic reduction throughout different maturity stages, until show a mean range of 348–2800 mg 100 g⁻¹ in red-ripe edible fruits, depending on the cultivar (Huang et al., 2022; Vilvert et al., 2024a). Thus, only about two or three acerolas are needed to meet the daily requirement for ascorbic acid ingestion (75 mg and 90 mg for women and men, respectively), following the Recommended Dietary Allowance (RDA) established by the U.S. Institute for Medicine (Institute for Medicine, 2000).

The content of total phenolic compounds of Junko acerolas was higher than the other cultivars across all harvesting seasons. In addition, this was the only cultivar affected by harvest season, with higher content in the spring. Previous quantitative and qualitative variations in phenolic profile of acerola cultivars were reported as a response to different cultivars (Carneiro Ferreira et al., 2021). Phenolic compounds represent a group of plant secondary metabolites, which are the most diverse and well-known among phytochemical molecules possessing antioxidant properties. Secondary metabolites are compounds not essential for plant growth and development but provide protection against both biotic and abiotic stresses (Li & Ahammed, 2023).

Phenolic compounds are applied for the treatment of several human ailments such as bacterial, protozoal, fungal, and viral infections, inflammation, diabetes, and cancer (Sun & Shahrajabian, 2023). Earlier investigations have demonstrated that dietary phenolic compounds exhibit pleiotropic behavior on important proteins, resulting in beneficial effects in numerous chronic conditions associated with oxidative stress, including aging, cancer, and inflammation. This is closely linked to the diverse array of biological activities such as antioxidant, antimicrobial, anti-allergic, anticancer, anti-inflammatory, and cardioprotective properties (Chaouch & Benvenuti, 2020).

The biosynthesis and storage of polyphenols and other secondary metabolites in plants are seen as evolutionary adaptations of two interlinked biochemical pathways, shikimic acid and phenylpropanoid pathways, to adverse environmental conditions. Therefore, to withstand environmental stresses such as drought, high temperature and excessive UV radiation, plants exhibit response by

adjusting their biosynthesis pathways through changes in gene expression or the regulation of enzyme activities, as well as by altering the production of secondary metabolites (Paul et al., 2023).

Phenolic-rich fruits with high anthocyanin concentrations tend to present the highest antioxidant activity. Furthermore, strong correlations have been demonstrated between phenolics and antioxidant capacity in berries (Skrovankova et al., 2015). Antioxidant activity of acerolas, measured with both ABTS and DPPH assays, was highly influenced by cultivar, while the harvest season had a low effect. Undoubtedly, fruits of Junko cultivar had the highest antioxidant activity, because of the high contents of bioactive compounds.

The relationship between different bioactive compounds and the antioxidant activity in horticultural crops have been investigated using different statistical approaches, including path analysis. Path analysis is a standardized partial regression statistical technique that partition the correlation coefficients into two types of effects, direct and indirect (Kumar et al., 2015). Thus, the contribution of each trait to a response variable is assessed. In agricultural experiments, path analysis is usually adopted to explore the relationships between yield-related traits (Zannat et al., 2023; Singh et al., 2024), such as the association between macro/micronutrients and yield in cherry tomato (He et al., 2024) and mango (Kumar et al., 2015).

Path analysis has been successfully applied to study the association between bioactive compounds and antioxidant activity in various fruits, including blackberry (Li et al., 2018), strawberry (Zebrowska et al., 2021), guava (Kherwar & Usha, 2016), and cornelian cherry (Hassanpour et al., 2011). In our study, we propose the first application of path analysis in the literature to assess the bioactive compounds responsible for the antioxidant activity in acerola. Our investigations found through this multivariate analysis that phenolic compounds and ascorbic acid are the major contributors to antioxidant activity of acerola, corroborating with previous research (Mezadri et al., 2008; Xu et al., 2020).

5.5 CONCLUSION

Acerola quality traits were jointly regulated by harvest season and cultivars. In contrast, the genotypic effect was much greater than the seasonal effect for the bioactive compounds and antioxidant activity.

Based on the findings, it should be feasible for breeding programs focused on developing advanced acerola cultivars rich in bioactive compounds to succeed, considering the greater influence of the cultivar compared to seasonal conditions on most of the bioactive components.

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FINAL CONSIDERATIONS

Although acerola is a tropical superfruit native to South America with good adaptability to Brazilian soil and climate conditions and high nutritional value, the national production of this fruit is below its potential, especially due to the high perishability and acidity of the fruits, which restrict their fresh consumption. Breeding methods focused on improving fruit quality traits are essential to the consumers' acceptability of acerola.

Our study broadens and deepens our knowledge about the genetic aspects related to fruit quality traits and bioactive compounds in an acerola breeding program. This thesis associates the areas of Fruit Postharvest with Plant Breeding and Statistics, aiming to optimize the improvement of acerola quality.

Statistical methods applied to acerola breeding, whether classic or advanced, have revealed important insights into different quality traits and antioxidant compounds, and elucidated genetic effects on final fruit quality.

The results obtained are primarily intended for the advancement of acerola production, especially in the Brazilian semi-arid region, responsible for $\frac{3}{4}$ of the Brazilian production, but extend to other potential producing regions in the country. Furthermore, our work seeks to awaken consumer interest in acerola nationally and internationally, increasing production to the potential that this superfruit offers.

ANNEXES

Review - Food/Feed Science and Technology

Genetic Diversity on Acerola Quality: A Systematic Review

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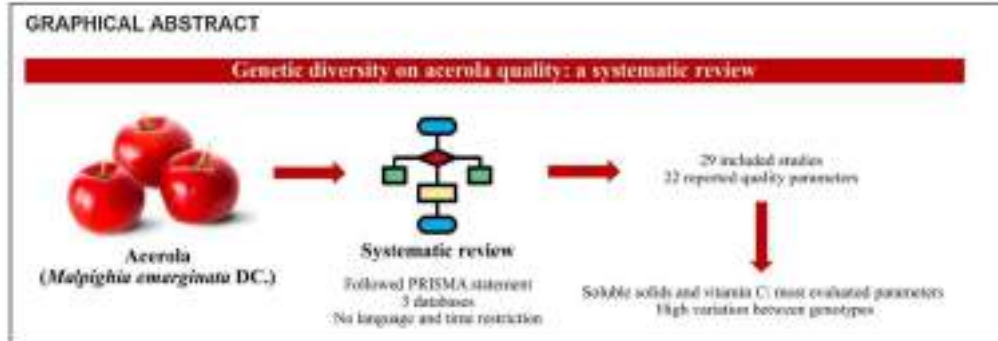
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HIGHLIGHTS

- Acerola is a tropical super fruit with high vitamin C content.
- Acerola genotypes present great variability in quality traits.
- Acerola genotypes present high variability for physicochemical and antioxidant properties.
- Genetic diversity is useful for breeding programs and germplasm conservation.

Abstract: Acerola (*Malpighia emarginata* DC.) is a super-fruit with high ascorbic acid content and its quality can be highly affected by the genetic and environmental conditions. This systematic review aims to provide an overview of the influence of genetic variability on acerola quality traits. It was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) recommendations. PubMed, Scopus and SciELO electronic databases were searched using terms acerola, selection, and genotypes. 29 studies were selected after assessing articles for eligibility criteria, which reported data on 22 quality parameters. A high genetic diversity was observed for all quality traits. Soluble solids and ascorbic acid contents are the main parameters determined in studies with acerola. Titratable acidity, SS/TA ratio and pH are also evaluated in most studies. Different studies have already identified possible genotypes for use in acerola breeding programs based on fruit quality, both for launching new cultivars and for use as parents in crosses. Our review is a useful basis for acerola breeding programs and germplasm conservation. Future studies are required to further identify and quantify bioactive compounds in acerolas of different genotypes.

Keywords: *Malpighia emarginata* DC.; plant breeding; genotypes; breeding methods; fruit quality; vitamin C.



INTRODUCTION

Acerola (*Malpighia emarginata* DC.), also known as Barbados cherry and West Indian cherry, is a tropical fruit native to Central and South America and the Caribbean Islands. It is considered a super-fruit due to its high ascorbic acid (vitamin C) content, which is up to 100 times higher than the content observed in orange and lemon [1], in addition to phytochemicals such as phenolic compounds and carotenoids.

Global demand for acerola has been increasing due to the fruit attractive color, pleasant flavor, and nutraceutical potential. With high potential as a functional food, acerola may be consumed fresh or used in the processing industry [2]. Acerola has a high appealing economic prospect for growers to reach the specific markets of natural health-promoting products that maintain health and prevent degenerative diseases [3].

Fruit quality is defined by appearance, flavor, texture, and nutritional properties, which might be affected by several factors, including environmental conditions, crop management practices, maturity stage at harvest, storage conditions and genotype [4]. Fruit quality is measured through physical and chemical properties, which can also be used to characterize maturity stage and indicate when fruit are ready for consumption [5].

Brazil is the largest producer, consumer, and exporter of acerola, especially in tropical regions with high temperatures and high sunlight incidence, ideal for production of high-quality fruit. In most of the Brazilian commercial orchards of acerola, trees have been propagated by sexual methods, which result in a high genetic variability and high orchard heterogeneity, since this crop has predominantly cross-pollination [6–8].

Genetic diversity is one of main factors that defines acerola quality traits, such as color, weight, soluble solids, acidity, and ascorbic acid [9]. According to Magalhães and coauthors [10], the need for acerola genetic breeding results from the lack of established commercial cultivars with high fruit quality and yield. The high genetic variability in acerola has stimulated the development of breeding programs [11], essential for the preservation of biodiversity and the maintenance of genetic variability for future development of new genotypes.

Considering the importance of acerola as a crop species, this systematic review aims to provide an overview about the influence of genetic variability on acerola quality traits.

MATERIAL AND METHODS

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [12]. Two reviewers independently performed a bibliographic search in PubMed, Scopus, and Scielo electronic databases. The literature search was performed using the keywords *acerola*, *selection*, and *genotypes*. The word *acerola* was accomplished by Boolean operator "OR" and its scientific name (i.e., *Malpighia emarginata*, synonyms *Malpighia glabra* and *Malpighia punicifolia*) and it was combined one-to-one with all other terms. References comprising both titles and abstracts were exported to Mendeley.

No restriction was applied to language and time limit on search. The last search was performed on July 2nd, 2022. Studies were included in the selection if they met all the following criteria: (1) were articles published on peer-reviewed scientific journals; (2) evaluated acerolas (whole fruits) in ripe maturity stage, i.e., fruits with the skin completely covered with red color; and (3) evaluated at least one identified genotype. Exclusion criteria for study selection consisted of: (1) reviews, systematic reviews, meta-analyses, letters, conference abstracts, book chapters, personal opinions and articles of non-peer-reviewed journals; (2) evaluates acerola by-products or fruits in early maturity stages (green or intermediate stages); and (3) do not identify the genotype.

Potentially relevant articles were screened independently by two reviewers, initially by abstract and then by full text. Disagreements among reviewers were resolved by a third reviewer. Two authors independently used a standard data form to extract the relevant study information, including: author(s), publication year, Köppen's climate in study area, number of genotypes, and main values of physicochemical and antioxidant traits evaluated in the studies.

RESULTS

Study selection and characterization

The initial search through three electronic databases resulted in 1462 journal articles. Records were reduced for 1182 when duplicates were discarded. Of these latter, 40 articles were selected for full-text reading, and 29 were included in final review (Figure 1). Journal articles excluded from reading the title were $n = 1095$ and the abstract were $n = 47$. Articles excluded after full text review were $n = 11$: six for not presenting the studied genotype, one for characterizing only the industrial waste (bagasse) and not the fruits, one for characterizing only the isoenzymatic data and not the fruits, two for studying the repeatability of characteristics of the acerola in plants of unidentified genotypes and one for evaluating unripe fruits.

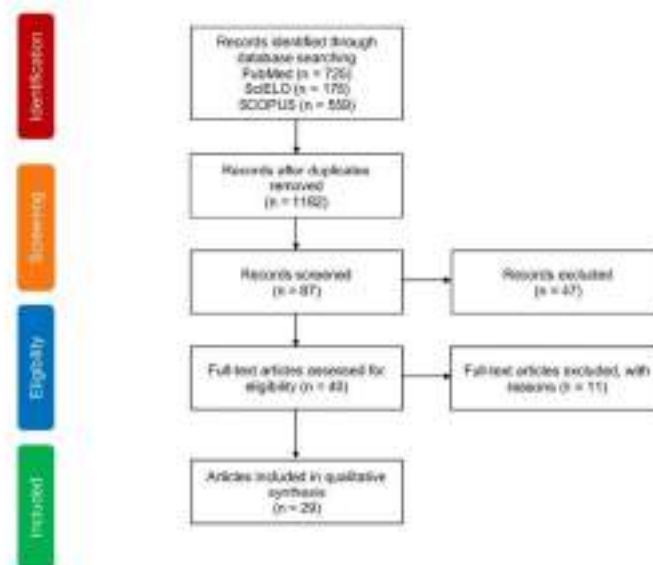


Figure 1. PRISMA flow chart showing the process of literature search and study selection.

Information on 22 physical, chemical and antioxidant properties of acerola were found and extracted from selected studies, namely soluble solids (SS), titratable acidity (TA), SS/TA ratio, pH, ascorbic acid, weight, diameter, length, color (expressed as lightness, a^* , b^* , chroma and hue angle), firmness, pulp yield, moisture, reducing sugars, anthocyanins, yellow flavonoids, carotenoids, total phenolic compounds, and total flavonoids (Table 1).

The number of genotypes identified in the studies varied between 1 and 103. 'Flor Branca', 'Okinawa' and 'Olivier' were the most evaluated genotypes, being present in seven, seven, and six studies, respectively. In the studies included in the systematic review, five types of climates were found, according to Köppen's classification: seven studies in As climate and six in Aw climate, both characterized as tropical savanna with drier season in summer (As) or in winter (Aw); six studies in Bsh climate (hot and dry semi-arid); six studies in Cwa climate and four studies in Cfa climate, both characterized by subtropical climates with hot summer, with dry winter (Cwa) or without dry season (Cfa).

Table 1. Characteristics of studies included in the systematic review assessing genetic diversity of acerola.

Reference	Study location	Climate	Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Adriano et al., 2011 [13]	Jaraguápolis, SP	Cwa	1	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Batista et al., 2015 [14]	Petrolina, PE	Bsh	4	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Beijra et al., 2018 [15]	Petrolina, PE	Bsh	4	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Caetano et al., 2012 [16]	Jaraguápolis, SP	Cwa	1	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Carpentieri-Pipolo et al., 2000 [17]	Londrina, PR	Cla	14	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Carpentieri-Pipolo et al., 2002 [18]	Londrina, PR	Cla	3	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Carralante et al., 2007 [6]	Jatobá, SP	Cwa	16	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Ferreira et al., 2021 [19]	Mal. Cândido Rondon, PR	Cla	103	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Ferreira et al., 2021 [20]	Petrolina, PE	Bsh	7	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Ferreira et al., 2022 [2]	Petrolina, PE	Bsh	35	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Gomes et al., 2000 [21]	Itapólis, SP	Aw	12	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Lima et al., 2000 [22]	Carpina, PE	Aw	8	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Lima et al., 2005 [23]	Carpina, PE	Aw	12	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Macedo et al., 2010 [24]	Carpina, PE	Aw	18	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Magalhães et al., 2018 [10]	Jatobá, MG	Cwa	24	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Martins et al., 2009 [25]	Cruz das Almas, BA	Aw	3	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Mariano-Nasser et al., 2017 [26]	Adrianópolis, SP	Cwa	8	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Masaura et al., 2001 [27]	Cruz das Almas, BA	Aw	12	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Moura et al., 2007 [6]	Fonseca, CE	Aw	45	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Musser et al., 2004 [28]	Carpina, PE	Aw	12	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Musser et al., 2005 [29]	Carpina, PE	Aw	12	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Nasser et al., 2018 [30]	Carpina, PE	Aw	12	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Nogueira et al., 2002 [31]	Adrianópolis, SP	Cwa	7	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Reis et al., 2017 [32]	Petrolina, PE	Bsh	1	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Ribeiro & Freitas, 2020 [33]	Petrolina, PE	Bsh	2	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Rosato & Mercadante, 2005 [34]	Campinas, SP	Cla	2	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Somersalo & Pereira, 2000 [35]	Aurópolis, GO	Aw	9	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Souza et al., 2014 [36]	Paragolus, CE	Aw	3	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Viana et al., 2021 [37]	Cruz das Almas, BA	Aw	3	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

* The x represents quality parameters evaluated in each included study: 1. Soluble solids (SS); 2. Titratable acidity (TA); 3. SS/TA ratio; 4. pH; 5. Ascorbic acid; 6. Weight; 7. Diameter; 8. Length; 9. Lightness; 10. a*; 11. b*; 12. Chroma; 13. Hue angle; 14. Firmness; 15. Pulp yield; 16. Moisture; 17. Reducing sugars; 18. Anthocyanins; 19. Yellow flavonoids; 20. Total flavonoids; 21. Total phenolic compounds; 22. Carotenoids.

Soluble solids, titratable acidity, SS/TA ratio, and pH

Soluble solids (SS), titratable acidity (TA), SS/TA ratio, and pH were evaluated in 83%, 66%, 52%, and 66% of the included studies, respectively (Table 1).

SS content of acerola genotypes varied between 5.2% and 15.8%, with mean values in each study ranging between 6.4% and 11.0% (Figure 2A). The lowest TA was 0.37 g of malic acid per 100 g, while the maximum TA was 3.82 g 100 g⁻¹. The mean TA in most of studies ranged between 0.78 and 1.70 g 100 g⁻¹, with exception of two studies (3.07 and 3.14 g 100 g⁻¹) (Figure 2B).

In the 15 studies that evaluated SS/TA ratio, minimum and maximum values for this parameter were 1.86 and 19.39, respectively. The mean SS/TA ratio ranged between 2.41 and 9.46 (Figure 2C). The pH of acerola genotypes varied between 2.34 and 4.26 in selected studies, with a range of mean pH values of 2.78–3.68 (Figure 2D).

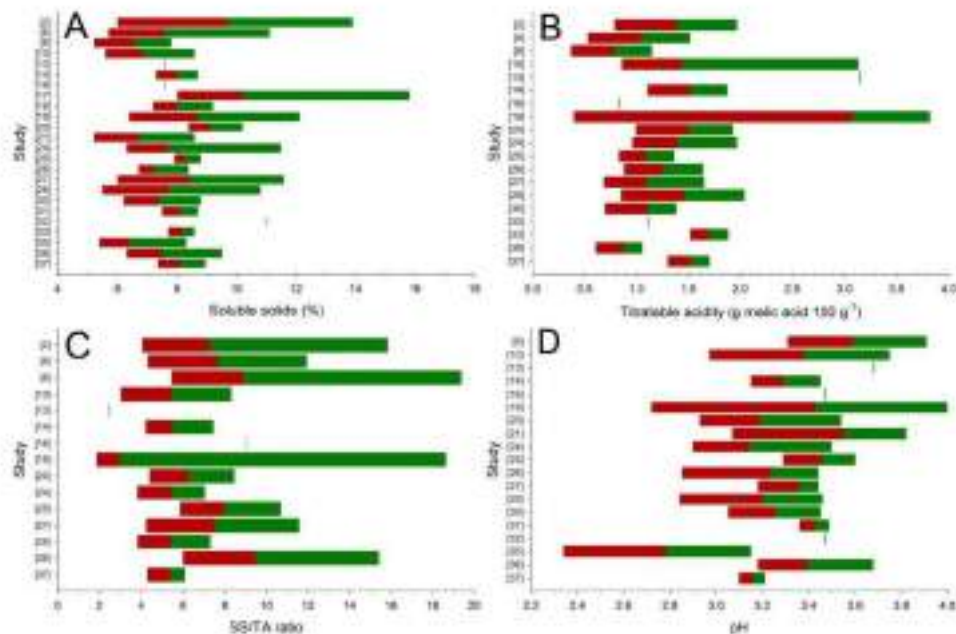


Figure 2. Soluble solids (SS) (A), titratable acidity (TA) (B), SS/TA ratio (C), and pH (D) of acerola genotypes as reported in selected studies. The red and green areas in the bars represent genotypes with lower and higher values than the general mean of the physicochemical property in each study, respectively. For studies with only one genotype evaluated, the mean is represented by a vertical line.

Ascorbic acid, weight, diameter, and length

The ascorbic acid content of acerolas was reported in 24 studies, which corresponds to 83% of included studies. Ascorbic acid content varied between 348 and 2800 mg per 100 g in acerola genotypes.

Weight, diameter, and length were the most evaluated physical parameters in acerola genotypes, with results reported in 14, 11, and 9 included studies, respectively (Table 1).

Weight of acerola genotypes ranged between 1.53 g and 15.02 g, which represents a maximum weight 9.8 times greater than the minimum value. Mean weight had a variation between 4.08 g and 9.99 g (Figure 3B). Diameter (also called transversal diameter) had minimum and maximum values between 0.83 cm and 3.00 cm, respectively, with a range of mean values of 1.21–2.83 cm (Figure 3C). Length (or longitudinal diameter) varied between 0.94 cm and 2.92 cm, with mean values between 1.51 cm and 2.18 cm (Figure 3D).

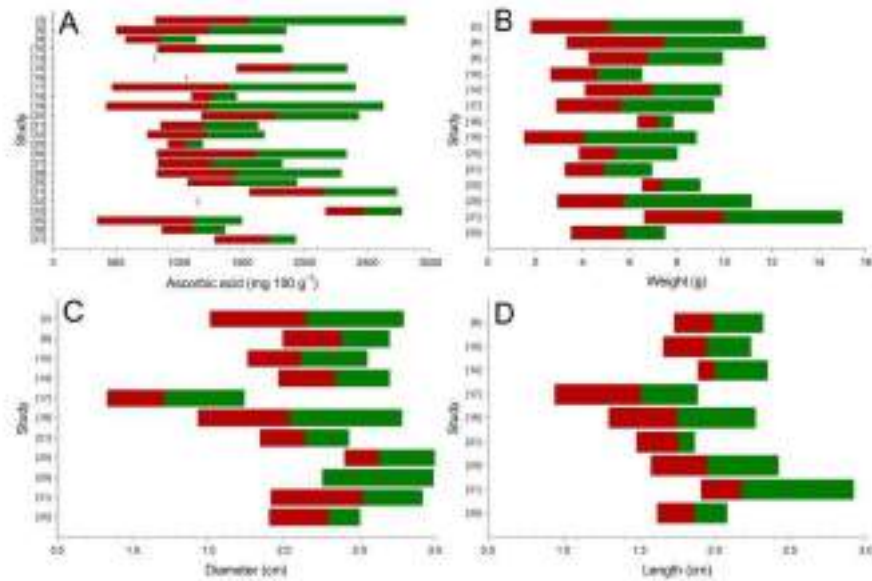


Figure 3. Ascorbic acid (A), weight (B), diameter (C), and length (D) of acerola genotypes as reported in selected studies. The red and green areas in the bars represent genotypes with lower and higher values than the general mean of the physicochemical property in each study, respectively. For studies with only one genotype evaluated, the mean is represented by a vertical line.

Color, firmness, pulp yield, and moisture

The lightness was the most reported color parameter in selected studies, with seven results indicating a range between 19.40 and 55.90. Chroma and hue angle of acerola genotypes were quantified in six and seven studies, respectively, with variations of 16.36–55.84 and 9.17–60.80. The color spaces a^* and b^* were evaluated in three studies, with ranges of 11.30–46.60 and 3.00–48.90, respectively (Table 2).

Table 2. Color parameters, firmness, pulp yield and moisture of acerola genotypes as reported in selected studies.

Ref	Lightness	a^*	b^*	Chroma	Hue angle	Firmness (N)	Pulp yield (%)	Moisture (%)
[2]	32.47–51.63	–	–	27.66–53.29	19.45–45.82	5.02–20.00	–	–
[6]	–	–	–	–	–	2.56–8.48	–	–
[8]	–	–	–	–	–	–	38.58–61.21	–
[10]	–	–	–	–	–	–	67.17–81.76	90.04–92.36
[13]	48.12	37.16	21.70	43.15	30.06	–	–	–
[14]	29.48–23.68	–	–	16.36–22.33	9.17–14.04	2.54–2.62	–	–
[16]	–	–	–	–	–	–	–	92.49
[17]	–	–	–	–	–	–	61.36–86.18	–
[18]	–	–	–	–	–	–	72.10–82.50	–
[19]	19.40–55.90	11.30–46.60	3.00–48.90	–	–	–	67.20–90.10	–
[20]	33.18–46.22	36.14–46.20	12.95–29.98	38.64–55.07	19.00–32.98	–	–	–
[21]	–	–	–	–	–	–	47.80–58.90	–
[24]	–	–	–	–	–	–	53.17–72.56	–
[25]	–	–	–	–	–	–	–	–
[26]	23.30–31.80	–	–	19.45–33.70	13.10–26.10	–	–	–
[28]	–	–	–	–	–	–	–	–
[31]	–	–	–	–	–	–	–	–
[32]	–	–	–	–	–	–	–	90.75
[33]	–	–	–	–	24.90–60.80	13.60–19.20	–	–
[34]	–	–	–	–	–	–	–	90.67–93.30
[37]	34.48–37.71	–	–	43.43–55.84	21.31–30.08	–	–	91.66–93.86

Dash-separated values indicate the minimum and maximum for each parameter. In lines without a dash, the value represents the mean in studies with only one genotype evaluated.

The pulp firmness of acerolas varied between 2.14 N and 20.02 N in four reported studies. Pulp yield and moisture had ranges of 36.56-90.10% and 80.04-93.86%, respectively, in the seven and five studies that reported these parameters (Table 2).

Reducing sugars and bioactive compounds

Reducing sugars content of acerola genotypes was evaluated in six reported studies, with a minimum value of 2.32% and a maximum of 6.03% (Table 3).

Table 3. Reducing sugars and bioactive compounds of acerola genotypes as reported in selected studies.

Ref	Reducing sugars (%)	Anthocyanins (mg 100 g ⁻¹)	Yellow flavonoids (mg quercetin equivalent 100 g ⁻¹)	Total flavonoids (mg quercetin equivalent 100 g ⁻¹)	TPC (mg 100 g ⁻¹)	Carotenoids (mg β-carotene 100 g ⁻¹)
[8]	-	1.52-28.47	-	-	-	0.34-8.41
[10]	-	-	-	-	-	-
[12]	5.73	-	-	-	-	-
[15]	-	7.03-13.60	6.86-10.73	-	850.26-1345.21 ^b	1.62-3.28
[16]	5.17	-	-	-	-	-
[18]	-	-	-	-	84.00-3196.00 ^a	-
[20]	2.57-3.80 ^a	14.99-68.23 ^a	-	3.92-5.96 ^a	1188.46-2590.40 ^a	-
[22]	-	14.06-50.98	-	9.31-20.22	-	-
[23]	-	-	-	-	737.00-1888.00 ^a	0.96-4.06
[24]	-	4.35-16.90	-	4.40-15.04	-	-
[26]	2.89-4.24	-	3.56-12.34	-	814.20-2428.30 ^a	-
[28]	-	2.40-55.80	-	6.90-22.20	-	-
[29]	-	-	-	-	-	-
[32]	-	6.78-7.28	-	-	-	-
[34]	-	-	-	-	-	3.71-18.81 ^a
[35]	-	-	-	-	-	-
[36]	2.32-6.03	5.07-12.37	7.36-9.82	-	1561.67-2631.34 ^a	-
[37]	5.56-4.97	-	-	-	410.82-784.22 ^a	0.45-1.58

TPC: Total phenolic compounds. ^aQuantified in high-performance liquid chromatography. ^bExpressed as gallic acid equivalent. ^cExpressed as catechin equivalent. Dash-separated values indicate the minimum and maximum for each parameter. In lines without a dash, the value represents the mean in studies with only one genotype evaluated.

For bioactive compounds, the number of studies per variable ranged from three for yellow flavonoids, to eight for anthocyanins. Even with the low number of studies reporting these variables, a large variation was observed between genotypes regarding the anthocyanins, yellow flavonoids, carotenoids, total flavonoids, and total phenolic compounds (Table 3).

Anthocyanin content varied between 1.52 mg 100 g⁻¹ and 68.23 mg 100 g⁻¹. Contents of yellow flavonoids and total flavonoids had ranges of 3.56-12.34 mg of quercetin equivalent 100 g⁻¹ and 3.92-22.20 mg of quercetin equivalent per 100 g⁻¹, respectively (Table 3).

Total phenolic compounds (TPC) content of acerola genotypes ranged between 84.00 and 3196.00 mg of equivalent gallic acid (GAE) per 100 grams. Carotenoids were quantified in five included studies and varied between 0.34 and 18.81 mg β-carotene 100 g⁻¹ (Table 3).

DISCUSSION

Among the tropical fruits, acerola is a highly attractive exotic fruit with high ascorbic acid content, adapted to different edaphoclimatic conditions with high potential for industrial use, which has been attracting the interest of fruit growers [31].

Brazil is the largest producer, consumer, and exporter of acerola. However, even with the production potential of this crop in Brazil, the implantation of commercial acerola orchards is considered recent, when compared to other fruit species, being formed mostly by plants with unidentified genetics, and which in most cases, do not present agronomic and commercial characteristics desirable for the fresh market. High genetic variability in commercial acerola orchards is a result of seed propagation, characterizing them as highly heterogeneous in terms of fruit quality and quantity, which takes place due to the lack of new genotypes with improved quality traits for fresh market [10].

Even with the importance of acerola as a crop species, this is the first time in the literature that data from quality of different acerola genotypes are gathered and discussed. From a systematic search and selection of peer-reviewed articles published in journals indexed to databases, we extracted information from 29 studies related to 22 physicochemical attributes and bioactive compounds of acerola from different genotypes.

In the selected studies, the number of evaluated genotypes varied between 1 and 103. 'Flor Branca', 'Okinawa' and 'Olivier' were the most present, in at least five studies each. 'Flor Branca' and 'Okinawa' are among the main acerola varieties produced in the São Francisco Valley (SFV) [38], which is the main acerola producing mesoregion in Brazil, occupying about 1/4 of the cultivated area with the crop in the country. Ideal edaphoclimatic conditions for acerola fruit development, including high solar radiation and high temperatures throughout the year, associated with the short cycle of 3–4 weeks between flowering and harvest, allow the production of up to eight annual crops in the SFV under irrigation [20].

'Flor Branca' is a cultivar with good productivity and high production regularity, characterized by low vigor plants and small fruits (<5 g). The fruits have low flesh firmness and high sensitivity to mechanical damage during handling and transport, as well as a very short shelf life [38]. 'Okinawa' acerolas are usually higher than other cultivars (5–9 g), with an attractive purple color and high ascorbic acid contents [39–40]; however, this cultivar presents low productivity and production irregularity as limiting factors.

Selected studies were classified in five different Köppen's climate conditions, including tropical savanna climate with a drier season in summer (As) or in winter (Aw), hot and dry semi-arid (Bsh), and subtropical climates with hot summer, with dry winter (Cwa) or without dry season (Cfa). Although the regions of the studies belong to three distinct climatic zones (A, B and C), all of them have high temperatures (≥ 18 °C) as a common factor, either throughout the year (A and B zones), or at least one month a year (C zone) [41]. As a typical species of tropical climate, acerola grows in temperatures between 15 °C and 32 °C and is well adapted to temperatures around 26 °C [42].

Soluble solids (SS) and ascorbic acid contents were the most reported quality traits, both present in 83% of selected studies. SS content represent substances that can be dissolved in water, mainly soluble sugars, influencing on sweetness perception by consumers. Thus, the selection of genotypes with high SS is essential in acerola breeding programs for fresh consumption [27]. Average SS of acerola genotypes in the selected studies varied between 8.4% and 11.0%, close when compared to other fruits rich in vitamin C, such as guava [43], lemon [44], and strawberry [45–46]. Some importing countries require a minimum SS content in acerolas, such as the European Union (7.0° Brix) and Japan (7.5° Brix) [47].

Titrateable acidity (TA) represents the organic acids that greatly affect fruit overall eating quality and flavor [48]. The major organic acid in acerolas is malic acid, although succinic, citric, and other acids are also detectable [20,49]. In selected studies, TA of genotypes ranged between 0.37 g and 3.82 g of malic acid 100 g⁻¹. Low pH values were observed in acerola genotypes, with a range of 2.34–4.36 in the selected studies. In general, acerola is a very acidic fruit, so low fruit acidity associated with high sugar content is essential for the release of successful commercial acerola cultivars for fresh consumption [3]. The combined perception of sweetness and sourness (acidity), expressed through the SS/TA ratio, is a useful practical indicative of flavor perception by consumers [8], whose minimum value of 10 is established for acerolas intended to fresh consumption [47].

A wide range of 1.86–19.39 was observed for SS/TA ratio in selected studies, but few genotypes showed a SS/TA ratio greater than 10. 'Florida Sweet' acerola evaluated by Souza and coauthors (2014) [36] showed a high SS/TA ratio of 15.42, in contrast to the values below 7 observed in the other evaluated cultivars ('Flor Branca' and 'BRS 366'). Ferreira and coauthors [2] observed that 'BRS Rubra' was the only genotype among 35 evaluated in two production cycles that presented SS/TA greater than 10, confirming a previous result by Mamede and coauthors [25]. 'Florida Sweet' is a well-established acerola cultivar selected in Florida, USA, and available since the mid-1950s, outstanding for its high sugar and low acid contents. In 2004, the Brazilian acerola cultivar 'BRS Rubra' was released with the same intention to meet the demand for fruits for fresh consumption with a high SS/TA ratio.

Mean values of ascorbic acid content in acerola genotypes were higher than 1000 mg 100 g⁻¹ in all studies, except in those by Adriano and coauthors (810 mg 100 g⁻¹) [13] and Cavalcante and coauthors (852 mg 100 g⁻¹) [8]. Ascorbic acid is an essential nutrient for the human diet, playing a key role on formation of blood vessels, cartilage, muscle, and bone collagen, in addition to improving the immune system and aiding in the absorption of iron [50]. Ascorbic acid is also one of the most important water-soluble antioxidants in fruits. Acerola is the second richest natural source of ascorbic acid in the world, only comparable to the native Amazonian fruit camu-camu (*Myrciaria dubia*, Myrtaceae) [51]. Siqueira and coauthors [52] compared the costs of nutrients provided by typical foods found in the Brazilian diet and found that vitamin C is the cheapest

nutrient in Brazil, considering that with only 3.3 Brazilian Real cents ($\approx$ USD 0.0067) it is possible to supply the whole daily needs of this nutrient by consuming acerola juice.

Weight of acerola genotypes ranged between 1.53 g and 15.02 g, which represents a maximum weight 9.8 times greater than the minimum value. For fruit diameter and length, also known as transversal and longitudinal diameters, the ranges were 0.83–3.00 cm and 0.94–2.92 cm, respectively. Acerola fruit is a thin-skinned, three-lobed small globose drupe [42]. High variations in acerola physical traits are intrinsic to genetic materials and their interaction with different edaphoclimatic conditions of the crop environments [10]. Fruit dimension is an important trait for acerola selection, since the larger the fruit, the easier and faster the harvesting, reducing required labor and production costs [14].

As an important quality trait for consumers and a major criterion used to assess fruit maturity, the color of acerola changes from bright glossy green to darkish-red or purple throughout fruit development due to chlorophyll degradation concomitant with a rise in the synthesis of carotenoids and anthocyanins [42]. The presence of these pigments in ripe fruit of different genotypes was reported by the high values of color spaces a^* (11.30–46.60) and b^* (3.00–48.90) and low hue (9.17–60.80°).

Pulp firmness of acerolas varied between 2.14 N and 20.02 N, which represents a difference of 9.4 times between genotypes with minimum and maximum firmness. Acerola is considered a highly perishable fruit, with high respiration rate and ethylene production [3]. Both factors trigger an intense fruit softening after harvest that reduce fruit quality [53]. Thus, the selection of acerola genotypes with higher pulp firmness and short time between harvesting the fruit and reaching the consumer are essential for the fruit to present high quality.

Pulp yield showed a high variation between genotypes (38.58–90.10%). The low pulp yield is not a characteristic that makes it impossible to use a fruit species for fresh consumption or industrial processing, although higher values are desirable to reduce cost/benefit ratio [24]. Fruit moisture was higher than 90% in all genotypes, while reducing sugars content ranged between 2.32% and 6.03%. Reducing sugars of acerola are mainly composed of fructose and glucose and are responsible for the sweetness of the fruit. Santos and coauthors [53] quantified sugars in acerola through nuclear magnetic resonance spectroscopy and found that glucose and fructose were predominant and accumulated in ripe fruit, while sucrose exhibited a slight increase during ripening.

Acerola is a 'superfruit' due to presence of phytochemicals with antioxidant, astringent, anti-viral, anti-carcinogenic, anti-inflammatory, antianemics, and antifungal properties [9]. Anthocyanin content had a large variation of 45 times between genotypes with minimum (1.52 mg 100 g⁻¹) and maximum (68.23 mg 100 g⁻¹) values. Large differences between studies regarding the anthocyanin content of acerola genotypes tend to be related to the procedures of laboratory analysis, since the determination of these pigments involves the use of reagents and equipment. Even so, the high intra-study variation in anthocyanin content confirms that the concentration of this pigment is highly genotype-dependent, as pointed out by Ferreira and coauthors [20]. The anthocyanin content directly impacts the color of red-ripe acerolas, which can vary from orange to dark purple, and the fruit antioxidant activity, since anthocyanins are the major phenolic compounds in ripe fruit, and therefore, can reduce oxidative damage caused by free radicals to the human organism.

High variations were also observed for contents of carotenoids (0.34–18.81 mg β -carotene 100 g⁻¹), yellow flavonoids (3.56–12.34 mg quercetin equivalent 100 g⁻¹), total flavonols (3.92–22.20 mg of quercetin equivalent 100 g⁻¹), and total phenolic compounds (TPC) (84.00–3196.00 mg of gallic acid equivalent 100 g⁻¹). Acerola is a remarkable functional food resource with high levels of phenolic compounds. Phenolic compounds are important for plant metabolism and have been stated as important molecules for human organism due to their health characteristics, particularly related to their antioxidant power [42].

High variability in acerola quality has been reported in Brazilian orchards, especially those propagated by seeds, which motivated the selection of superior genotypes with desired agronomic traits and fruit quality [7]. Acerola quality is highly associated with genetic factors, but also by environmental factors such as precipitation, solar radiation, and temperature, and by preharvest factors such as irrigation, fertilization, and control of pests and diseases [42].

Studies carried out with acerola clones and accessions using different molecular markers, including Inter Simple Sequence Repeats (ISSR) [54] and Random Amplified Polymorphic DNA (RAPD) [55–56] primers, revealed a high genetic variability.

CONCLUSION

With a continuous rise in global demanding of acerolas, there is a need for the characterization of their key quality traits. In the present review, we systematically identified a high genetic diversity on all acerola quality traits. Soluble solids and ascorbic acid contents are the main parameters determined in studies with acerola. Titratable acidity, SS/TA ratio and pH are also evaluated in most studies. Different studies have already identified possible genotypes for use in acerola breeding programs based on fruit quality, both for launching new cultivars and for use as parents in crosses. Our review is a useful basis for acerola breeding programs and germplasm conservation. Future studies are required to further identify and quantify bioactive compounds in acerolas of different genotypes.

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Phenolic compounds in acerola fruit and by-products: an overview on identification, quantification, influencing factors, and biological properties

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Abstract

Acerola (*Molipighia esarguinum* DC) is a cherry-like tropical fruit well-known for being a rich source of ascorbic acid (vitamin C) and phenolic compounds. This review provides a complete overview on aspects related to phenolic compounds of acerola fruit and by-products, comprising the identification and quantification of phenolic compounds, influencing factors, and biological properties. Scientific evidences suggest that the acerola is a promising superfruit with great potential in the food and pharmaceutical industries. At least 76 phenolic compounds were identified using high performance liquid chromatography in acerolas, including 55 flavonoids (anthocyanins, flavan-3-ols, flavonols, flavones, flavanones, isoflavones and chalcones) and 21 non-flavonoids (phenolic acids, stilbenes and lignans). Phenolic compounds in acerola show several biological properties, including antioxidant, antibacterial, antihyperglycemic, antihyperlipidemic, anti-inflammatory, and hepatoprotective activities. However, studies are further required to assess the seasonal and genotypic influence on the phenolics of acerola and their bioaccessibility. Acerola is an anthocyanin-rich fruit with high potential for pigment extraction, but stabilization of anthocyanins in juice and pulp should be further elucidated and improved.

Keywords *Molipighia esarguinum* DC · Polyphenols · Phytochemicals · Antioxidant activity · Biological properties

Introduction

In recent years, global demand for fruit and vegetables is steadily increasing, as a result of healthier eating behavior and lifestyle by consumers, since their consumption have been associated with lower incidence of mortality caused by non-communicable chronic diseases [1, 2]. Red fruit, also called berries, are very popular due to their attractive appearance, pleasant taste and aroma and high nutritional value, as sources of vitamins,

carotenoids, phenolic compounds, fibers, phytochemicals, and other bioactive compounds with well-known biological properties, including antioxidant, anti-inflammatory, anti-cancer, antimutagenic, antimicrobial, antiproliferative, anti-neurodegenerative, antihyperglycemic, hypolipidemic, cardio-protective, and neuroprotective properties [3–6].

In tropical countries, there are several native red fruit species that are underexploited when compared with traditional ones such as strawberry, grape, blueberry, raspberry, and blackberry, but which have high potential for use in food and pharmaceutical industries [7]. One of these species is acerola (*Molipighia esarguinum* DC), a cherry-like tropical fruit that is one of the richest natural sources of ascorbic acid (AsA, or vitamin C) in the world, representing up to 100 times the levels found in orange and lemon [8].

AsA together with phenolic compounds such as flavonoids, phenolic acids, and stilbenes are the main responsible for the high antioxidant activity of acerolas, which can help reducing the oxidative damages of free radicals to human body and strengthening the immune system.

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In spite of its high nutrient profile and appeal for functional food, acerola is underutilized worldwide and information related to bioactive compounds and the factors that influence the synthesis of these compounds are still scarce [9]. This review provides a complete overview on aspects related to phenolic composition of acerola fruit and by-products, comprising identification, quantification, influencing factors and biological properties.

Research methodology and data collection

A search was performed on the Scopus database including the keywords *acerola* OR *Barbados cherry* OR *West Indian cherry* OR *Guarani cherry* OR *Surinam cherry* OR *Malpighia* OR *Malpighia emarginata* OR *Malpighia glabra* OR *Malpighia paniculata* AND phenolic compounds OR polyphenols, resulting in 171 published documents between January 2002 and December 2022 (Fig. 1). The main results were related to identification and quantification of phenolic compounds in whole fruit (skin and pulp), juice or residuals from pulp processing (seeds), extraction methods of phenolic compounds, and influencing factors. We addressed and discussed results from articles published in high impact scientific journals between 2013 and 2022.

Information on phenolic compounds studied in this review, as well as the 2D structures of the main phenolics in acerola fruit and by-products, were based on PubChem (<https://pubchem.ncbi.nlm.nih.gov/>).

Botanical description and cultivation of acerola

Acerola, also known as Barbados cherry, West Indian cherry, and Guarani cherry, is a tropical fruit belonging to the Malpighiaceae family. It is native from Central America, South

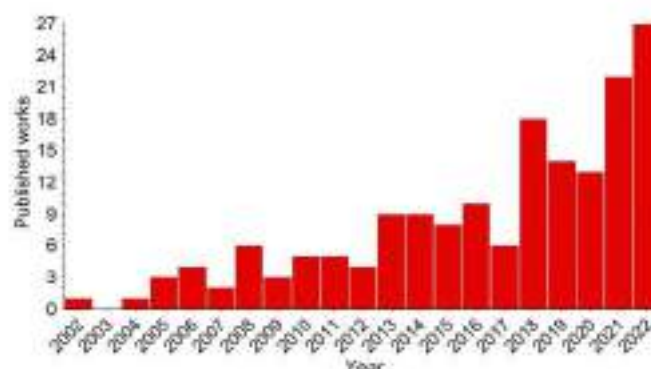
American and the Caribbean islands, and is also found in tropical and subtropical areas of Asia and Africa [10].

The taxonomic classification of acerola is not well established in the literature, since some researchers defend that the acerola originates from two species, *Malpighia paniculata* and *M. glabra*, while others argue that both are not distinct species, but different botanical forms. In Brazil, acerola orchards were established from *M. paniculata* seeds imported from Puerto Rico in the 1950s, characterized by causing strong skin irritation due to the presence of hairs on the leaves, but the presence of *M. glabra* is also confirmed due to the occurrence of glabrous plants (without hairs), which do not cause irritation [11]. Gomes et al. [12] indicate *M. emarginata* is the proper nomenclature for designating acerola. The Integrated Taxonomic Information System establishes *M. glabra* and *M. emarginata* as accepted nomenclatures for acerola, while *M. paniculata* is considered misapplied (non-accepted) [13].

Acerola (Fig. 2) tree is a shrub with small to medium height (2–3 m), the leaves are oval to elliptical-shaped with dark and glossy greenish color and height of 2.5–7.5 cm, and the inflorescences are sessile or have short peduncles, with five-fringed light or dark pink petals [10]. Ripe fruit is a globose drupe with 1–4 cm of diameter, a thin and fragile pericarp of reddish or dark purplish color, a mesocarp (pulp) that represents around 60–80% of total fruit weight, and one seed surrounded by a reticulated, tri-lobbed endocarp [14].

Acerola is a juicy, acid to sub-acid (0.46–1.10% malic acid, 0.05–0.09% succinic acid, titratable acidity of 0.87–1.97 g of malic acid 100 g⁻¹) and sweetish fruit (1.23–1.87% fructose, 1.24–1.83% glucose, 0.06–0.13% maltose, soluble solids of 6.03–13.87%), whose flavor is highly dependent on genotype [15, 16]. Acerola is well-known for its high AsA content, with ranges from 400 to 2,800 mg 100 g⁻¹ in red-ripe fruit and up to 4,000 mg 100 g⁻¹ in unripe green fruit [17, 18].

Fig. 1 Number/year of published works on phenolic composition of acerola fruit and by-products



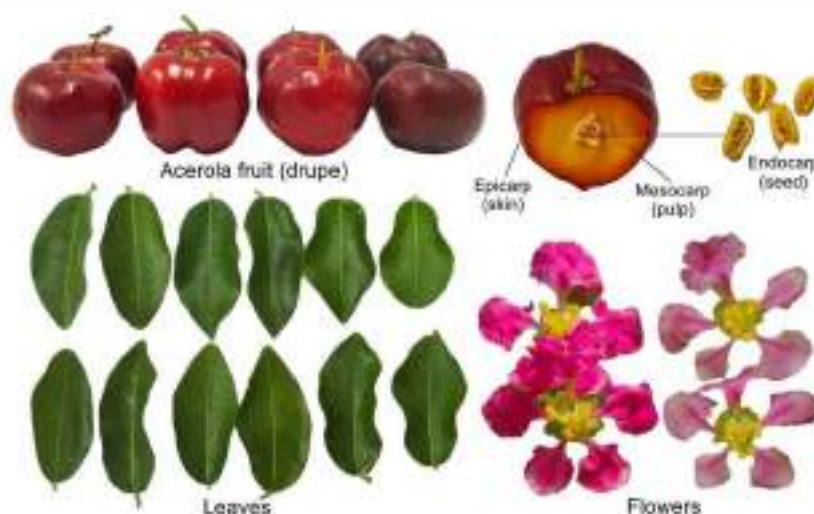


Fig. 2 Leaves of the tree and structure of the acerola (*Malpighia emarginata* DC.) fruit

Acerola flowering and fruiting can occur concomitantly when temperatures are close to or greater than 26 °C and the season is rainy or water is made available through irrigation [10]. Fruit formation occurs very quickly, with an average period of 17–25 days between anthesis and harvest, which may vary depending on the genotype, temperature, solar radiation, and water availability.

Short production cycle of acerola enables the occurrence of up to eight harvests per year in irrigated orchards in the Brazilian Northeast under Semi-arid climate conditions, between August and April, when climatic conditions are favorable for fruit growth and development. Furthermore, acerola has little damage from pests and diseases when compared to other fruit species, which reduces the use of pesticides and enables the production of organic fruit, whose value is up to 50% more than that paid for fruit produced in the conventional system [11].

The commercial cultivation of acerola in Brazil has been growing every year, with a planted area of 5,753 hectares and production of 60,966 tons of fruit in 2017, according to official data by the Brazilian Institute of Geography and Statistics [19]. Acerola market is divided in two main purposes: green fruit for AsA extraction and red fruit for fresh consumption or processing.

The great AsA content in green acerolas motivated their use for the production of concentrates for food enrichment or pharmaceuticals, as an alternative to synthetic AsA in

dietary supplements [20, 21]. In Brazilian Northeast, several plantations are exclusively destined for AsA extraction in green fruit through partnerships between rural producers and multinational companies [22]. The acerola extract market is steadily expanding, with an expected market value of US\$ 17.5 billion until 2026 [23], as a result of higher absorption of acerola AsA by human body when compared to synthetic AsA, besides its ability to act as a food preservative.

Acerolas at red maturity stage are commonly commercialized for fresh consumption or for processing of value-added products, including frozen pulp, juice, marmalade, frozen concentrate, jam, yogurt, and liquor [20]. As a naturally very acidic fruit, new acerola varieties have been developed in order to reach a higher consumer acceptance of fruit, characterized by high diameter, mass, firmness and sugar content, and low acidity [16]. Even though, high natural perishability and intense metabolic activity can limit the shelf life in acerolas, making processing an alternative to avoid postharvest fruit losses.

Scientific investigations on phenolic composition and health-promoting properties of native and exotic fruit as yet unknown or under-exploited species is important for their adding value, serving as a basis for social and economic development of local population and future source of income for small farmers [24].

Phenolic compounds in acerola: identification, quantification, and influencing factors

Phenolic compounds are phytochemicals produced by plants as secondary metabolites, i.e., compounds not required for plant growth or development but produced for its defense against biotic and abiotic stresses, such as salinity, exposure to high-energy radiation, drought, extreme temperatures, flood, attacks of insects, nematodes and phytopathogenic fungi, bacterial infection, and disease progression. In addition to defense responses, phenolic compounds perform a wide array of antioxidant, structural, attractant, ultraviolet (UV) screen, and signal functions in plants [25]. These compounds are synthesized by the shikimate and phenylpropanoid pathways, and are characterized by their structures with at least one aromatic ring, with single or multiple hydroxyl (-OH) and methyl (-CH₃) groups linked, ranging from simple structures to highly polymerized compounds [25–27].

From the discovery of phenol in 1834 to the present day, more than 10,000 phenolic compounds are known, and many of them have been studied in relation to their functional properties [28]. The interest in phenolic compounds is mainly related to their antioxidant activity, preventing and reducing oxidative damages by free radicals to vital cellular constituents such as lipids, proteins and nucleic acids. Free radical scavenger potential of a phenolic compound depends on its reducing properties as hydrogen- or electron-donating agents, which in turn varies according to the number of hydroxyl groups and their position in relation to the carbonyl functional group [29, 30].

Phenolic compounds are categorized according to their structural differences, source of origin, and biological activity [28]. In general, they can be divided in two major classes, flavonoids and non-flavonoids. Flavonoids are the main group of phenolic compounds, characterized by a polyphenolic structure (C6–C3–C6) constituted of two aromatic rings (A and B) linked by a three-carbon bridge (frequently as a heterocyclic ring, named C). Flavonoids are subdivided into different subgroups, according to the substitution pattern (degree of unsaturation and oxidation) in C rings. Additionally, the sites of hydroxyl and methyl groups attached to flavonoid structure can result in glycoside modifications, thus flavonoids are found in both aglycone and glycoside forms. The main subgroups of phenolic compounds are anthocyanidins, flavan-3-ols, flavonols, flavones, flavanones, isoflavones, and chalcones [30–32].

Biosynthesis of phenolic compounds is regulated by several intrinsic and extrinsic factors, including plant hormones, developmental phase, water, temperature, minerals, salinity, UV radiation, and visible light, and can be stimulated by agricultural practices such as the application of plant growth

regulators and the postharvest application of UV radiation [33–35].

Many flavonoids are often responsible alongside the carotenoids for color pigmentation of fruit and flowers in most angiosperm families [31, 36]. In red fruit and berries, major flavonoids are composed of anthocyanins, water-soluble pigments with pink, red, blue and purple hues [4]. The color provided by anthocyanin depends on its hydroxylation, methylation, and glycosylation; higher number of hydroxyl groups attached to B-ring increases blue coloration, while methylation results in reddish hues [37].

The use of fruit anthocyanins to enhance and provide color to food products has been increasing, since natural ingredients are associated by consumers with the health benefits and improvement in life quality, in contrast with critics to synthetic dyes and their insecurity for human health [38]. However, anthocyanins are highly susceptible to degradation, depending on temperature, pH and light, which leads to changes in their color to yellow or even colorless degradation under these conditions [37].

Methods for assessment of acerola phenolics: from spectrophotometry to smartphone-based sensor and high-performance liquid chromatography (HPLC)

Classical method

Folin-Ciocalteu colorimetric method is the most widespread analytical technique for estimation of total phenolic compounds (TPC) content in plant extracts by the oxidation-reduction reaction of phenols with a mixture of phosphomolybdate and phosphotungstate (Folin-Ciocalteu reagent) to form a blue complex able to be quantified by spectrophotometry [39].

Since the first study on quantification of phenolic compounds in acerola, using the classical colorimetric Folin-Ciocalteu method [40], this fruit has shown to have an incredibly high content of these compounds. A comparative study of 18 non-traditional fruits from Brazil was carried out by Rufino et al. [41] and demonstrated that this fruit had the second highest content of polyphenols [1063 mg of gallic acid equivalents (GAE) per 100 g of fresh mass (fm)], slightly lower than that observed in camu-camu (*Myrciaria dubia*, Myrtaceae) (1,176 mg GAE 100 g⁻¹ fm), a little known Amazonian fruit also recognized as one of the richest natural sources of AsA worldwide.

Acerola (593.77 mg GAE 100 g⁻¹ fm) preseeded together with the paná (*Annona coriiflora*) (902.18 mg GAE 100 g⁻¹ fm), uçá (*Euterpe oleracea*) (708.22 mg GAE 100 g⁻¹ fm) and jaboticaba (*Myrciaria cauliflora*) (626.57 mg GAE 100

g^{-1} fw) the highest TPC content in frozen pulps between 44 traditional and exotic Brazilian fruit species [42].

Innovations on Folin–Ciocalteu-based approaches for assessment of phenolic content in acerola

As alternatives to the use of spectrophotometer in determination of TPC content, which has high cost and little portability and that is limited to the laboratory routine, some innovative approaches have been proposed using acerolas. These new techniques mainly aim to make the assessment of plant-based phenolic content cheaper and more accessible, ceasing to be exclusive to the laboratory and accessible to industries.

Miranda et al. [43] designed a low-cost system for measuring the concentration of TPC and flavonoids in twelve fruit species, including acerola. Briefly, the system consists of an optical sensor, which requires a light emitting diode, an optical filter, a cuvette holder, a photodiode, an electronic board and a mechanical structure. Measurements were collected in transmittance and compared with those from a commercial spectrophotometer. The results did not indicate significant ($p > 0.05$) differences between the methods for determination of TPC ($48.16 \mu\text{g} \mu\text{L}^{-1}$ for the spectrophotometer and $60.27 \mu\text{g} \mu\text{L}^{-1}$ for the sensor) and flavonoids ($31.41 \mu\text{g} \mu\text{L}^{-1}$ for the spectrophotometer and $68.61 \mu\text{g} \mu\text{L}^{-1}$ for the sensor) in acerola, as well for the other fruit evaluated in the study.

Martins et al. [44] developed a simple and fast system based on colorimetry for quantification of phenolic compounds in acerola using acquisition of images by mobile devices followed by image conversion into the quantifiable RGB color system, i.e., a mathematical model that represents simultaneously red (R), green (G) and blue (B) colors as number values. Both spectrophotometry and smartphone-based method were in close agreement (145.0 and 146.0 mg GAE g^{-1} dm, respectively). Additionally, the proposed method reduced in five times the reagent consumption and waste generation, being an efficient and greener approach for determination of the phenolic content.

HPLC: detailed phenolic composition of acerola

Recently, technological advances on food composition analysis have allowed an efficient and sensible separation, identification and quantification of phenolic compounds, whose biological and health properties are structure-dependent [45]. HPLC and mass spectrometry (MS) are undoubtedly recognized as the most advanced techniques for the elucidation and determination of phenolic compounds by the scientific community [46].

Phenolic compounds in acerola were separated by chromatography for the first time by Vendramini and Trugo

[47]. The authors identified nine phenolic compounds in red-ripe acerolas including three anthocyanins (malvidin-3,5-diglucoside, cyanidin 3-glucoside and pelargonidin), four phenolic acids (*p*-coumaric acid, ferulic acid, caffeic acid and chlorogenic acid), and two flavonols (kaempferol and quercetin). Since this period, at least 76 phenolic compounds were identified using HPLC in acerolas, including 55 flavonoids (anthocyanins, flavan-3-ols, flavonols, flavones, flavanones and isoflavones) (Table 1) and 21 non-flavonoids (phenolic acids, stilbenes and lignans) (Table 2). Different classes of phenolic compounds previously reported in acerola are discussed in the next topics.

The sensitivity of HPLC in detection of phenolic compounds is generally based on their purification and pre-concentration from matrices of plant extracts (i.e., acerola fruit and by-products). Furthermore, HPLC combined with MS detectors is highly sensitive and show high power for identification achieve high specificity due to the mass selectivity of detection [36].

The column selection also influences the success of the processes of identification and quantification of phenolic compounds in plant extracts. In studies with acerola and by-products, as with other fruit crops, a normal phase C_{18} or reversed phase (RP- C_{18}) column is employed, with 10–25 cm of length, 2.1–4.6 mm i.d., and 3–5 μm particle size [15, 48–53]. New types of columns, with 10 cm length, 2.1 mm i.d., and 1.7–1.8 μm particle size have been used in advanced HPLC methods of phenolic compounds identification, such as UPLC (ultra performance liquid chromatography) [54, 55]. Identification of acerola phenolic compounds has been carried out with ambient column temperature (30–40 °C), although higher temperatures can be adopted to new columns and techniques [36]. The running time is another crucial factor that influences the HPLC determination of phenolics, ranging from 15 to 90 min in studies with acerola. The flow rate varied between 0.35 and 1 mL/min and the volume injected was 4–40 μL .

Successful and accurate identification and quantification of acerola phenolic compounds with HPLC are confirmed by validation parameters, including calibration curves from external standards of the analyzed phenolics with excellent adjustment of linear regression, as well as low values for limit of detection (LOD) and limit of quantification (LOQ). Ferreira et al. [15] quantified 17 phenolic compounds in acerola fruit of seven varieties using HPLC–DAD, with LOD < 0.0027 $\text{mg}/100 \text{ g}$, LOQ < 0.0102 $\text{mg}/100 \text{ g}$, and $R^2 > 0.996$ for all evaluated compounds. In another study with acerola fruit using HPLC–DAD, low LOD (0.0039 $\text{mg}/100 \text{ g}$) and LOQ (0.0128 $\text{mg}/100 \text{ g}$) were found for nine phenolic compounds in fruit of three maturity stages [49].

In recent years, sophisticated systems of liquid chromatography combined with modern detectors have been employed

Table 1 Individual flavonoids (anthocyanins, flavan-3-ols, flavones, flavonols, flavanones, isoflavones and chalcones) reported in açai fruit and by-products

Flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
Anthocyanins							
Cyanidin 3-rutinoside	102901165	Skin and pulp	Ripe	HPLC-DAD	Ethanol	14.99–68.29 ^a	[13]
		Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	47–1152 ^b	[50]
Cyanidin 3-rutinoside	29251	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	12–67 ^b	[50]
Cyanidin 3,5-diglucoside	441688	By-product	Ripe	HPLC-DAD	Ethanol	0.3 ^a	[58]
Delphinidin	68265	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Malvidin 3-glucoside	11249520	By-product	Ripe	HPLC-DAD	Ethanol	8.9 ^b	[58]
Pelargonidin	440832	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	0.4–7.0 ^b	[50]
Pelargonidin 3-glucoside	443648	By-product	Ripe	HPLC-DAD	Ethanol	1.3 ^a	[58]
Pelargonidin 3-rutinoside	44296998	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	28–155 ^b	[50]
Pennisetin-5-xylopyranoside	NL	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	6–130 ^b	[50]
Pennisetin 3-glucoside	443651	By-product	Ripe	HPLC-DAD	Ethanol	14.7 ^a	[58]
Phloretin	4786	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Tiliquin	3492231	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Flavan-3-ols							
Monomer catechins							
(-)-Epicatechin	9064	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.64 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	0.8 ^b	[51]
		Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.68–2.51 ^a	[13]
		Skin and pulp	Ripe	HPLC-DAD	Methanol	871 ^a	[49]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	396 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Methanol	115 ^a	[49]
		Seed	–	UPLC-MS	Ethanol	1.57 ^b	[48]
		Seed	–	UPLC-MS	Acetone	3.12 ^b	[48]
		Seed	–	HPLC-UV	Water	1.09 ^b	[59]
		Seed	–	HPLC-UV	Methanol	1.13 ^b	[59]
		By-product	Ripe	HPLC-DAD	Ethanol	35.1 ^a	[58]
		Non-ponace	Green	U/HPLC-MS/MS	Water	1.26–22.08 ^b	[54]
		Juice	Ripe	HPLC-DAD	NS	0.15 ^b	[52]
		Juice	Green	U/HPLC-MS/MS	Water	0.42 ^a	[54]
		Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.63 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	0.7 ^a	[51]
		Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.003 ^a	[15]
		Skin and pulp	Ripe	HPLC-DAD	Methanol	704 ^a	[49]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	253 ^a	[49]
(x)-Epicatechin	72276	Skin and pulp	Green	HPLC-DAD	Methanol	521 ^a	[49]
		Seed	–	UPLC-MS	Ethanol	189.3 ^b	[48]
		Seed	–	UPLC-MS	Acetone	33.1 ^b	[48]
		Seed	–	HPLC-UV	Water	1.17 ^b	[59]
		Seed	–	HPLC-UV	Methanol	9.19 ^a	[59]

Table 1 (continued)

Flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Concent. (mg 100 g ⁻¹)	Reference
(-)-Epigallocatechin gallate	107905	Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.25–0.72 ^a	[15]
(-)-Epigallocatechin gallate	65064	Skin and pulp	Green	HPLC-DAD	Ethanol	5.83 ^b	[90]
		Seed	–	HPLC-UV	Methanol	0.91 ^a	[99]
		By-product	Ripe	HPLC-DAD	Ethanol	3.8 ^c	[58]
Procyanidin							
Procyanidin A2	124025	Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.52–0.93 ^a	[15]
		By-product	Ripe	HPLC-DAD	Ethanol	3.8 ^c	[58]
Procyanidin B1	1129013	Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.09–1.78 ^a	[15]
		By-product	Ripe	HPLC-DAD	Ethanol	20.9 ^c	[58]
Procyanidin B2	122738	Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.25–0.83 ^a	[15]
		By-product	Ripe	HPLC-DAD	Ethanol	39.1 ^c	[58]
Haricetin							
Isorhamnetin	528884	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	23.2 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	43.2 ^b	[51]
		Skin and pulp	Ripe	HPLC-DAD	Ethanol	1.31–3.29 ^a	[15]
		Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	0.5–2.8 ^b	[90]
		By-product	Ripe	HPLC-DAD	Ethanol	15.8 ^c	[58]
Isorhamnetin	5281654	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.62 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	0.99 ^b	[51]
		Skin and pulp	Ripe	HPLC-DAD	Ethanol	2.08–4.79 ^a	[15]
		Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	1.1–1.3 ^b	[90]
		Skin and pulp	Green	HPLC-DAD	Ethanol	10.13–11.42 ^b	[60]
		Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	2.7–3.0 ^b	[90]
Isorhamnetin 3-glucuronide	13245586	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	17–43 ^b	[90]
Isorhamnetin 3-glucoside	5318645	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	17–43 ^b	[90]
Kaempferol	528883	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	2.39 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	1.61 ^b	[51]
		Skin and pulp	Ripe	HPLC-DAD	Methanol	162 ^a	[49]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	251 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Ethanol	6.16–6.30 ^b	[60]
		Skin and pulp	Green	HPLC-DAD	Methanol	125 ^a	[49]
		Seed	–	UPLC-MS	Ethanol	2.50 ^b	[48]
		Seed	–	UPLC-MS	Methanol	3.26 ^b	[48]
		Seed	–	UPLC-MS	Acetone	4.15 ^b	[48]
		Juice	Ripe	HPLC-DAD	NS	0.37 ^a	[52]
Kaempferol 3-rutinoside-7-alpha-L-arabinofuranoside	44258992	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	1.5–3.5 ^b	[90]
Kaempferol 3-glucoside	5282102	Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.34–0.83 ^a	[15]
		Skin and pulp	Green	HPLC-DAD	Ethanol	8.50–32.68 ^b	[60]
		By-product	Ripe	HPLC-DAD	Ethanol	12.0 ^c	[58]
Kaempferol 3-neohesperidoside	5485985	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	3.1–7.0 ^b	[90]
Kaempferol 3-arabinofuranoside	5318717	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	10.5–18.1 ^b	[90]
Myricetin	5281672	Juice	Ripe	HPLC-DAD	NS	0.09 ^a	[52]

Table 1 (continued)

Flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Concentration (mg 100 g ⁻¹)	Reference
Myricetin 3-glucuronide	44259442	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	0.1–14.3 ^a	[50]
Myricetin 3-glucuronide	44259426	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	0.1–25.1 ^b	[50]
Myricetin 3-rhamnoside	5352000	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	76–190 ^b	[50]
Quercetin	5280343	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	12.0 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	12.1 ^b	[51]
		Skin and pulp	Ripe	HPLC-DAD	Methanol	118 ^a	[49]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	113 ^b	[49]
		Skin and pulp	Green	HPLC-DAD	Methanol	601 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Ethanol	6.63–8.13 ^b	[60]
		Seed	–	HPLC-UV	Water	0.007 ^a	[59]
		Seed	–	HPLC-UV	Methanol	0.03 ^a	[59]
		Juice	Ripe	HPLC-DAD	NS	1.36 ^a	[52]
Quercitrin	5280459	Skin and pulp	Ripe	HPLC-DAD	Methanol	213 ^a	[49]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	580 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Methanol	193 ^a	[49]
Quercitrin-2"-O-gallate	NL	Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	0.8–1.0 ^b	[50]
Rutin	5280805	Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.06–0.48 ^b	[15]
		Skin and pulp	Ripe	HPLC-DAD	Methanol	1057 ^a	[49]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	1034 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Methanol	1019 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Ethanol	2.35 ^b	[60]
		Seed	–	UPLC-MS	Ethanol	3.62 ^b	[48]
		Seed	–	UPLC-MS	Methanol	7.07 ^a	[48]
		Seed	–	UPLC-MS	Acetone	8.29 ^b	[48]
		Non-phenolic	Green	UHPLC-MS/MS	Water	1.54–2.48 ^b	[54]
		By-product	Ripe	HPLC-DAD	Ethanol	1.3 ^a	[58]
		Juice	Green	UHPLC-MS/MS	Water	0.28 ^a	[54]
Flavones							
Acaatin	5280442	Seed	–	UPLC-MS	Ethanol, methanol or acetone	<1.0Q	[48]
Apigenin	5280443	Skin and pulp	Ripe or fully ripe	HPLC-MS/MS	Methanol	<1.0Q	[51]
		Seed	–	UPLC-MS	Ethanol, methanol or acetone	<1.0Q	[48]
Chrysin	5281607	Seed	–	UPLC-MS	Ethanol, methanol or acetone	<1.0Q	[48]
Luteolin	5280445	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.05 ^b	[51]
		Juice	Ripe	HPLC-DAD	NS	0.04 ^a	[52]
		Seed	–	UPLC-MS	Ethanol, methanol or acetone	<1.0Q	[48]
Malonylquercetin	5280803	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Pinobanksin	73302	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.15 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	0.32 ^b	[51]
Vincetin	5280444	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
7,8,4'-Trihydroxyflavone	688853	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Flavonones							
Hesperetin	72261	By-product	Ripe	HPLC-DAD	Ethanol	32.6 ^a	[58]

Table 1 (continued)

Flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
Hesperidin	10621	Skin and pulp	Ripe	HPLC-DAD	Ethanol	1.02–3.20 ^a	[15]
Naringenin	932	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.11 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	0.28 ^b	[51]
		Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.22–1.73 ^a	[15]
		Seed	–	UPLC-MS	Ethanol	47.88 ^b	[48]
		Seed	–	UPLC-MS	Methanol	33.73 ^b	[48]
		Seed	–	UPLC-MS	Acetone	118.82 ^b	[48]
		By-product	Ripe	HPLC-DAD	Ethanol	16.7 ^a	[50]
Naringin	442428	Skin and pulp	Ripe or fully ripe	HPLC-MS/MS	Methanol	<LOQ	[51]
2,4,7-Trihydroxy-isoflavone	13953272	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Isoflavones							
Biochanin A	5280373	Seed	–	UPLC-MS	Ethanol, methanol or acetone	<LOQ	[48]
Daksoin	5281708	Seed	–	UPLC-MS	Ethanol, methanol or acetone	<LOQ	[48]
6,7,8-Trihydroxy-isoflavone	5284649	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Formononetin	5283378	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Genistein	5289961	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Chalcones							
Naringenin chalcone	5289960	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]

NS, not found on PubChem; NS not shown; LOQ limit of quantification

^aExpressed in fresh mass basis; ^bExpressed in dry mass basis

for the identification of acerola phenolics, including UPLC-q-TOF-MS (ultra performance liquid chromatography coupled to a quadrupole-time-of-flight mass spectrometer) [54, 55]. This system consists in a powerful and reliable analytical approach to trace qualitatively and quantitatively the phenolic profile of plant extracts, outperforming the conventional HPLC method, which may present limitations in the detection of compounds in very small amounts, besides disturbance of high background noise [56]. However, these systems are still very expensive and limited to a few labs around the world, which justifies the wide use of DAD coupled to the liquid chromatography in the identification of phenolics, as a low cost, sensitive, efficient, and flexible system [57].

Identification and quantification of flavonoids in acerola

Anthocyanins

The majority of polyphenols found in acerola are anthocyanins, responsible for the reddish/purplish fruit color, similarly to other red fruit. The anthocyanin profile of acerola

includes cyanidins, delphinidins, malvidins, petalunidins, peonidins, and perunidins (Table 1). These anthocyanin subclasses are the six most widespread in nature [61]. Cyanidin is the main anthocyanin found in acerolas, corroborating with Olivas-Aguirre et al. [62] as the most widely distributed anthocyanin in red and blue fruit.

Anthocyanins are a large class of water-soluble pigments with a wide range of color diversity, present in vacuoles of different plant tissues, including the fruit [63]. Fruit color is not only the main indicator of sensory quality, but also correlated with nutritional quality [64]. Anthocyanins isolated from acerola show strong ability as free radical scavengers [65] and exhibit several health-promoting effects.

Anthocyanins extracted from fruit are promising for use as food colorant. However, they are unstable and highly susceptible for degradation, depending on conditions such as pH, light, oxygen, and temperature. In this sense, techniques can be adopted for anthocyanin stabilization. In acerola, the stability of anthocyanins seems to be severely compromised by the very high AsA content in the fruit, which condensates on carbon 4 of anthocyanins, resulting in losses of both compounds [66].

Table 2 Individual non-flavonoids (phenolic acids, stilbenes and lignans) reported in acerola fruit and by-products

Non-flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Content (mg 100 g ⁻¹)	Reference
Phenolic acids							
<i>Hydroxybenzoic acids</i>							
Ellagic acid	5281855	Skin and pulp	Ripe	HPLC-DAD	Methanol	253 ^a	[49]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	172 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Methanol	130 ^a	[49]
Gallic acid	370	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.4 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	0.38 ^b	[51]
		Skin and pulp	Ripe	HPLC-DAD	Methanol	536 ^a	[49]
		Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	1.3–2.0 ^b	[50]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	451 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Methanol	470 ^a	[49]
		Seed	–	HPLC-UV	Water	0.47 ^a	[59]
		Seed	–	HPLC-UV	Methanol	0.53 ^a	[59]
Protocatechuic acid	72	Juice	Ripe	HPLC-DAD	NS	0.00 ^a	[52]
		Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.39 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	0.31 ^b	[51]
Salicylic acid	338	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.24 ^b	[51]
Syringic acid	10742	Seed	–	HPLC-UV	Water	4.61 ^a	[59]
		Seed	–	HPLC-UV	Methanol	3.72 ^a	[59]
Vanillin	1187	Skin and pulp	Ripe	HPLC	Methanol	0.24 ^a	[53]
<i>Hydroxycinnamic acids</i>							
Caffeic acid	689043	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.73 ^b	[51]
		Skin and pulp	Ripe	HPLC-MS/MS	Methanol	0.99 ^b	[51]
		Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.07–0.29 ^b	[15]
		Skin and pulp	Ripe	HPLC-DAD	Methanol	682 ^a	[49]
		Skin and pulp	Intermediate	HPLC-DAD	Methanol	565 ^a	[49]
		Skin and pulp	Green	HPLC-DAD	Methanol	429 ^a	[49]
		Non-processed	Green	UHPLC-MS/MS	Water	1.26–3.44 ^b	[54]
		By-product	Ripe	HPLC-DAD	Ethanol	0.9 ^a	[58]
		Juice	Green	UHPLC-MS/MS	Water	0.49 ^a	[54]
		Juice	Ripe	HPLC-DAD	NS	0.05–0.10 ^a	[52]
		Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.34–0.45 ^a	[15]
Cinnamic acid	6441597	By-product	Ripe	HPLC-DAD	Ethanol	19.4 ^a	[58]
		Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.17–0.38 ^b	[15]
Chlorogenic acid	1794427	Seed	–	UPLC-MS	Methanol	0.28 ^b	[48]
		Seed	–	UPLC-MS	Acetone	4.25 ^b	[48]
		By-product	Ripe	HPLC-DAD	Ethanol	6.5 ^a	[58]
		Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
<i>o</i> -Coumaric acid	637541	Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.79 ^b	[51]
<i>p</i> -Coumaric acid	637542	Skin and pulp	Ripe	HPLC-MS/MS	Methanol	0.96 ^b	[51]
		Skin and pulp	Green	HPLC-DAD	Ethanol	28.85 ^b	[60]
		Seed	–	UPLC-MS	Ethanol	4.22 ^a	[48]
		Seed	–	UPLC-MS	Methanol	6.06 ^a	[48]
		Seed	–	UPLC-MS	Acetone	8.34 ^a	[48]
		Seed	–	HPLC-UV	Methanol	0.12 ^a	[59]
		Seed	–	HPLC-UV	Methanol	0.24 ^b	[59]

Table 2 (continued)

Non-flavonoid	PubChem CID	Fruit fraction	Fruit maturity stage	Identification method	Extraction solvent	Concentr. (mg 100 g ⁻¹)	Reference
<i>p</i> -Coumaroylquinic acid	441280	Juice	Ripe	HPLC-DAD	NS	0.33–0.84 ^a	[52]
		Skin and pulp	Intermediate	HPLC-MS/MS	Methanol	3.9–8.1 ^b	[50]
Ferulic acid	345858	Skin and pulp	Ripe	HPLC-MS/MS	Methanol	1.27 ^a	[51]
		Skin and pulp	Fully ripe	HPLC-MS/MS	Methanol	0.62 ^b	[51]
		Skin and pulp	Green	HPLC-DAD	Ethanol	0.34–1.63 ^b	[60]
		Seed	–	UPLC-MS	Ethanol	4.10 ^b	[48]
		Seed	–	UPLC-MS	Methanol	5.41 ^b	[48]
		Seed	–	UPLC-MS	Acetone	6.53 ^b	[48]
		Non-porous	Green	UHPLC-MS/MS	Water	2.96–3.44 ^b	[54]
		Juice	Green	UHPLC-MS/MS	Water	0.61 ^a	[54]
		Juice	Ripe	HPLC-DAD	NS	0.04–0.45 ^a	[52]
Isotiflorogenic acid	6436237	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
<i>n</i> -Fenylglyoxaline Stilbenes	5280527	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
<i>trans</i> -Resveratrol	445154	Skin and pulp	Ripe	HPLC-DAD	Ethanol	0.23–0.38 ^b	[15]
		Juice	Ripe	HPLC-DAD	NS	0.24 ^b	[52]
		By-product	Ripe	HPLC-DAD	Ethanol	8.1 ^b	[58]
<i>cis</i> -Resveratrol	1548910	By-product	Ripe	HPLC-DAD	Ethanol	2.8 ^b	[58]
Chastin	323	Skin and pulp	Green	HPLC-DAD	Ethanol	1.69–4.08 ^b	[60]
Lignans							
Lariciresinol	332427	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Sesamol	94672	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]
Secoisolariciresinol monoglucoside	101138945	Skin and pulp	Green and ripe	UPLC-MS/MS	Methanol	NS	[55]

NS, not listed on PubChem; NS, not shown; LOD, limit of quantification

^aExpressed in fresh mass basis; ^bExpressed in dry mass basis

The use of inorganic host materials as additives to increase stability of acerola anthocyanins was suggested by Ribeiro et al. [67]. These authors demonstrated that the incorporation of montmorillonite (Mnt) clay into clarified acerola juice at 4% (dry basis) was enough for intercalation of major anthocyanins into Mnt interlayers, resulting in variations from pale red to darker and redder shades after 60 days, and thus preserving the red color of the juice.

Innovative and advanced approaches for stabilization of anthocyanin have been proposed, including co-pigmentation, acylation with various organic acids, complexation metal ions, and microencapsulation, but their application in acerola is still incipient.

Flavan-3-ols: monomeric catechins and proanthocyanidins

(+)-Catechin and (–)-epicatechin were the major flavan-3-ols previously reported in acerola, besides the isomers (–)-epicatechin gallate and (–)-epigallocatechin gallate were also observed in fruit and by-product (Table 1). Catechin content in acerola is comparable to that found in other fruit such as grape [68], apple [29, 69], pear [70], and cherry [71, 72], known as some of major sources of catechin in the world.

Catechins are colorless and astringent, responsible for the fruit bitter taste. Their extraction in different plant tissues is a hard task, due to polymerization with other molecules and high susceptibility to degradation by oxygen, high temperature, light and alkaline conditions. In this way, encapsulation and protected coating are strategies to stabilize catechin molecules [73].

Several beneficial properties has been attributed to catechin and derivatives, including anticancer, anti-obesity, antidiabetic, antidiarrhoeal, anti-infectious, hepatoprotective, and neuroprotective effects [74]. Furthermore, the antioxidant activity of catechins occurs through the scavenging of free radicals, chelation of redox active transition-metal ions, inhibition of redox active transcription factors, inhibition of pro-oxidant enzymes, and induction of antioxidant enzymes [75]. Studies with catechin from apple after ingestion have shown that this phenolic compound remains stable during gastric digestion, besides being rapidly absorbed from the small intestine [76].

Concerning the presence of proanthocyanidins (or condensed tannins) in acerola fruit and by-products, three were previously reported, namely procyanidin A2, procyanidin B1 and procyanidin B2 (Table 1). These compounds, together, accounted for 1.7–6.0% of total phenolic compounds analyzed by RP-HPLC/DAD in ripe acerolas of different cultivars [15].

Proanthocyanidins are synthesized by the polymerization or oligomerization of flavan-3-ols units (catechins and/or epicatechins). Proanthocyanidins are formed in the flavonoid pathway, sharing the same upstream pathway with anthocyanins, including the function of the anthocyanidin synthase as key role in the biosynthesis of both [77].

Proanthocyanidins are mainly produced in fruit at earlier maturity stages and are responsible for their bitterness [78]. They are synthesized as end products of the flavonoid pathway and are involved in the browning of plant tissues, including fruit, as substrates of polyphenol oxidases, causing higher browning than other phenolics [79, 80].

Flavonols

Flavonols were the phenolic class with the highest diversity of compounds in acerola, with 17 flavonols previously found, including the aglycones kaempferol, myricetin, quercetin, quercitrin, isoquercitrin, isorhamnetin, and rutin, as well as some of their glycosylated forms (Table 1). Glucose was the most frequent sugar type linked to flavonols in acerola, but other mono- and di-saccharides were also found, such as galactose, arabinofuranose, ribinobiose, neohesperidose, and rhamnose. Other compounds attached to flavonols in acerola were glucosiduronic acid (in myricetin-3-O-glucuronide) and gallic acid (in quercitrin-2''-O-gallate).

Flavonols is usually the main class of phenolic compounds in acerolas at green maturity stage, when the anthocyanins are absent [60]. In that case, kaempferol, isorhamnetin, and quercetin have been reported as the main flavonols in unripe green fruit [49, 51, 60].

Flavonols are flavonoids with a ketone group and a hydroxyl at 3-position on the C ring, where glycosylation

generally occur [31]. Quercetin and kaempferol are the major classes of flavonols, differing from each other by one extra OH group at the C3 of the B ring in quercetin (Fig. 3) [81]. Both were the flavonols reported with the greatest variety of glycosylations in acerola, with five distinct forms besides aglycone for quercetin, considering isoquercitrin, quercitrin, and rutin are glycoside derivatives of quercetin, and four distinct glycosylated forms for kaempferol (Table 1). At least 347 and 279 different glycosidic combinations are possible for kaempferol and quercetin, respectively [82].

Flavonol glycosides and aglycones are involved in several functions in plants, including UV photoprotection, reproduction, and internal regulation of cell physiology, besides acting as a free radical scavenger and contributor to plant immune system [83].

Flavones and flavanones

Eight flavones (acacetin, apigenin, chrysin, luteolin, malonylapiin, pinobanksin, vitexin and 7,8,4'-trihydroxyflavone) and five flavanones (hesperetin, hesperidin, naringenin, naringin and 2,4,7-trihydroxyisoflavone) were found in acerolas (Table 1). These classes have less participation in the phenolic composition of acerolas than other flavonoid classes [15, 51].

Flavones and flavanones are generally colorless or pale yellow. Flavones are structurally similar to flavonols, with a double bond between positions 2 and 3 and oxidized at position 4 of the C ring, differing only in the absence of hydroxyl group at C3 in flavones. The flavanones, in turn, have a similar structure to the flavones, but with the absence of double bond between C2 and C3 (Fig. 3) [27, 80, 84].

Naringenin was the major flavanone reported in acerola (Table 1). Naringenin is the first product resulted from the synthesis of flavonoids, from which many other flavonoids are formed [78].

Isoflavones and chalcones

The literature search indicated that, so far, at least five isoflavones and one chalcone have been identified in acerola fruit, but without quantification of these phenolic compounds. The isoflavones biochanin A and daidzein were reported in seed of acerola agro-industrial residues [48], while formononetin, genistein, 6,7,8'-trihydroxyisoflavone and the chalcone 2',4,6'-trihydroxychalcone (naringenin chalcone) were found in skin and pulp of green and red-ripe acerolas [55].

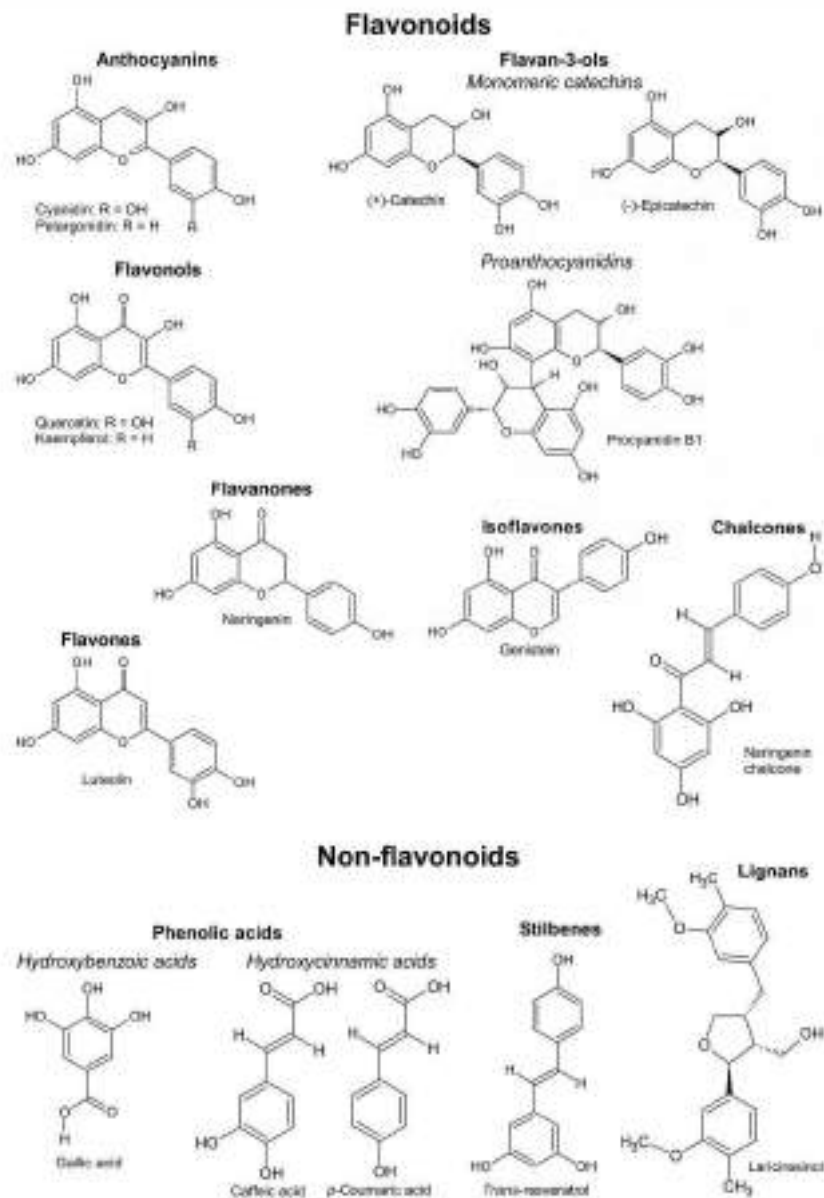


Fig. 3 Chemical structures of representative phenolic compounds in acerola fruit and by-products

Identification and quantification of non-flavonoids in acerola

Phenolic acids

Phenolic acids are classified in two major substituted acid derivatives, hydroxybenzoic and hydroxycinnamic acids. The main differences between both classes of phenolic acids are related to the methoxylation and hydroxylation positions of their aromatic rings and the number of carbons: C6–C1 basic skeleton for hydroxybenzoic acids, and C6–C3 basic skeleton for hydroxycinnamic acids [71].

A total of six hydroxybenzoic (ellagic, gallic, protocatechuic, salicylic and syringic acids, and vanillin) and nine hydroxycinnamic acids (caffeic, cinnamic, chlorogenic, *o*-coumaric, *p*-coumaric, *p*-coumaroylquinic, ferulic and isochlorogenic acids, and *o*-feruloylglycine) were found in studies with acerola fruit and by-products (Table 2).

Stilbenes and lignans

Stilbenes described in acerola fruit and by-products include resveratrol and coumarins (Table 2). Resveratrol was reported for the first time in acerola in its *trans* form by Nowak et al. [52], in cold-pressed juices prepared from whole fruit without addition of water and sugar, in a level of $0.34 \text{ mg } 100 \text{ mL}^{-1}$. Afterwards, *trans*-resveratrol was found in the skin and pulp of ripe fruit ($0.23\text{--}0.38 \text{ mg } 100 \text{ g}^{-1} \text{ fm}$) [15] and in by-products of fruit pulp processing ($8.1 \text{ mg } 100 \text{ g}^{-1} \text{ fm}$) [58], where *cis* form of resveratrol was also observed ($2.5 \text{ mg } 100 \text{ g}^{-1} \text{ fm}$).

Stilbenes are characterized by two aromatic rings joined by an ethylene bridge. Resveratrol has one of the simplest structures among the stilbenes, and present two forms: *trans* form is not sterically hindered, while *cis* form is sterically hindered and therefore less stable [68, 80]. Resveratrol is an antimicrobial and antifungal phytoalexin produced in the plants as a response to injury or infection [26]. It is the most studied stilbene and one of the most popular phenolic compounds, especially for its high concentration in wines, whose moderated and continuous ingestion plays a key role on the prevention of chronic heart diseases [57].

Even if the skin of red grapes (and consequently red wines) are the most known sources of resveratrol in human diet, its presence was also noted in several red fruits other than acerola, such as açai ($0.38 \text{ mg } 100 \text{ g}^{-1} \text{ fm}$), blackberry ($6.48 \text{ mg } 100 \text{ g}^{-1} \text{ fm}$), blueberry ($1.58 \text{ mg } 100 \text{ g}^{-1} \text{ fm}$) and raspberry ($1.12\text{--}3.85 \text{ mg } 100 \text{ g}^{-1} \text{ fm}$) [4].

A total of three lignans (lariciresinol, sesaminol and secoisolariciresinol monoglucoside) were identified in the

skin and pulp of both green unripe and red ripe acerolas, but without quantification [55]. Lignans are less common and less studied in fruit and vegetables when compared to flavonoids, but are involved in important pathways of cell signaling, which arouses the interest in future investigations about its usefulness [26, 27].

Bioavailability and bioaccessibility of phenolic compounds in acerola

In recent years, the importance of research related to the bioavailability and bioaccessibility of phenolic compounds was raised, once the biological activities and health-promoting properties of these compounds are not only based on the absolute quantity of intake, but also their behavior in the gastrointestinal system [76]. The ambiguity in the concepts and the undifferentiated use require clear definitions of both terms [85]. Bioavailability is defined as the fraction of a phenolic compound (or any phytochemical or nutrient) that is absorbed, i. e. that reaches the bloodstream, and is available for use in metabolic processes. Bioaccessibility refers to the amount of phenolic compounds released from the food matrix during digestion, becoming absorbable (available for intestinal absorption) [86]. Bioavailability is part of the bioaccessibility, thus the bioaccessible fraction is always equal to or higher than the bioavailable fraction [85].

Studies on bioavailability and bioaccessibility of phenolic compounds of acerola are scarce. For the best of our knowledge, there are no reports in the literature related to the assessment on phenolic content of acerola fruit after gastrointestinal digestion. Bioaccessibility in acerola was previously reported only in frozen pulp, which was compared with other Brazilian fruit pulps. Stafusa et al. [87] found that acerola pulp exhibited remarkably high content of phenolic compounds ($13,890.90 \text{ mg GAE } 100 \text{ g}^{-1} \text{ dm}$) and high antioxidant activity by DPPH ($727.86 \text{ } \mu\text{M Trolox g}^{-1} \text{ dm}$) and DPPH ($958.20 \text{ } \mu\text{M Trolox g}^{-1} \text{ dm}$) methods, outperforming other phenolic-rich fruit such as açai and jaboticaba. Furthermore, a considerable reduction of phenolic content and antioxidant activity occurred for frozen pulp of all species after *in vitro* digestion in gastric and intestinal stages; bioaccessibility of phenolic compounds in acerola after both phases was 13.74%, while 8.35% of antioxidant activity was bioaccessible after gastrointestinal digestion [87].

Assis et al. [88] assessed seven flavonoids, three phenolic acids and two stilbenes in acerola purees after oral, gastric, and intestinal digestion and found great variations; *cis*-resveratrol (6.79%) and chlorogenic acid (7.18%) presented the lowest bioaccessibility, while rutin (52.24%) and catechin (52.19%) were the most bioaccessible. Additionally, fermentation with probiotic cultures (*Lactobacillus* spp.) increased

the bioaccessibility of all phenolic compounds, except cis-resveratrol and kaempferol 3-glucoside.

In acerola juices prepared with frozen pulps diluted in water (1:3, w/v), Fonteles et al. [89] reported a bioaccessibility of 13.3% for phenolic compounds, which was increased with high-temperature short time (22.8%) or ultra-high temperature (22.3%) processing. Acerola by-products (skins and seeds) extracted with water were assessed for phenolic compounds, and presented a bioaccessible fraction of 10.2% [90]. Additionally, the authors found a reduction in bioaccessibility to 4.8% when samples were ultrasonically extracted.

Factors affecting phenolic composition of acerola

Genotype/cultivar

It is very clear that the genotype of acerola plant greatly influences the fruit postharvest quality and chemical composition [16–18, 91]. Likewise, the phenolic composition of acerola fruit is also affected by the choice of cultivar. In the last decade, first studies were accomplished by research groups in different regions of Brazil to investigate genetic influence on the phenolic compounds in edible portion of acerola fruit by the classical Folin-Ciocalteu method [92–94].

'Flor Branca', one of the major acerola cultivars produced in the Brazilian Northeast Region due to high production regularity, showed less phenolic content when compared to other acerola varieties [92, 94]. The cultivar 'Okinawa' was compared to other varieties in at least three studies, and has been noted for its high content of AsA, but not of phenolic compounds [92, 93, 95].

Studies by Mariano-Nasser et al. [93] and Ferreira et al. [15] assessed the same four acerola cultivars (Apodi, Cereja, Roxinha and Protacot) and conducted similar results; 'Cereja' acerola stood out in terms of phenolic compounds, in addition to its high AsA content, and is a great choice for processing and production of frozen pulp, once its high acidity and low sugar content is not recommended in fruit for fresh consumption.

Recently, the use of HPLC revealed both quantitative and qualitative variations on acerola phenolic composition, depending on the genotype. In seven Brazilian acerola varieties, the sum of phenolic compounds quantified individually ranged between 237.02 mg kg⁻¹ fw (cv. Jabura) and 845.89 mg kg⁻¹ fw (cv. Roxinha), which represents a difference of almost four times between the varieties with the highest and lowest phenolic concentration [15]. The study also revealed that variation in types of phenolic compounds is genotype-dependent, like the 16 phenolic compounds found in 'Apodi' acerolas, in contrast to 31 found in 'BRS Cabota'. Furthermore, only five of the 17 studied phenolic compounds were reported in all genotypes.

In the same way, the anthocyanin content is also influenced by the genotype, which reflects in diversity of skin color in acerola cultivars, from dark orange/light-red to dark-purple fruit. Cyanidin content in 'Flor Branca' ripe acerolas was 520.76 mg 100 g⁻¹ dm, 262% higher than that found in 'Jabura' in the same study [94]. Besides that, pelargonidin content was 97.04 mg 100 g⁻¹ dm in 'Flor Branca', but this anthocyanin was absent in 'Jabura' acerolas.

Fruit maturity stage

Throughout acerola maturity stages, several physical, biochemical, and physiological changes are accomplished in the fruit, visually represented by color shift from light green to final reddish hues, which serve as an indicator of harvest time. Thus, a large increase is observed in the anthocyanin content during acerola ripening, in the same way as observed in most red fruit crops [96]. In acerolas harvested at four maturity stages, cyanidin content was 0.49–1.15 mg 100 g⁻¹ dm in small-green fruit, increasing in big-green (9.47–21.63 mg 100 g⁻¹ dm) and intermediate (44.87–111.84 mg 100 g⁻¹ dm) stages and reaching maximum levels in red-ripe fruit (143.65–520.76 mg 100 g⁻¹ dm) [94].

Nascimento et al. [49] assessed phenolic acids and flavonols in green, intermediate and red acerolas by HPLC-DAD, and reported rutin as major phenolic compound in green (101.8 mg 100 g⁻¹ fw) and ripe (16.57 mg 100 g⁻¹ fw) fruit, in contrast to quercetin (11.27 mg 100 g⁻¹ fw) as the main polyphenol in fruit at intermediate stage.

Most of anthocyanins and flavones, as well as chlorogenic and isochlorogenic acids, one flavanone, one lignan, and one chalcone predominantly accumulated in the red acerola fruit rather than in green unripe ones [55]. Meanwhile, ferulic acid, *o*-coumaric and feruloylglycine were found in higher amounts in unripe fruit than in ripe ones.

Betta et al. [51] determined phenolic composition of acerolas in two red stages, named ripe and fully ripe. Most of the phenolic acids, flavan-3-ols, flavonols, and flavanones assessed individually showed highest concentrations in the ripe stage rather than in fully ripe. A decrease in flavanol content throughout fruit ripening was also related in blueberries, strawberries and cranberries (*Vaccinium macrocarpon* Ait.) [97]. This behavior may be a consequence of the use of phenolics from other classes for biosynthesis of anthocyanins in late ripening stages, like the conversion of flav-2-en-3-ol 3-O-glucoside (a flavanol glycoside) to cyanidin 3-rutinoside as a probable phenomenon in the seed-coat of black soybean (*Phaseolus vulgaris* L.), a cyanidin-rich crop [98].

Extraction methods

Extraction is the first procedure to obtain bioactive compounds from fruit or other parts of plants. Different approaches have been applied to separate phenolic compounds from acerola fruit and by-products [99]. Considering the diversity on structure of phenolic compounds, the extraction method influences directly on the recovery rate of phenolic compounds from fruit samples [5, 100]. The use of different organic solvents such as acetone, ethanol, methanol, and their aqueous solutions affect the extraction efficiency, based on the solubility, polarity, and affinity to the solute. Furthermore, the application of physical forces and thermal treatment may optimize the phenolic extraction [4].

Conventional extraction methods such as maceration and Soxhlet methods have been applied in the extraction of phenolic compounds in fruit crops, for their low cost, high accessibility in most laboratories and satisfactory recovery. However, the high volume of solvents and manual procedures required in conventional methods, as well as the concernment about solvent toxicity and environmental pollution, led to the development of emergent extraction approaches [101, 102].

Emergent unconventional extraction techniques reported in acerola include subcritical water extraction (SWE), enzyme-assisted extraction (EAE) and ultrasound-assisted extraction (UAE), as described below.

SWE is an eco-friendly technique that uses only water in a liquid state at a temperature between 100 and 374 °C, under high pressure. In acerola by-product (seeds and pomace), SWE had a higher extraction efficiency and AOX in fresh and dry samples when compared to Soxhlet method. Additionally, an increase in temperature of SWE from 70 to 130 °C improved the extraction yield, phenolic content, and antioxidant activity [99].

EAE involves the use of cell wall degrading enzymes to weaken cell wall and ensure the extraction of cellular content. In acerola residues, the combination of skin and EAE method using the protease/peptidase enzyme showed the best result of total phenolics when compared to conventional method and seed [103].

UAE method applies ultrasound energy by implosion of cavitation bubbles, resulting in damages to cell wall with increased contact between phenolic compounds and solvents. This process reduces the processing time, energy and solvent consumption [100]. Carvalho Gualberto et al. [48] assessed the combination of extraction methods (UAE and conventional shaking) and solvents (ethanol, methanol and acetone), and found that UAE and acetone were the best conditions for extraction of phenolic compounds in acerola residues. In acerola wastes evaluated by Silva et al. [104], the UAE yielded better results than those obtained with the conventional solid-liquid extraction.

Biological activities and health benefits of phenolic compounds in acerola fruit and by-products

The first study on biological activity of acerola was published in 2005 by Hanamura et al. [105]. These authors isolated three phenolic compounds (cyanidin 3-rhamnoside, pelargonidin 3-rhamnoside and quercetin-3-rhamnoside) from acerola fruit and reported effects of these compounds on *in vitro* O₂^{•−} scavenging activity, as well as an inhibitory effect on diabetes-related molecules (α -glucosidase and advanced glycation end products).

Since then, several health-promoting properties have been attributed to phenolic compounds extracted from acerola. Most of the works published about phenolic compounds in acerola in the 2000s and early 2010s were focused on quantification of antioxidant activity in fruit, juice and frozen pulp by the widely used methods ABTS and DPPH [106–108]. In the last ten years, residues generated as by-products from acerola processing industry, including seeds and residual skin and pulp, began to be investigated as a source of antioxidants for food and nutraceutical applications [109, 110].

Antioxidant activity

In vitro antioxidant activity is the most evaluated biological property attributed to phenolics of acerola fruit and by-products, due to remarkable advances in analytical methods and tools for its quantification during the last few decades. It is known that acerola is rich in various bioactive compounds with antioxidant properties, although phenolic compounds and AsA have the strongest antioxidant capacity [108].

Antioxidants acts in different cellular processes, including direct scavenging of ROS, activation of antioxidant enzymes, metal-chelating activity, increase in levels of α -tocopherol and uric acid, inhibition of NADPH oxidases, mitigation of oxidative stress by NO, and increase in the antioxidant properties of low-molecular-weight antioxidants [32].

Antioxidant potential is variable between different phenolic compounds, depending on substitutions in aromatic rings and arrangements in molecules related to hydrogenation, hydroxylation, methylation, malonylation, sulfation, and glycosylation [33]. The presence of two hydroxyl groups in the ring tends to improve the antioxidant potential of a phenolic compound, since hydrogens and electrons are donated to stabilize free-radicals. C2–C3 double bonds associated with 4-carbonyl groups also increase antioxidant activity by providing planarity, electron expansion,

and displacement between adjacent rings. Conversely, the presence of 3-OH in a phenolic compound tends to suppress its antioxidant action. Furthermore, *O*-glycosylation seems to reduce the free radical scavenger property of a flavonoid when compared to its corresponding aglycone [30, 111].

In vitro antioxidant activity of natural extracts can be determined by a range of assays with different mechanisms of action, whose results may vary between them. The lack of consensus on the most convenient and applicable method reflects the need for multiple assays to consistently determine the antioxidant capacity of a plant material. In acerola fruit and by-products, most used methods are based on the capacity of plant extract to neutralize the organic free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Table 3). These colorimetric assays are widely disseminated in scientific research worldwide due to their low cost, easy operation and good reproducibility when compared to other methods, despite depending on a spectrophotometer [112].

A very high antioxidant activity was observed in green acerola fruit (skin and pulp) assessed by the DPPH assay, in both ethanolic (1,910.87–2,154.93 $\mu\text{M Trolox g}^{-1}$ dm) [60] and methanolic (95.0–251.0 mM Trolox kg^{-1} fm) [113] extracts. Advancement in ripening results in a small reduction in antioxidant activity of acerola, as observed in ripe (1120.4 mg Trolox g^{-1} dm) and fully ripe (963.3 mg Trolox g^{-1} dm) fruit [51]. Poletto et al. [22] evaluated the composition of by-products generated in the acerola processing of acerola and reported the following DPPH free radical scavenging activity (IC_{50}): 38.17 $\mu\text{g mL}^{-1}$ for bagasse (seed and peel), 6.87 $\mu\text{g mL}^{-1}$ for non-pomace (from the juice clarification step), and 4.24 $\mu\text{g mL}^{-1}$ for juice powder. Additionally, DPPH assay was also adopted for measurement of antioxidant activity of acerola juice prepared with green (67.4 $\mu\text{M Trolox mL}^{-1}$) [54] and ripe (5149.0 mg Trolox L^{-1}) [52] fruit (Table 3).

Results of antioxidant activity in fruit skin and pulp using ABTS assay corroborated with the DPPH method, reflecting the reduction on antioxidant activity during acerola ripening, from 3,628–14,004 $\mu\text{M Trolox 100 g}^{-1}$ fm in green fruit, to 4,104–4,617 and 3,293–4,236 $\mu\text{M Trolox 100 g}^{-1}$ fm in intermediate and ripe stages, respectively [94]. In agro-industrial residues of acerola composed of seeds, the antioxidant activity showed oscillations depending on the solvent and extraction method, ranging from 5,556.22 $\mu\text{M Trolox 100 g}^{-1}$ dm (using methanol and ultrasonic bath) and 25,493.45 $\mu\text{M Trolox 100 g}^{-1}$ dm (using acetone and shaker) [48]. In addition, the antioxidant activity of a water extract of acerola juice prepared with ripe fruit was also reported in the literature (21.4 mM Trolox) [52] (Table 3).

The Ferric Reducing Antioxidant Power (FRAP) is other colorimetric method that has also been considered

for determining the antioxidant capacity of acerola [15, 51, 53, 60]. FRAP is a single electron transfer method based on the measurement of the reduction in the colorless complex of ferric iron and tripyridyltriazine (TPTZ) (Fe^{3+} -TPTZ) to the intensely blue ferrous complex (Fe^{2+} -TPTZ) by the plant extract containing antioxidants [114].

Ethanolic and methanolic extracts of ripe acerola evaluated by the FRAP assay presented antioxidant activity of 29.3–53.5 $\text{mM Fe}^{2+} 100 \text{ g}^{-1}$ fm [15] and 2.2 $\text{mM Fe}^{2+} 100 \text{ g}^{-1}$ fm [53], respectively. Antioxidant capacity of acerola seeds showed a variation between 17,105.57 and 86,045.58 $\mu\text{M ferrous sulfate 100 g}^{-1}$ dm by the FRAP assay, depending on the extraction method and solvent [48] (Table 3).

The Oxygen Radical Absorbance Capacity (ORAC) assay was also adopted in previous reports for determination of antioxidant capacity in acerola [22, 60, 113]. ORAC is based on the ability of the radical chain reaction by antioxidants through monitoring the inhibition of peroxyl radical-induced oxidation [114]. ORAC method showed a slight reduction in antioxidant activity of fruit (skin and pulp) from green (43.5–79.0 $\mu\text{M Trolox kg}^{-1}$ fm) to intermediate (36.5–62.0 $\mu\text{M Trolox kg}^{-1}$ fm) and ripe (36.2–53.0 $\mu\text{M Trolox kg}^{-1}$ fm) stages. In addition, Poletto et al. [22] used ORAC assay for determining antioxidant capacity (IC_{50}) of by-products generated in the acerola processing, and reported 2.13 $\mu\text{g mL}^{-1}$ for non-pomace from the juice clarification step, 7.86 $\mu\text{g mL}^{-1}$ for bagasse, and 2.44 $\mu\text{g mL}^{-1}$ for juice powder (Table 3).

Antibacterial activity

Acerola bagasse flour was tested against the microorganisms *Listeria monocytogenes* ATCC 19117, *Escherichia coli* ATCC 11229, *Providencia aeruginosa* ATCC 15442, and *Salmonella choleraesuis* ATCC 6539 [115].

Staphylococcus aureus was inhibited by contact with acerola puree extract (minimum inhibitory concentration (MIC) = 6.25 mg mL^{-1}), which was attributed to the high content of anthocyanins, especially pelargonidin, and rhamnosides of cyanidin and pelargonidin [87]. Phenolic compounds extracted from different portions of acerola fruit were tested against *S. aureus* by Delva and Goodrich-Schneider [113]. The antimicrobial activity of flavonoids from the edible fraction (skin and pulp) was clear in green fruit and moderate in red fruit, while the non-edible fraction (seeds) had a clear activity against *S. aureus*. Additionally, phenolic acids from seeds also showed moderate antimicrobial activity.

In several plant species, the antimicrobial activity has been attributed to their secondary metabolites, including phenolic compounds and alkaloids [116].

Table 1 Antioxidant activity measured by different assays (DPPH, ABTS, FRAP and ORAC) in avocado fruit and by-products

Method	Contents	Fruit fraction	Maturity stage	Extraction solvent	References
DPPH	1,010.83–2,154.93 μM Trolox g^{-1} dm	Skin and pulp	Green	Ethanol	[60]
	38.33 μg fm g^{-1} DPPH (IC_{50})	Skin and pulp	Green	Methanol	[49]
	95.0–251.0 mM Trolox kg^{-1} fm	Skin and pulp	Green	Methanol	[113]
	54.7–162.0 mM Trolox kg^{-1} dm	Skin and pulp	Intermediate	Methanol	[113]
	39.57 μg fm g^{-1} DPPH (IC_{50})	Skin and pulp	Intermediate	Methanol	[49]
	1,120.4 mg Trolox g^{-1} dm	Skin and pulp	Ripe	Methanol/acetone	[51]
	138.1–200.0 mM Trolox kg^{-1} fm	Skin and pulp	Ripe	Ethanol	[15]
	3,276.83–7,738.05 g fm g^{-1} DPPH (IC_{50})	Skin and pulp	Ripe	Methanol/acetone	[95]
	40.4–101.0 mM Trolox kg^{-1} fm	Skin and pulp	Ripe	Methanol	[113]
	46.20 μg fm g^{-1} DPPH (IC_{50})	Skin and pulp	Ripe	Methanol	[49]
	40.0 μg fm g^{-1} DPPH (IC_{50})	Skin and pulp	Ripe	Methanol	[53]
	963.3 mg Trolox g^{-1} dm	Skin and pulp	Fully ripe	Methanol/acetone	[51]
	2,226.24–2,305.27 μM Trolox 100 g^{-1} dm	Seed	–	Ethanol	[48]
	2,221.39–2,305.85 μM Trolox 100 g^{-1} dm	Seed	–	Methanol	[48]
	2,214.16–2,242.86 μM Trolox 100 g^{-1} dm	Seed	–	Acetone	[48]
	67.4 μM Trolox mL^{-1}	Juice	Green	Water	[54]
	5149.0 mg Trolox L^{-1}	Juice	Ripe	Not mentioned	[52]
	88–208 μM Trolox g^{-1}	Non-poncax	Green	Water	[54]
	6.87 μg mL^{-1} (IC_{50})	Non-poncax (pressure)	Green	Ethanol	[22]
	38.17 μg mL^{-1} (IC_{50})	Bagasse (seed and peel)	Green	Ethanol	[22]
	4.29 μg mL^{-1} (IC_{50})	Juice powder	Green	Ethanol	[22]
ABTS	7,475.86–8,613.54 μM Trolox g^{-1} dm	Skin and pulp	Green	Ethanol	[60]
	3,628–14,004 μM Trolox 100 g^{-1} fm	Skin and pulp	Green	Methanol/acetone	[94]
	4,104–4,617 μM Trolox 100 g^{-1} fm	Skin and pulp	Intermediate	Methanol/acetone	[94]
	3,293–4,236 μM Trolox 100 g^{-1} fm	Skin and pulp	Ripe	Methanol/acetone	[94]
	2.72–5.84 μM Trolox g^{-1} fm	Skin and pulp	Ripe	Methanol/acetone	[95]
	135.7–208.3 mM Trolox kg^{-1} fm	Skin and pulp	Ripe	Ethanol	[15]
	5,256.22–12,035.18 μM Trolox 100 g^{-1} dm	Seed	–	Ethanol	[48]
	7,943.29–16,752.68 μM Trolox 100 g^{-1} dm	Seed	–	Methanol	[48]
	14,404.44–25,483.45 μM Trolox 100 g^{-1} dm	Seed	–	Acetone	[48]
	21.4 mM Trolox	Juice	Ripe	Not mentioned	[52]
FRAP	204–380 μM Trolox g^{-1}	Non-poncax	Green	Water	[54]
	824.23–1,447.97 μM Trolox g^{-1} dm	Skin and pulp	Green	Ethanol	[60]
	28.5–85.3 mM Fe^{2+} 100 g^{-1} fm	Skin and pulp	Ripe	Ethanol	[19]
	506.8 μM Trolox g^{-1} dm	Skin and pulp	Ripe	Methanol/acetone	[51]
	2.2 mM Fe^{2+} 100 g^{-1} fm	Skin and pulp	Ripe	Methanol	[53]
	338.3 μM Trolox g^{-1} dm	Skin and pulp	Fully ripe	Methanol/acetone	[51]
	33,789.66–86,045.58 μM FS 100 g^{-1} dm	Seed	–	Ethanol	[48]
	17,105.57–66,941.90 μM FS 100 g^{-1} dm	Seed	–	Methanol	[48]
ORAC	33,480.00–57,171.28 μM FS 100 g^{-1} dm	Seed	–	Acetone	[48]
	1,090.10–2,454.42 μM Trolox g^{-1} dm	Skin and pulp	Green	Ethanol	[60]
	43.5–79.0 μM Trolox kg^{-1} fm	Skin and pulp	Green	Methanol	[113]
	36.5–62.0 μM Trolox kg^{-1} fm	Skin and pulp	Intermediate	Methanol	[113]
	36.2–55.0 μM Trolox kg^{-1} fm	Skin and pulp	Ripe	Methanol	[113]
	2.13 μg mL^{-1} (IC_{50})	Non-poncax (pressure)	Green	Ethanol	[22]
	7.86 μg mL^{-1} (IC_{50})	Bagasse (seed and peel)	Green	Ethanol	[22]
	2.44 μg mL^{-1} (IC_{50})	Juice powder	Green	Ethanol	[22]

FS: fat-free saline

Antihyperglycemic, antihyperlipidemic, anti-inflammatory and hepatoprotective activities

A methanolic extract of bagasse flour from acerola agro-industrial residue containing phenolic compounds (gallic acid, catechin, epicatechin gallate, epicatechin, syringic acid, *p*-cumaric acid and quercetin) was able to inhibit *in vitro* digestive enzymes α -amylase and α -glucosidase, and can help in the treatment of obesity, associated comorbidities and type 2 diabetes [117].

In a study by Dias et al. [118], the effect of acerola juice intake was investigated in mice with cafeteria diet-induced obesity. The results showed that acerola juice prevents weight gain (based on body weight and adiposity) and dyslipidemia (based on triglycerides), and restores inflammation to a normal range, which was attributed by the authors to the presence of phenolic compounds in the juice.

Phenolic-rich lyophilized extract of acerola bagasse was tested against the toxic action of carbon tetrachloride (CCl_4) in Wistar rats by Marques et al. [59]. According to the results, there was a decrease in the activity of aspartate aminotransferase, alanine aminotransferase and gamma glutamyl transferase, and an increase in superoxide dismutase, total antioxidant capacity and albumin content in relation to control treatment, confirming the hepatoprotective action of acerola extract.

Applications of phenolic compounds from acerola in food and pharmaceutical industries

In tropical regions such as Brazil, several native and exotic unknown or underexploited fruit species present high potential for use in the agroindustry, including acerola [41]. Acerola is a fruit with intense metabolism, characterized by very high respiration rate and ethylene-mediated molecular, biochemical and physiological changes, resulting in a high perishability and fragility after harvest [119]. Thus, the fruit processing is key to reduce acerola postharvest losses.

Fresh consumption of acerola is actually restricted to some varieties selected for high fruit sweetness and low acidity, as well as to regions close to the production area. In order to overcome limitations due to short fruit postharvest life, acerolas have been processed in several products (Fig. 4), including frozen pulp, juice, marmalade, frozen concentrate, jam, and liquor [20].

The production of frozen fruit puree is the main destination for ripe acerolas (Fig. 4). Previous studies confirmed the high amounts of phenolic compounds in acerola frozen purees, which presented the highest antioxidant activity ($\text{DPPH} = 7433.37 \mu\text{mol Trolox}/100 \text{ g fm}$ and $\text{ABTS} = 8511.84 \mu\text{mol Trolox}/100 \text{ g fm}$) among the 44 fruit pulps evaluated [42]. The processing of frozen fruit is promising in acerola industries, which enables its global distribution to consumers interested in phenolic-rich products. In recent years, the consumption of acerola frozen pulp acerola has increased in Japan, United States, and Europe [20].

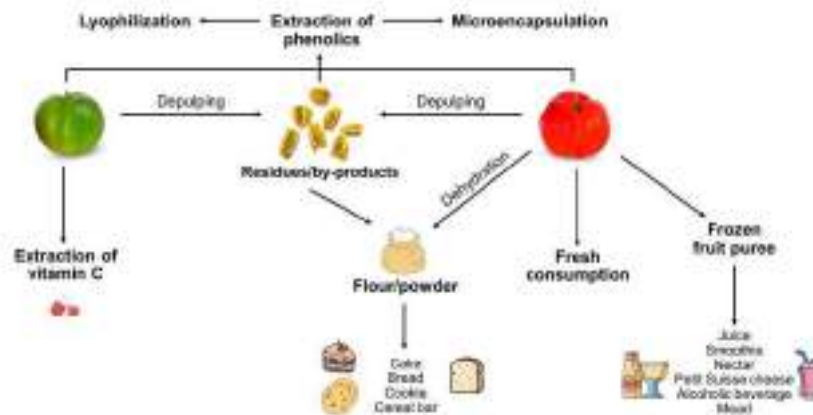


Fig. 4 Illustrative representation of potential technological processing of acerola fruit and its residues

In addition to being an alternative to reduce fruit post-harvest losses, the acerola frozen purees are versatile for the preparation of phenolic-rich products, including juices [52], smoothies [120], nectars [121], Petit Suisse cheese [122], alcoholic beverages [123], and meads [124]. Acerola is widely consumed worldwide as juice, prepared with acerola fruit [54] or in blends with other tropical fruit [125, 126]. A high diversity of phenolic compounds has been reported in acerola juices, including phenolic acids, flavan-3-ols, flavonols, and flavones, as well as resveratrol (stilbene) [52].

In recent years, some approaches have been proposed to stabilize phenolic compounds and other antioxidant compounds such as ascorbic acid and carotenoids in acerola pulps and juices, which are highly susceptible to degradation when exposed to light, moisture, heat, and oxygen. Microencapsulation is a technique that has shown positive effects in conservation phenolic compounds in acerola, using maltodextrin and gum Arabic as encapsulating agents, and dried by spray and freeze-drying [126, 127]. This technology successfully preserved bioactive compounds and antioxidant activity in acerola pulp for a period of at least 180 days [128], which demonstrates its high potential for application in functional and nutraceutical foods.

The industrial extraction of vitamin C from green unripe fruit represents an important portion of the global acerola market. With contents that can exceed 4,000 mg/100 g, green acerolas have high potential for the production of extracts, supplements, and concentrated powders of vitamin C for use in food and pharmaceutical purposes. In addition to the high ascorbic acid content, green acerolas contain several phenolic compounds, such as flavonols, flavan-3-ols, iso-flavones, phenolic acids, stilbenes, and lignans [49, 55, 60]. Together, these bioactive compounds make acerola an excellent alternative for dietary supplementation and development of new functional foods. For this, processes such as microencapsulation and lyophilization can be applied to extracts of unripe acerola fruit, in order to increase the stability of these compounds, which are suitable as an antioxidant ingredient in food emulsions [8].

Acerola-based products manufacturing results in the discarding of fruit skin and seeds, constituting a residue. These fractions (also named by-products) represent between 10 and 60% of total fruit weight [91] and concentrate high levels of phenolic compounds [54, 99], suggesting their potential use in the food and pharmaceutical industries. Recent researches have focused on potential technological processes that affect phenolic composition of acerola residues, adding value to these by-products. The industrial application of acerola

residuals is an important alternative to reduce both environmental impacts and production costs [129].

The processing of acerola by-products into flour and powder results in a product with several antioxidant compounds, destined for nutritional enrichment of cakes, breads, cookies, and cereal bars [115, 130]. Some characteristics found in the flour produced from acerola residues, such as high content of dietary fibers, emulsion stability, and water and oil absorption capacity, make it ideal for incorporation into meat and bakery products [131].

The extraction of phenolic compounds from acerola by-products for use as active compounds in edible coatings is emerging in postharvest researches focusing on fruit conservation. A chitosan solution containing phenolic compounds extracted from acerola by-products was applied on guavas, being able to delay fruit ripening, preserving firmness and maintaining green color [132].

Extracts of acerola flour demonstrated high potential for several biotechnological applications, especially in the cosmetic industry, due to the presence of triterpene saponins with good surface activity and emulsifying capacity [133]. Furthermore, extracts of acerola fruit reduced the cellular melanin level and inhibited tyrosinase activity, suggesting a potential as a component in skin-whitening cosmetics [134].

Conclusions and future perspectives

Scientific evidences suggest that the acerola is a promising superfruit with great potential in the food and pharmaceutical industries. At least 76 phenolic compounds were identified using high performance liquid chromatography in acerolas, including 55 flavonoids (anthocyanins, flavan-3-ols, flavonols, flavones, flavanones, isoflavones and chalcones) and 21 non-flavonoids (phenolic acids, stilbenes and lignans).

Phenolic compounds in acerola show several biological properties, including antioxidant, antibacterial, antihyperglycemic, antihyperlipidemic, anti-inflammatory, and hepatoprotective activities. However, studies are further required to assess the seasonal and genotypic influence on the phenolics of acerola and their bioaccessibility.

Acerola is an anthocyanin-rich fruit with high potential for pigment extraction, but stabilization of anthocyanins in juice and pulp should be further elucidated and improved.

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Data availability Data availability is not applicable to this article as no new data were created or analysed in this study.

Declarations

Competing of interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Bayesian and classical approaches for the estimation of genetic parameters and coefficients of repeatability of acerola quality traits

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ABSTRACT. Although acerola (*Malpighia emarginata* DC.) is a tropical fruit of high interest due to its high ascorbic acid content and attractive sensory attributes, fruit production is characterized by high genetic variability. Additionally, the use of new biometric tools for acerola breeding is scarce. This study aimed to estimate genetic parameters and the coefficient of repeatability, as well as determine the optimal number of fruits for quality trait analyses in different acerola genotypes, using different approaches. Twenty-three (Experiment I) and thirty-five (Experiment II) genotypes were evaluated in a randomized block design with four replicates and three plants per plot. Twenty fruits per plant were harvested and evaluated for the following quality traits: diameter, mass, skin color (lightness, chroma and hue), firmness, soluble solids (SS), titratable acidity (TA), SS/TA ratio, and ascorbic acid content. The genetic parameters and the coefficient of repeatability were estimated for each experiment using classical and Bayesian methods. Both approaches achieved similar results on estimating variance components, genetic parameters and the coefficient of repeatability. Genetic parameters showed favorable conditions for acerola selection. The coefficient of repeatability was high for all acerola quality traits. A total of 17 fruits are required for the effective selection of acerola genotypes with an accuracy of 95%.

Keywords: *Malpighia emarginata* DC.; analysis of variance; genetic variability; fruit quality; repeated measures.

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Introduction

Acerola (*Malpighia emarginata* DC.) is a tropical fruit well-known for its high ascorbic acid (vitamin C) content, attractive red color and high antioxidant activity (Delva & Schneider, 2013; Prakash & Baskaran, 2018). Acerola is usually consumed as fresh fruit or processed products (Ferreira et al., 2022; Gualberto et al., 2021).

Currently, Brazil is the global largest producer, consumer and exporter of acerola, which is cultivated in all states, but it is mainly concentrated in the Northeast region, which has tropical climatic conditions that favor the growth and development of acerola trees (Ferreira et al., 2021). Acerola commercial cultivation in Brazil started in the 1980s and is still characterized by high genetic variability that results in irregular fruit production, low productivity and poor fruit quality as a result of propagation by seeds (Ritzinger, Kobayashi, & Oliveira, 2003).

With the rising global appeal for fruit consumption, there is a need for genetic breeding programs to select genotypes with high yield and fruit quality to meet the demands of consumers, growers and exporters. In the breeding programs of fruit crops, the determination of physicochemical quality traits and their related genetic parameters is indispensable to improve the efficiency of the selection of superior genotypes and to efficiently preserve germplasm resources (Liu, Qi, Song, Li, & Li, 2018; Zaouay & Mars, 2014). Furthermore, the use of biometric tools optimizes genetic selection and allows the estimation of experimental accuracy.

In this context, the coefficient of repeatability has been extensively adopted in genetic breeding programs of several fruit species (Alcoforado, Pedroni, Meyer, & Lima-Primo, 2019; Andrade Júnior et al., 2020; Catarina et al., 2020; Diel et al., 2020; Jesus, Lima, Souza, & Girardi, 2021; Mallickowski et al., 2021). The coefficient of repeatability indicates whether multiple measurements of a quality trait in a genotype are the same over time or

space, based on the contribution of the genotype to the total variance (Cruz, Regazzi, & Carneiro, 2012), which allows the estimation of an optimal number of fruits to ensure high accuracy in quality trait analysis.

In acerola, a previous study by Lopes, Bruckner, Cruz, Lopes, and Freitas (2001) estimated the coefficient of repeatability in some quality traits of sixteen genotypes and found values between 0.03 and 0.94 using four classical methods of determination (analysis of variance, principal component analysis based on the correlation and covariance matrices and structural analysis). However, there are no studies estimating genetic parameters and the coefficient of repeatability in acerola using Bayesian inference.

Bayesian inference is a data analysis approach based on Bayes' theorem that has been shown to be more informative and flexible in obtaining the components of variance to estimate genetic parameters than classical approaches because it allows the assessment of small samples and the inclusion of prior information, increasing selection accuracy (Evangelista et al., 2022; Valadares et al., 2022).

This work aimed to estimate genetic parameters and the coefficient of repeatability, as well as determine the optimal number of fruits for quality trait analyses in different acerola genotypes, using different approaches.

Material and methods

Plant material and experimental conditions

Two experiments were carried out in an experimental orchard in Petrolina, Pernambuco State, Brazil, situated at 9°09' S latitude and 40°22' W longitude at an altitude of 365 m. The local climate is BSh (semi-arid), according to Köppen's classification (Alvares, Staple, Sentelhas, Gonçalves, & Sparovek, 2013), and the soil is classified as a dystrophic Yellow Argisol (Santos et al., 2018). The orchard was established in 2012, and cultural practices including micro-sprinkler irrigation, fertilization, pruning, and control of weeds, pests and diseases were carried out according to technical recommendations (Ritzinger et al., 2003).

Both experiments were conducted under a randomized complete block design, with four replications each. The experimental unit was composed of three plants, which were grown at a spacing of 4.0 × 3.0 m. Experiments I and II were composed of 25 and 35 genotypes, respectively, which were cultivated in the Active Germplasm Bank at the Brazilian Agricultural Research Corporation (Tropical Semi-Arid Embrapa), Petrolina, Pernambuco State, Brazil.

A total of 20 acerolas at the red-ripe maturity stage were collected per plant in September 2018 (Experiment I) and September 2019 (Experiment II). The climatic data during the fruit set period, i.e., 20–25 days from anthesis to harvest, depending on the genotype (Maranhão Ribeiro et al., 2018; Santos et al., 2019), were monitored at the experimental orchard weather station. Meteorological data for September 2018 and September 2019 were as follows: minimum/mean/maximum temperature 21.1/27.6/34.8°C and 21.3/27.4/34.9°C, rainfall 0 and 0.04 mm, relative humidity 56.7 and 55.3%, global solar radiation 26.0 and 24.4 MJ m⁻² day⁻¹, and reference evapotranspiration of 6.9 and 6.8 mm day⁻¹, respectively.

After harvest, the fruit was washed, sanitized, dried and selected based on uniformity of color, size, shape, and absence of damage and fungal infection. Ten fruit quality traits were analyzed, as described below.

Acerola quality traits

Fruit diameter (mm) was determined with 0.01 mm precision using a digital caliper model CD-6 CS (Mitutoyo Corp., Japan). The weight (g) was evaluated with an accuracy of 0.1 g using a semi-analytic balance model VI 2400 (Acculab, USA).

Skin color was analyzed with a colorimeter model CR-400 (Konica Minolta, Japan), according to the three-dimensional CIEL*a*b* space, which records the following measurements: lightness (L*), varying from black (0) to white (100); chroma (C*), which indicates the color intensity; and hue angle (h°), which indicates the color, where 0/360° represents red, and 90°, 180°, and 270° represent yellow, green, and blue, respectively (Pathare, Opara, & Al-Said, 2013).

Pulp firmness (N) was measured with a texture analyzer (model TA.XT Plus, Stable MicroSystem, UK) equipped with a P/75 compression plate. Firmness was determined using the compression test, which recorded fruit resistance against 10% deformation in its volume.

Soluble solid (SS) content (%) was measured with an accuracy of 0.2% using a digital refractometer model PAL-1 (Atago, Japan). Titratable acidity (TA) (% of malic acid) was determined by titrating 5 mL of juice with 0.1 M NaOH until reaching a pH of 8.1. The SS/TA ratio was calculated for each sample by dividing the SS value by its respective TA value.

The ascorbic acid content (AsA, in $\text{mg } 100 \text{ g}^{-1}$) was determined using Tillman's method according to AOAC method no. 942.15 (Association of Official Analytical Chemists [AOAC], 2016). One milliliter of juice was diluted in 100 mL of 0.5% oxalic acid, which was titrated with 0.02% 2,6-dichlorophenol indophenol (DFI).

Frequentist and Bayesian analysis

In each experiment, genetic parameters were estimated using the frequentist method, i.e., analysis of variance (ANOVA) and Bayesian inference. The model for the frequentist approach was $Y_{ij} = \mu + g_i + a_i + e_{ij}$, where Y_{ij} is the observation of the i -th genotype in the j -th environment; μ is the overall mean; g_i is the random effect of the i -th genotype under the influence of the permanent environment; a_i is the fixed effect of the temporary environment in the j -th measurement; and e_{ij} is the experimental error established by the temporary environmental effect of the j -th measurement in the i -th genotype (Cruz et al., 2012).

Bayesian inference was applied using Monte Carlo via Markov chains (MCMC) through the Gibbs sampling algorithm. Bayesian inference analysis was carried out using the MCMCglmm package (Hadfield, 2010) of R (R Foundation for Statistical Computing, Austria). A total of 1,500,000 iterations were generated per trait. A burn-in of 100,000 and a thin sampling interval of 1,000 iterations, where the variance components were obtained (a posteriori distribution), were used. Different degrees of freedom (ν) (0.002, 0.02, 2, 4, and 7) were adopted to test informative priors (Resende & Alves, 2020). Chain convergence was tested using the Geweke test without autocorrelation ($p > 0.05$) in the boa package (Smith, 2007). After confirming these criteria for each quality trait, genetic parameters and the coefficient of repeatability were estimated.

Estimation of genetic parameters and coefficient of repeatability

The following genetic parameters were estimated: genetic coefficient of variation ($CV_g = \sqrt{\sigma_g^2} / \mu \times 100$); residual (experimental) coefficient of variation ($CV_e = \sqrt{\sigma_e^2} / \mu \times 100$); variation index ($I_v = CV_g / CV_e$); phenotypic variance (σ_p^2); genetic variance (σ_g^2); environmental (residual) variance (σ_e^2); and heritability ($h^2 = \sigma_g^2 / \sigma_p^2 \times 100$). The phenotypic and genotypic correlations were evaluated using a t -test at a 5% probability level.

The coefficient of repeatability (ρ) was estimated with four classical methods, according to Cruz et al. (2012): a) analysis of variance (ANOVA) ($\rho = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$); b) principal component analysis based on a correlation matrix (PCA-Cor); c) principal component analysis based on a covariance matrix (PCA-Cov) (Abeywardena, 1972); and d) structural analysis based on a correlation matrix (SA-Cor) (Mansour, Nordheim, & Rutledge, 1981). The coefficient of repeatability from Bayesian inference was calculated using variance components and the same formula as the ANOVA method.

After the estimation of the coefficient of repeatability in all methods, for all quality traits, the minimum required number of fruit (n_0) for prediction of the real value of the individuals was calculated based on predefined R^2 values of 0.80, 0.85, 0.90, and 0.95, as follows:

$$n_0 = \frac{h^2(i) - p_i}{(1 - R^2)p}$$

Results

Genetic parameters of acerola genotypes determined by the frequentist method, represented by the genetic coefficient of variation, environmental coefficient of variation, variation index, phenotypic variance, genetic variance, environmental variance and heritability are shown in Table 1.

In Experiment I, the CVg ranged between 5.90 and 31.59%, while those for Experiment II were between 7.62 and 35.28%. In both experiments, lightness was the trait with the lowest CVg, while pulp firmness and fruit mass had the highest CVg in Experiments I and II, respectively. The CVg was higher in Experiment II for all quality traits, except for the pulp bae. In 80% of quality traits (considering both experiments together), CVg was higher than 10%, considering the desired variability for genotype selection.

The CVe ranged between 2.13 and 6.20% in Experiment I and between 2.19 and 5.32% in Experiment II. All CVe values were considered low (<10%). Fruit mass and pulp firmness were the traits with the lowest and highest CVe, respectively, in both experiments.

The heritability was very high (>80%) for all quality parameters. Fruit lightness had the lowest h^2 in Experiment I (84.58%) and hue in Experiment II (89.06%). The highest h^2 values were observed for fruit mass in both experiments, at 96.84 and 98.60% in Experiments I and II, respectively.

Table 1. Estimates of genetic parameters of 10 postharvest physicochemical fruit traits from acerola genotypes using the frequentist method.

Parameter	FD	FM	L	C	h	PF	SS	TA	SS/TA	AsA
Experiment I (23 genotypes)										
CV _g	8.04	25.47	5.90	10.08	18.30	31.39	8.85	14.31	17.54	19.05
CV _e	2.13	4.60	2.52	3.77	5.57	6.20	5.58	3.75	4.75	5.78
CV _g /CV _e	3.77	5.53	2.34	2.67	3.29	5.10	2.47	3.82	3.71	3.30
σ_p^2	3.58	1.79	5.82	19.26	26.19	12.99	0.78	0.86	1.42	105894
σ_e^2	3.53	2.70	4.92	16.89	23.98	12.51	0.67	0.845	1.32	96968
σ_g^2	0.25	0.09	0.98	2.56	2.21	0.48	0.11	0.805	0.18	8926
h^2	93.42	96.84	84.58	87.72	91.56	96.30	85.90	95.59	93.22	91.57
Experiment II (35 genotypes)										
CV _g	11.06	35.28	7.62	12.92	13.62	33.79	18.88	19.96	23.35	28.84
CV _e	2.19	4.21	2.54	3.09	4.78	5.52	5.08	3.97	4.28	4.98
CV _g /CV _e	5.23	8.38	3.00	4.18	2.85	6.35	6.15	5.05	5.48	5.79
σ_p^2	6.31	5.46	10.06	35.80	16.93	11.99	2.85	0.880	2.42	181331
σ_e^2	6.29	7.41	9.06	31.97	15.08	11.70	2.77	0.877	2.34	175504
σ_g^2	0.22	0.05	1.01	1.83	1.83	0.29	0.07	0.903	0.08	6027
h^2	96.60	98.60	89.99	94.59	89.06	97.54	87.40	96.20	96.70	96.68

FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; AsA: ascorbic acid. CV_g: genetic coefficient of genetic variation; CV_e: residual (experimental) coefficient of variation; σ_p^2 : phenotypic variance; σ_e^2 : genetic variance; σ_e^2 : environmental (residual) variance; h^2 : heritability.

Genetic parameters were also estimated using Bayesian inference and are shown in Table 2. The estimates of the variance components (σ_g^2 and σ_e^2) were higher using the Bayesian method, with a slight increase in CV_g and CV_e when compared to the frequentist method. CV_g ranges of acerola genotypes were 6.02–33.14% (Experiment I) and 7.92–36.50% (Experiment II) using the Bayesian approach.

Table 2. Estimates of genetic parameters of 10 postharvest physicochemical fruit traits from acerola genotypes using the Bayesian method.

Parameter	FD	FM	L	C	h	PF	SS	TA	SS/TA	AsA
Experiment I (23 genotypes)										
CV _g	8.52	26.22	6.02	10.48	19.16	33.14	9.04	14.52	18.06	19.45
CV _e	2.16	4.70	2.56	3.08	5.77	6.26	5.65	3.76	4.80	5.84
CV _g /CV _e	3.96	5.58	2.35	2.70	3.32	5.29	2.48	3.86	3.76	3.33
σ_p^2	4.03	2.95	6.04	20.75	28.50	14.25	0.82	0.05	1.50	109951
σ_e^2	3.78	2.86	5.12	18.24	26.13	13.76	0.70	0.044	1.40	108647
σ_g^2	0.25	0.09	0.92	2.51	2.57	0.49	0.11	0.805	0.18	9104
h^2	93.71	96.89	84.70	87.92	91.70	96.56	86.92	93.71	93.80	91.72
Experiment II (35 genotypes)										
CV _g	11.84	36.50	7.92	13.17	13.76	34.79	19.33	20.84	24.30	27.37
CV _e	2.26	4.50	2.65	3.15	4.92	5.45	5.10	4.03	4.39	5.15
CV _g /CV _e	5.23	8.34	2.98	4.18	2.80	6.38	6.35	5.17	5.53	5.30
σ_p^2	6.72	5.70	10.88	35.15	17.33	12.70	2.99	0.09	2.61	188765
σ_e^2	6.48	5.65	9.78	33.23	15.38	12.40	2.91	0.084	2.53	182306
σ_g^2	0.23	0.05	1.10	1.90	1.96	0.50	0.07	0.885	0.08	6443
h^2	96.47	98.58	89.00	94.50	89.68	97.61	87.50	96.40	96.05	96.50

FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; AsA: ascorbic acid. CV_g: genetic coefficient of genetic variation; CV_e: residual (experimental) coefficient of variation; σ_p^2 : phenotypic variance; σ_e^2 : genetic variance; σ_e^2 : environmental (residual) variance; h^2 : heritability.

In both experiments, h^2 was slightly higher when estimated by Bayesian inference for most quality traits. In Experiment I, the lowest and highest h^2 were 84.70 (lightness) and 96.89% (fruit mass), respectively. In Experiment II, h^2 varied between 88.68 (lightness) and 98.58% (hue).

The phenotypic and genotypic correlations for the ten quality traits in acerola genotypes are shown in Table 3. A total of 9 and 11 significant phenotypic correlations were found in Experiments I and II, respectively, with coefficient ranges (in modulus) of 0.41–0.96 and 0.34–0.90. Of these, two were strong correlations ($0.90 < |r| < 1.00$), nine were strong correlations ($0.60 < |r| < 0.90$), and nine were medium correlations ($0.30 < |r| < 0.60$). When considering genotypic correlations, nine significant correlations were found in Experiment I and eleven in Experiment II. Most significant genotypic correlations were classified as strong (50%) or medium (40%).

For both experiments, fruit mass and diameter had strong and positive phenotypic (0.96 and 0.90) and genotypic (0.97 and 0.91) correlation coefficients. All correlations between color traits (L^* , C^* , and h^*) were significant and positive, and most of them were strong. Other significant phenotypic and genotypic correlations in both experiments included FM $\times$ (PF and SS) and L $\times$ AsA. As expected, the SS/TA ratio was

positively correlated with TA and negatively correlated with SS. In Experiment II, AsA was negatively correlated with SS/TA ratio, considering phenotypic (-0.47) and genotypic (-0.48) correlations.

Table 3. Estimates of phenotypic (upper diagonal) and genotypic (lower diagonal) correlations for 10 postharvest physicochemical fruit traits from acerola genotypes.

	FD	FM	L	C	h	PF	SS	TA	SS/TA	AsA
Experiment I (25 genotypes)										
FD		0.96 ^{***}	-0.19	-0.23	-0.22	0.33	-0.36	-0.12	-0.08	-0.25
FM	0.97 ^{***}		-0.15	-0.24	-0.09	0.45 [*]	-0.41 [*]	-0.05	-0.17	-0.28
L	-0.22	-0.18		0.77 ^{***}	0.78 ^{***}	0.15	-0.18	-0.02	-0.12	-0.52 [*]
C	-0.72	-0.58	0.75 ^{***}		0.58 ^{***}	-0.06	0.05	-0.05	0.01	-0.12
h	-0.25	-0.10	0.77 ^{***}	0.57 ^{***}		0.33	-0.23	0.14	-0.25	-0.28
PF	0.36	0.44 [*]	0.10	-0.09	0.32		-0.36	-0.16	-0.04	-0.05
SS	-0.38	-0.44 [*]	-0.14	0.12	-0.22	-0.38		-0.15	0.61 ^{***}	0.24
TA	-0.15	-0.04	-0.04	-0.04	0.04	-0.18	-0.39		-0.85 ^{***}	0.57
SS/TA	-0.06	-0.17	-0.10	0.05	-0.24	-0.05	0.62 ^{***}	-0.88 ^{***}		-0.10
AsA	-0.27	-0.31	-0.62 ^{***}	-0.16	-0.28	-0.05	0.38	0.40	-0.11	
Experiment II (35 genotypes)										
FD		0.90 ^{***}	-0.11	-0.26	0.05	0.34 [*]	-0.25	-0.16	-0.08	-0.25
FM	0.91 ^{***}		-0.04	-0.24	0.18	0.56 [*]	-0.26	-0.09	-0.15	-0.09
L	-0.12	-0.04		0.71 ^{***}	0.72 ^{***}	-0.31	-0.14	-0.18	0.07	-0.14
C	-0.27	-0.25	0.75 ^{***}		0.68 ^{***}	-0.41	0.05	-0.14	0.16	-0.05
h	0.06	0.20	0.74 ^{***}	0.70 ^{***}		-0.11	-0.12	-0.18	0.10	-0.11
PF	0.55 [*]	0.36 [*]	-0.34 [*]	-0.43	-0.15		-0.08	0.34	-0.51	0.35
SS	-0.25	-0.26	-0.14	0.04	-0.11	-0.07		0.59 [*]	0.47 ^{***}	0.24
TA	-0.18	-0.08	-0.18	-0.15	-0.20	0.26	0.59 [*]		-0.60 ^{***}	0.72 [*]
SS/TA	-0.08	-0.16	0.08	0.18	0.12	-0.52	0.48	-0.80 ^{***}		-0.47 ^{***}
AsA	-0.23	-0.09	-0.14	-0.05	0.12	0.24	0.24	0.74 ^{***}	-0.48 ^{***}	

FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; AsA: ascorbic acid; * and ** significant correlations at 5 and 1% by the t-test, respectively.

Variation between quality traits was observed for the coefficient of repeatability, whose values ranged from 0.58 for lightness in Experiment I to 0.95 for fruit mass in Experiment II. Estimates of the coefficient of repeatability in our study are considered high since all quality traits except lightness in Experiment I showed a coefficient of repeatability greater than or equal to 0.60 (Table 4).

Table 4. Estimates of the coefficient of repeatability (ρ) and its coefficient of determination (R^2) for 10 postharvest physicochemical fruit traits from acerola genotypes.

Quality trait	ANOVA		PCA-Cor		PCA-Cov		AS-Cor		Regression	
	ρ	R^2	ρ	R^2	ρ	R^2	ρ	R^2	ρ	R^2
Experiment I (25 genotypes)										
FD	0.78	95.43	0.88	95.96	0.79	95.86	0.79	95.85	0.78	95.39
FM	0.88	96.84	0.90	97.24	0.90	97.22	0.90	97.22	0.88	96.79
L	0.58	86.58	0.62	86.90	0.58	84.89	0.61	86.39	0.59	81.99
C	0.64	87.72	0.68	89.44	0.67	88.97	0.67	89.09	0.61	86.27
h	0.73	91.56	0.73	92.47	0.75	92.38	0.75	92.29	0.72	90.91
PF	0.87	96.30	0.87	96.46	0.87	96.38	0.87	96.42	0.87	96.43
SS	0.60	85.90	0.64	88.45	0.61	86.13	0.62	87.90	0.56	85.74
TA	0.78	95.59	0.85	96.98	0.81	94.62	0.82	94.86	0.78	95.28
SS/TA	0.77	95.22	0.81	96.45	0.79	95.94	0.81	94.30	0.77	92.94
AsA	0.73	91.57	0.75	92.47	0.77	92.90	0.75	92.19	0.72	90.97
Experiment II (35 genotypes)										
FD	0.88	96.69	0.88	96.82	0.88	96.81	0.88	96.79	0.87	96.54
FM	0.95	98.60	0.95	98.66	0.95	98.63	0.95	98.66	0.94	98.56
L	0.60	89.44	0.72	91.29	0.70	90.13	0.72	90.99	0.66	88.77
C	0.81	94.59	0.82	94.95	0.82	94.84	0.82	94.84	0.80	94.29
h	0.67	89.86	0.79	90.25	0.69	89.92	0.68	89.65	0.63	87.23
PF	0.91	97.58	0.92	97.84	0.92	97.94	0.92	97.82	0.91	97.53
SS	0.90	97.40	0.91	97.61	0.91	97.61	0.91	97.59	0.90	97.44
TA	0.86	96.20	0.87	96.34	0.86	96.21	0.87	96.30	0.87	96.26
SS/TA	0.88	96.78	0.89	96.89	0.88	96.85	0.89	96.85	0.88	96.73
AsA	0.88	96.68	0.88	96.82	0.88	96.73	0.88	96.79	0.87	96.10

ANOVA: analysis of variance; PCA-Cor: principal component analysis based on correlation matrix; PCA-Cov: principal component analysis based on covariance matrix; AS-Cor: structural analysis based on correlation matrix; FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; AsA: ascorbic acid.

Classical methods for estimating the coefficients of repeatability showed very similar values. Bayesian estimation resulted in similar coefficients of repeatability for most quality traits. In a few quality traits, the Bayesian approach revealed values slightly lower than the other methods for this parameter, such as for SS in the first experiment (0.56 in Bayesian inference + 0.66 in PCA-Cor method).

Based on classical methods for estimating the coefficient of repeatability, the minimum required number of measurements for prediction of the real value of the individuals was 13.86, 11.46, 13.62, and 12.07 fruit in the first experiment, and 9.34, 8.21, 8.52, and 8.78 in the second experiment, considering ANOVA, PCA-Cor, PCA-Cov and SA-Cor methods, respectively, with an R^2 of 95%. For the same accuracy, the number of measurements estimated by Bayesian inference was 16.75 and 11.12 fruit in Experiments I and II, respectively. The diameter, mass, pulp firmness, titratable acidity and SS/TA ratio determined by the PCA-Cor and PCA-Cov approaches required the smallest number of fruit (<6) to predict the real value with a 95% accuracy for both experiments independent of the adopted approach (Figure 1).

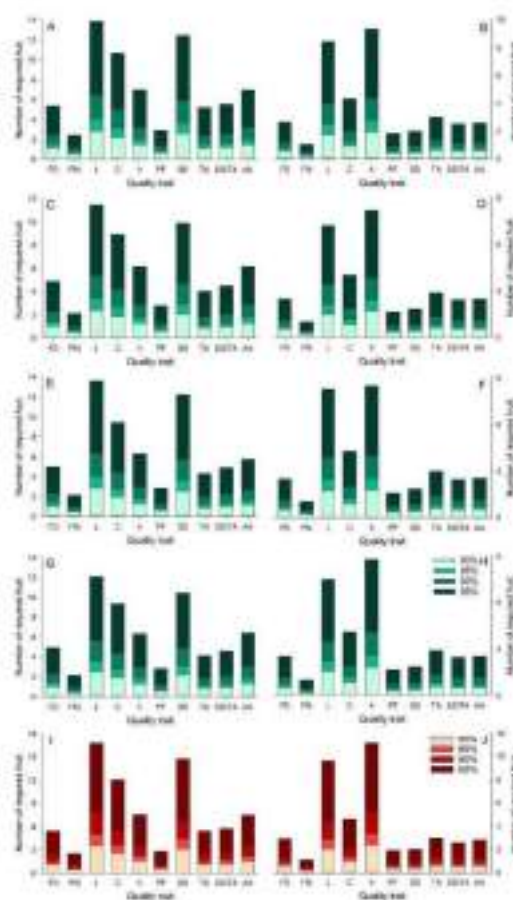


Figure 1. Number of fruit required for different R^2 values (90, 95, 99, and 99%) estimated by analysis of variance (A and F), principal component analysis based on correlation matrix (B and G), principal component analysis based on covariance matrix (C and H), structural analysis based on correlation matrix (D and I), and Bayesian inference (E and J) for 10 postharvest physicochemical traits of fruit from 55 *acmoli* genotypes. Figures on the left represent Experiment I (25 genotypes), and those on the right represent Experiment II (55 genotypes). FD: fruit diameter; FM: fruit mass; L: lightness; C: chroma; H: hue angle; PP: pulp firmness; SS: soluble solids; TA: titratable acidity; AA: ascorbic acid.

Discussion

The commercial cultivation of acerola in Brazil started in the late 1980s and was mostly focused on the production of ascorbic acid (vitamin C) for the food and pharmaceutical industries. At that time, acerola trees were mainly propagated by seeds (sexual method), which resulted in a high genetic variability in Brazilian acerola orchards (Ritzinger, Ritzinger, Fonseca, & Machado, 2017) that has been observed until the present day and reported in studies that have evaluated the fruit quality of different acerola genotypes cultivated in different regions (Farinelli et al., 2021; Ferreira et al., 2021; Ferreira et al., 2022; Magalhães et al., 2018). In this context, the use of biometric tools is key for genotype characterization to optimize selection.

The assessment of genotypes in fruit breeding programs requires time, labor and expenses. Therefore, the study of experimental parameters, such as the coefficient of correlation and repeatability, can optimize genotype selection by estimating an ideal number of measurements required to ensure high quality characterization accuracy (Alcoforado et al., 2019; Jesus et al., 2021).

In this study, the high values of genetic coefficients of variance (CV_g) (> 10%) and high heritability (h^2) (> 60%) for most quality traits indicate favorable conditions for selection as a result of high genetic variability (Jesus et al., 2021). The environmental coefficient of variation (CV_e) was lower than 10% for the ten studied quality traits. When assessing quality parameters of eight acerola genotypes, Mariano-Nasser et al. (2017) found low CV_e values (3.0–7.9%) for skin lightness, soluble solids, titratable acidity and ascorbic acid and medium CV_e values (13.8–19.9%) for skin chroma and hue. Furthermore, the relative CV (CV_g/CV_e) was higher than 1 for all quality traits, which is recommended for genotype selection (Azevedo et al., 2021; Vasconcelos et al., 2020).

Fruit mass and pulp firmness presented the highest CV_g among all quality traits assessed in both experiments, confirming exploitable and desirable genetic variability. Larger and firmer fruit are strongly desirable in acerola breeding programs to improve pulp yield and facilitate processing, as well as to reduce fruit losses due to higher resistance to postharvest handling, storage, and shipping (Ferreira et al., 2022). However, lightness showed the lowest CV_g in both experiments. Lightness is part of the CIEL*a*b* color space system that can range from dark (0) to light (100), which varies less than other color parameters, such as chroma and hue angle (Lazaro, Boada, Villarino, & Girbau, 2019).

Some significant correlations in acerola quality traits include those between both physical traits (diameter and mass) and between pairs of skin color parameters (lightness, chroma and hue), confirming previous results observed in 103 acerola trees (phenotypes) evaluated by Farinelli et al. (2021) and in 24 genotypes evaluated by Magalhães et al. (2018). The genetic coefficients of correlation for acerola were higher than phenotypic ones, which confirms the high genetic variability in the studied genotypes.

The coefficient of repeatability was estimated for each quality trait in each experiment using four classical methods and Bayesian inference. In all methods, the variance components (genetic and environmental variances) were the basis for calculating the coefficient of repeatability. A higher repeatability coefficient (close to 1) means that multiple measurements of a quality trait in one evaluated genotype are consistent in different times or spaces (Cruz et al., 2012), and thus, not many fruit are required to assess this trait with high accuracy in genetic breeding programs. A high coefficient of repeatability (>0.60) was observed for the ten quality traits, similar to Lopes et al. (2001) with 16 acerola genotypes evaluated for similar traits, including fruit diameter (0.88), mass (0.92), titratable acidity (0.81) and ascorbic acid (0.84).

The estimates of genetic parameters found in this study indicate similar results for different approaches (Bayesian + classical methods), which agrees with studies on kale (Brito et al., 2019) and physic nut (*Jatropha curcas* L.) (Evangelista et al., 2022). Some advantages of Bayesian inference in relation to frequentist determination of variance components (and consequently coefficients of repeatability) include the feasibility of using previous data information (*priori*), the lower sensitivity to outliers and lower data assumptions, such as experimental balance and high sample size (Singh, L-Yassin, & Omer, 2015), resulting in more accurate estimates of variance, as reported in guava (Silva et al., 2020) and soybean (Volpato et al., 2019).

Despite several studies on the genotypic variability of acerola quality, only a few studies have involved the evaluation of repeated measures in acerola breeding. Furthermore, Bayesian inference was applied for the first time as an alternative method for estimating genetic parameters in acerola.

Conclusion

Frequentist and Bayesian approaches achieved similar results in estimating variance components and genetic parameters of acerola quality traits. The coefficient of repeatability was high for all acerola quality

traits and varied little when estimated using five different methods. A total of 17 fruits are required for the effective selection of acerola genotypes with an accuracy of 95%.

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