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Research Article

Influence of Different Solvents on the Extraction of Bioactive Compounds from Acerola Processing Residues

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Abstract

The search for effective strategies to reduce food contamination and the incidence of foodborne diseases has increased interest in natural bioactive compounds as alternatives to synthetic food preservatives. Among these, fruit processing by-products represent promising and sustainable sources of antioxidant compounds. This study evaluated the extraction of bioactive compounds from a seed-rich acerola (*Malpighia emarginata* DC.) processing residue using different extraction solvents and assessed the antioxidant and antimicrobial properties of the resulting extracts. Hydroethanolic solvents, particularly 50% ethanol, provided the best overall extraction performance, yielding high concentrations of total phenolic compounds (35.26 ± 0.38 mg GAE/100 mL) and vitamin C (1.79 ± 0.05 mg ascorbic acid/100 mL), which were associated with greater antioxidant capacity (1118.22 and 2551.29 µmol TE/L by the DPPH and ABTS assays, respectively). Protein recovery was negligible under the evaluated conditions. Among all extracts, only the acidified 70% ethanol extract exhibited a slight antimicrobial effect against *Lactococcus lactis* ATCC 19435. Overall, hydroethanolic extraction represents an effective and sustainable approach for recovering antioxidant compounds from seed-rich acerola processing residues. Although the extracts demonstrated considerable antioxidant potential, they exhibited negligible antibacterial activity under the conditions evaluated, supporting their application primarily as antioxidant-rich natural food ingredients.

Keywords: Agro-Industrial By-Products; Biotechnology; *Malpighia emarginata* DC.; Sustainability; Antimicrobial Activity; Antioxidant Capacity

Abbreviations

GAE: Gallic Acid Equivalents; TE: Trolox Equivalent; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ABTS: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ATCC: American Type Culture Collection.

Introduction

Foodborne diseases (FBDs) remain a major global public health concern, causing substantial morbidity, mortality, and economic losses worldwide. According to recent estimates from the World Health Organization (WHO), approximately 866 million cases of foodborne illness occurred in 2021. Of these, about 666 million were attributed to diarrheal pathogens, whereas 194 million resulted from invasive enteric and parasitic pathogens. Among the etiological agents, non-typhoidal *Salmonella enterica* was responsible for the greatest disease burden, contributing substantially to both diarrheal and invasive infections [1].

In Brazil, epidemiological surveillance data have consistently shown that the highest incidence of foodborne diseases occurs in the Southeast region, accounting for approximately 45% of confirmed cases and 42% of FBD-related deaths nationwide. Similar to the global epidemiological scenario, *Salmonella* spp. is the leading etiological agent, followed by Rotavirus and *Escherichia coli*. These pathogens are widely distributed in food production environments and are associated with severe infections, particularly among vulnerable populations such as children, older adults, and immunocompromised individuals [2].

Beyond the burden of foodborne diseases, antimicrobial resistance has emerged as one of the most pressing global public health threats, primarily driven by the excessive and inappropriate use of antibiotics. Epidemiological projections indicate that, by 2050, infections caused by antimicrobial-resistant microorganisms may account for approximately 1.91 million deaths annually, with South Asia, Latin America, and the Caribbean expected to be the most affected regions [3].

Against this background, the search for effective compounds capable of reducing food contamination and preventing foodborne outbreaks has become a major priority for the food industry. At the same time, consumers have become increasingly discerning regarding the composition and quality of the foods they purchase. Population-based studies indicate that more than half of adults routinely examine food labels and associate this practice with the search for healthier products offering additional health benefits [4]. Consequently, growing efforts have been devoted to the bioprospecting of natural alternatives capable of extending food shelf life while ensuring microbiological safety, thereby meeting the expectations of an increasingly informed and health-conscious consumer market.

Within this context, the recovery of natural compounds from fruits and their processing by-products has emerged as a sustainable strategy for food preservation. Fruit processing residues, including peels, seeds, endocarps, and pulp residues, constitute valuable sources of bioactive compounds such as carotenoids, phenolic compounds, proteins, minerals, vitamins, as well as structural polysaccharides including cellulose, hemicellulose, and pectin [5].

Among these fruit species, *Malpighia emarginata* DC., commonly known as acerola, has attracted considerable attention. Acerola is one of the most widely cultivated fruits in Brazil and is recognized for its exceptionally high vitamin C content, ranging from 1,500 to 4,500 mg/100 g, which is up to 80-fold higher than that reported for citrus fruits such as oranges and lemons. In addition, acerola is an important source of anthocyanins, carotenoids, flavonoids, and phenolic compounds, which exhibit antioxidant and anti-inflammatory properties and have been associated with the prevention of cardiovascular diseases, certain types of cancer, and degenerative disorders [6-8].

Due to its high perishability, acerola is commonly processed into pulp and other value-added products. However, industrial processing generates up to 41% by-products, whose disposal not only poses environmental challenges but also represents a considerable loss of potentially valuable raw materials [9]. In response to the growing demand for sustainable technologies, increasing attention has been directed toward the valorization of these residues as high-value by-products [10-12]. This approach not only contributes to waste reduction and mitigation of environmental impacts but also enables the production of value-added ingredients, in line with the principles of sustainability and the circular economy.

The recovery of bioactive compounds from plant materials is influenced by several factors, including the extraction technique, the characteristics of the raw material, and the nature of the

extraction solvent. Ultrasound-assisted extraction (UAE) has emerged as one of the most promising non-conventional extraction techniques because it is environmentally friendly and economically viable compared with conventional methods. In addition to reducing energy consumption, UAE enables the use of alternative solvents while producing safe, high-quality extracts [12,13]. The technique relies on ultrasonic waves to induce acoustic cavitation, during which microscopic bubbles repeatedly expand and collapse, generating shear forces that disrupt plant cell walls and facilitate the release of intracellular bioactive compounds [14].

The efficiency of extracting plant-derived bioactive compounds is also strongly influenced by the physicochemical properties and polarity of the extraction solvent, as its dielectric characteristics determine the selective recovery of different classes of compounds. Therefore, careful solvent selection, combined with an appropriate extraction technique, is essential for optimizing the extraction process [8]. Supporting this premise, the effectiveness of ethanol in the ultrasound-assisted extraction of antioxidant compounds from acerola processing residues has been demonstrated [15]. Likewise, hydroethanolic extracts obtained from freeze-dried acerola residues inhibited the growth of *E. coli*, highlighting the biological potential of this agro-industrial by-product [16].

Therefore, considering the growing demand of the food industry for sustainable bioactive ingredients, the present study aimed to evaluate the influence of different organic solvents on the extraction of bioactive compounds from a seed-rich acerola processing residue using ultrasound-assisted solid-liquid extraction. In addition, the antioxidant and antimicrobial activities of the resulting extracts were investigated to identify the most effective extraction conditions and to support the sustainable valorization of acerola processing residues as a source of antioxidant-rich natural ingredients for potential food applications.

Materials and Methods

Raw material and preparation of the seed-rich acerola processing residue

Ripe acerola (*Malpighia emarginata* DC.) fruits were manually harvested in the municipality of Angatuba, São Paulo State, Brazil, and used as the raw material to obtain the processing residue evaluated in this study.

The fruits were sanitized by immersion in a 2% sodium hypochlorite solution for 15 min, followed by rinsing under running water. They were then manually depulped using a sieve, and the resulting processing residue was collected and stored under freezing conditions until further processing.

The residue, composed predominantly of seeds with small amounts of residual peel and pulp, was dried in a forced-air circulation oven (Lucadema LUCA-82/480, Brazil) at 40 °C for 24 h. The dried material was subsequently ground using a food processor (Philco Turbo Chef, Brazil) until a homogeneous powder was obtained. Moisture content was determined by the conventional oven-drying method [17,18].

Preparation of extracts

Extract preparation was carried out in two sequential stages. In the first stage, a preliminary solvent screening was performed to identify the most effective extraction solvent. Absolute ethanol, acetone, and distilled water were evaluated as pure extraction solvents.

Based on the results obtained during this screening, a second extraction stage was conducted using ethanol solutions at concentrations of 100%, 70%, and 50%, either non-acidified or acidified to pH 3.0 with glacial acetic acid. The extraction conditions are summarized in Table 1.

All extractions were performed using two independent biological replicates, each analyzed in duplicate (technical replicates). The dried residue was mixed with the extraction solvent at a 1:10 (w/v) ratio and homogenized thoroughly. Ultrasound-assisted extraction was then performed in an ultrasonic bath operating at 40 kHz and 160 W (SolidSteel, Brazil) for 1 h.

Following extraction, the suspensions were filtered and centrifuged at 2,318 × g for 15 min using an Excelsa II centrifuge (Fanem Model 206 BL, Brazil). The pellet was discarded, whereas the supernatant was collected and stored under refrigeration until further analyses.

Determination of total phenolic compounds

Total phenolic compounds (TPC) were quantified using the Folin–Ciocalteu colorimetric method, with minor modifications

Stage	Sample	Solvent	Concentration (%)	Acidified
1	E100	Ethanol	100	No
	Ac100	Acetone	100	No
	W100	Distilled water	100	No
2	W100-A	Distilled water	100	Yes
	E100-A	Ethanol	100	Yes
	E70	Ethanol	70	No
	E70-A	Ethanol	70	Yes
	E50	Ethanol	50	No
	E50-A	Ethanol	50	Yes

Table 1: Extraction solvent compositions and sample identification.

[19]. Prior to analysis, the extracts were appropriately diluted. Aliquots of 500 μL were mixed with 4.5 mL of distilled water and 500 μL of Folin–Ciocalteu reagent. After standing for 5 min, 5 mL of 7% (w/v) sodium carbonate solution was added, and the reaction mixture was vortexed for 10 s. The samples were incubated at room temperature, protected from light, for 90 min before absorbance was measured at 750 nm using a UV–Vis spectrophotometer (NI-1600UV, Biosuprimentos, Brazil). Gallic acid solutions (40–200 μg/mL) were used to construct the calibration curve, and results were expressed as mg gallic acid equivalents per 100 mL of extract (mg GAE/100 mL).

Determination of vitamin C

Vitamin C content was determined using the titrimetric method [17,18], with minor modifications, based on the reduction of 2,6-dichlorophenolindophenol (DCPIP) by ascorbic acid. For calibration, a standard solution containing 2 mL of ascorbic acid solution (10 mg/100 mL), 3 mL of 2% oxalic acid solution, and 25 mL of distilled water was prepared. The solution was titrated with DCPIP until a persistent light pink color was observed for 15 s. Calibration was performed in triplicate. The blank consisted of 5 mL of oxalic acid solution and 25 mL of distilled water and was titrated in duplicate.

For sample analysis, 6 mL of extract was mixed with 6 mL of oxalic acid solution. Subsequently, a 5-mL aliquot of this mixture was transferred to an Erlenmeyer flask, followed by the addition of 25 mL of distilled water. The mixture was titrated with DCPIP until a persistent light pink color was maintained for 15 s, and the volume of DCPIP consumed was recorded.

Vitamin C concentration was calculated using the correction factor of the DCPIP solution, taking the blank value into account, according to the following equation:

where:

C = vitamin C concentration (mg/100 mL);

V = volume of DCPIP consumed during titration (mL);

FC = correction factor of the DCPIP solution (mg of ascorbic acid corresponding to each milliliter of DCPIP solution);

A = sample volume used for titration (mL).

Determination of antioxidant activity

DPPH radical scavenging assay

The antioxidant activity of the extracts was evaluated using the DPPH radical scavenging assay, with minor modifications [20]. The reaction medium consisted of a 0.06 mM DPPH solution prepared in 80% ethanol. The solution was protected from light and used on the same day of preparation. A calibration curve was constructed using Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] at concentrations ranging from 50 to 800 μM.

Aliquots of 100 μL of each extract or Trolox standard (all samples were analyzed in duplicate) were mixed with 3.9 mL of DPPH solution and homogenized using an orbital shaker. The reaction mixtures were incubated for 1 h at room temperature in the absence of light. Absorbance was recorded at 515 nm using a UV–Vis spectrophotometer (NI-1600UV, Biosuprimentos, Brazil), previously calibrated with analytical-grade ethanol.

ABTS radical cation decolorization assay

Antioxidant activity was also determined using the ABTS radical cation assay, with minor modifications [20]. The ABTS radical solution was prepared 16 h before analysis by mixing equal volumes of 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The mixture was incubated in the dark until complete radical formation. Before analysis, the solution was diluted with analytical-grade ethanol to obtain an absorbance of 0.752 at 734 nm. A Trolox calibration curve was prepared using standard solutions at concentrations of 100, 500, 1000, 1500, and 2000 μM.

Aliquots of 90 μL of each extract or Trolox standard (all samples were analyzed in duplicate) were mixed with 9 mL of the ABTS radical solution and vortexed. After incubation for 6 min in the dark, absorbance was measured at 734 nm using a UV–Vis spectrophotometer (NI-1600UV, Biosuprimentos, Brazil), calibrated with analytical-grade ethanol.

The antioxidant capacity determined by both assays was expressed as μmol Trolox equivalents per liter of extract (μmol TE/L).

Determination of protein content

Protein concentration was determined using the Bradford method [21]. A 1% (w/v) bovine serum albumin (BSA) stock solution was prepared and subsequently diluted to construct the protein calibration curve (0.2–0.8 mg/mL). Bradford reagent was diluted at a 1:4 ratio and filtered before use.

For protein quantification, 100 μL of each sample or BSA standard was mixed with 5 mL of Bradford reagent, vortexed, and incubated at room temperature for 5 min. Absorbance was measured at 595 nm using a UV–Vis spectrophotometer (NI-1600UV, Biosuprimentos, Brazil). Protein concentration was determined from the BSA calibration curve and expressed as mg protein/mL of extract.

Determination of antimicrobial activity

Lactococcus lactis ATCC 19435, a well-established reference strain frequently employed as a preliminary screening microorganism in antimicrobial susceptibility studies, was selected as the indicator strain to enable standardized comparison of the antibacterial activity among the different extracts [22,23]. Cultures were grown in M17 broth supplemented with 0.5% glucose (GM17) and incubated at 37 °C for up to 24 h before the antimicrobial assays.

The antimicrobial activity of the extracts obtained from the seed-rich acerola processing residue was evaluated using the agar well diffusion method according to the Clinical and Laboratory Standards Institute [24], with minor modifications. Circular wells (5 mm in diameter) were punched into GM17 agar plates previously inoculated with approximately 5×10^5 CFU/mL of *L. lactis*. Subsequently, 50 μL of each extract was dispensed into the wells. The corresponding extraction solvents were included as experimental controls. Following incubation, the plates were examined for the presence of inhibition zones surrounding the wells. Inhibition halos with diameters ≥ 6 mm were considered indicative of positive antimicrobial activity. The diameter of each inhibition zone was measured in millimeters using a graduated ruler and compared with the respective solvent control.

All assays were performed in duplicate using two independent biological replicates.

Statistical analysis

Data were subjected to Analysis of Variance (ANOVA), and mean comparisons were performed using Tukey's multiple comparison test with Statistica Six Sigma software. Differences were considered statistically significant at $p < 0.05$. For the antimicrobial assay, inhibition zone diameters were compared between each extract and its corresponding extraction solvent control. Statistical comparisons were not performed when both the extract and its corresponding solvent control showed identical inhibition zones (0 mm), as no measurable treatment effect was observed.

Results and Discussion

Preparation of extracts from the seed-rich acerola processing residue

The moisture content of the fresh and dried acerola processing residue used for extract preparation was determined prior to extraction. The fresh residue exhibited a moisture content of 80.56%, which decreased to 21.06% after drying at 40 °C for 24 h. This value is consistent with values previously reported [25], who evaluated different drying temperatures for the production of flour from acerola residue containing predominantly seeds and obtained moisture contents ranging from 15.68% to 21.69% after

drying at 80 °C and 60 °C, respectively. The mass balance and dry residue yield are presented in Table 2.

Initial fresh residue (g)	Final dry residue (g)	Yield (%)	Drying time (h)	Drying temperature (°C)
609.78	246.96	40.5	24	40

Table 2: Dry yield of the seed-rich acerola processing residue.

The drying process resulted in a dry residue yield of 40.5%. Considering that the subsequent grinding step is associated with minimal material loss, the overall flour yield obtained from the dried residue was estimated to be approximately 89%. These results indicate that the processing residue, composed predominantly of seeds, can be efficiently converted into a stable dry material suitable for subsequent extraction procedures, supporting its potential as a valuable raw material for the recovery of bioactive compounds.

Characterization of the extracts

Total phenolic compounds

The total phenolic compound (TPC) content of the extracts was evaluated in two sequential stages. The first stage consisted of a preliminary solvent screening to identify the most effective extraction solvent. Based on these results, the second stage focused on environmentally friendly extraction systems by evaluating aqueous ethanol solutions at different concentrations and assessing the effect of solvent acidification. The results are presented in Table 3.

Stage	Non-acidified extracts	TPC (mg GAE/100 mL)*	Acidified extracts	TPC (mg GAE/100 mL)*
	E100	15.68 ± 2.06^{c}	Not applicable	
1	Ac100	26.98 ± 1.08^{b}		
	W100	33.54 ± 2.39^{a}		
2	W100	33.54 ± 2.39^{aA}	W100-A	34.65 ± 4.73^{aA}
	E50	35.26 ± 0.38^{aA}	E50-A	37.52 ± 0.16^{aB}
	E70	34.71 ± 1.60^{aA}	E70-A	35.03 ± 0.06^{aA}
	E100	15.68 ± 2.06^{bA}	E100-A	17.15 ± 0.54^{bA}

Table 3: Total phenolic compound (TPC) content of extracts obtained from the seed-rich acerola processing residue.

*Values are expressed as mean ± standard deviation. Different lowercase letters indicate significant differences among extracts within the same column according to Tukey's test ($p < 0.05$). Different uppercase letters indicate significant differences between non-acidified and acidified extracts within the same row according to Tukey's test ($p < 0.05$).

During the preliminary screening, significant differences ($p < 0.05$) were observed among the three extraction solvents. Distilled water (W100) produced the highest TPC content (33.54 mg GAE/100 mL), followed by acetone (Ac100) (26.98 mg GAE/100 mL), whereas absolute ethanol (E100) yielded the lowest phenolic concentration (15.68 mg GAE/100 mL).

The efficiency of phenolic extraction is influenced by several factors, including the physicochemical characteristics of the plant matrix, particle size, extraction technique, solvent composition, pH, temperature, and extraction time [26]. Among these variables, solvent polarity plays a particularly important role because the extraction efficiency depends on the ability of the solvent to establish hydrogen-bond interactions with phenolic compounds,

thereby enhancing their solubilization and diffusion into the extraction medium [27].

Similar findings have been reported for other plant matrices. Acetone has been shown to provide the highest recovery of phenolic compounds from *Baccharis dracunculifolia*, yielding between 29.22 and 31.84 mg GAE/g (dry basis) [28]. Likewise, a TPC value of 28.46 mg GAE/100 g was reported for flour produced from acerola processing residue [28], which is comparable to the values obtained in the present study. Furthermore, 80% aqueous acetone was identified as the most effective solvent for recovering phenolic compounds during ultrasound-assisted sequential extraction of pectin and phenolics from Granny Smith apple peel [29].

Although acetone exhibited high extraction efficiency, the second experimental stage prioritized the use of water and ethanol because of their lower toxicity and greater environmental sustainability. Water is widely regarded as one of the most environmentally friendly extraction solvents due to its availability, non-toxicity, recyclability, and low cost [30]. Ethanol is also considered safe for food applications by the European Food Safety Authority, whereas acetone is generally not recommended for food-grade extracts because of its toxicity [31,32].

Among the non-acidified extraction systems evaluated during the second stage, W100, E50, and E70 exhibited the highest TPC values, ranging from 33.54 to 35.26 mg GAE/100 mL, with no significant differences among them ($p > 0.05$). In contrast, absolute ethanol (E100) yielded significantly lower TPC values (15.68 mg GAE/100 mL; $p < 0.05$). These results indicate that the addition of water substantially improved the extraction efficiency, with 50% ethanol providing the highest phenolic recovery. Furthermore, E50 was the only extraction system that showed a significant increase in TPC following acidification.

The beneficial effect of aqueous ethanol has been consistently reported in the literature. A 50% aqueous ethanol solution has been identified as the most effective solvent for extracting phenolic compounds from the pericarp and seed coat of *Macadamia integrifolia*, outperforming both pure ethanol and aqueous acetone [33]. Similarly, a 20% hydroethanolic solution was reported to maximize phenolic extraction from brewer's spent grain, whereas pure water promoted faster extraction kinetics [34].

The improved extraction efficiency observed with aqueous ethanol is attributed to complementary mechanisms. Water promotes tissue swelling, increasing solvent penetration and facilitating mass transfer within the plant matrix, whereas ethanol weakens interactions between phenolic compounds and structural macromolecules, thereby enhancing their release into the extraction medium [34-37]. In addition, moderate ethanol concentrations help minimize protein denaturation, reducing interactions that may otherwise limit phenolic extraction [38,39].

The influence of solvent acidification on phenolic extraction remains controversial. One study reported no significant effect of pH on the ultrasound-assisted extraction of phenolic compounds from *Morinda citrifolia* biomass [40]. Conversely, a modest increase in polyphenol recovery from grape pomace was observed when acidified methanolic solutions were used [41]. These contrasting findings are consistent with the mechanism proposed in a recent study, which suggested that acidification may modify the hydrophobicity and solubility of phenolic compounds, thereby affecting extraction efficiency depending on the composition of the plant matrix [42].

Vitamin C

Based on the TPC results, extracts obtained with distilled water (W100) and 50% ethanol (E50), both in acidified and non-acidified forms, were selected for subsequent determination of vitamin C content and antioxidant activity. The vitamin C results are presented in Table 4.

Non-acidified extracts	Vitamin C (mg/100 mL)*	Acidified extracts	Vitamin C (mg/100 mL)*
W100	0.57 ± 0.00^{bA}	W100-A	1.29 ± 0.20^{aB}
E50	1.79 ± 0.05^{aA}	E50-A	2.16 ± 0.47^{aA}

Table 4: Vitamin C content of extracts obtained from the seed-rich acerola processing residue.

*Values are expressed as mean ± standard deviation. Different lowercase letters indicate significant differences within the same column according to Tukey's test ($p < 0.05$). Different uppercase letters indicate significant differences between non-acidified and acidified extracts within the same row according to Tukey's test ($p < 0.05$).

The extract obtained with 50% ethanol (E50) exhibited a significantly higher vitamin C concentration ($p < 0.05$) than the extract obtained with distilled water (W100), reaching the highest mean value (1.79 mg ascorbic acid/100 mL). Acidification of the extraction medium did not significantly affect vitamin C recovery from the hydroethanolic extract. In contrast, acidification significantly increased the vitamin C content of the aqueous extract, indicating that lowering the extraction pH enhanced ascorbic acid recovery when water was used as the extraction solvent.

The superior extraction performance observed with 50% ethanol is likely related to the intermediate polarity of the hydroethanolic mixture, which improves solvent penetration into the plant matrix while maintaining the high solubility and stability of ascorbic acid during the extraction process.

Previous studies evaluating acerola processing by-products have reported similarly low or undetectable concentrations of vitamin C in seed- and pomace-rich fractions. For example, extraction using gas-expanded liquids yielded no detectable ascorbic acid in the pomace fraction, composed primarily of seeds and peels, whereas substantially higher concentrations were found in the non-pomace fraction and acerola juice powder [44]. These findings are consistent with the present results and reinforce that acerola seed-rich residues contain considerably lower levels of vitamin C than fresh fruit tissues.

The lower vitamin C concentrations observed in seed-rich residues are expected, as ascorbic acid is predominantly concentrated in the edible pulp of acerola fruits [43] and is progressively reduced during fruit ripening and industrial processing [16]. Consequently, the vitamin C recovered from the present extracts represents only a small fraction of the bioactive compounds remaining in this agro-industrial by-product.

Although vitamin C recovery was limited, hydroethanolic extraction efficiently recovered phenolic compounds, which likely contributed to the antioxidant activity observed. However, the individual phenolic compounds present in the extracts were not characterized in the present study. Therefore, further chromatographic analyses are required to identify the compounds responsible for the observed antioxidant activity.

Antioxidant activity

Based on the total phenolic compound (TPC) results, only the extracts exhibiting the highest phenolic contents (W100, W100-A, E50, and E50-A) were selected for antioxidant activity determination because phenolic compounds are recognized as major contributors to the antioxidant capacity of plant extracts [45]. The results are presented in Table 5.

Assay	Non-acidified extracts	Trolox equivalent (µmol TE/L)*	Acidified extracts	Trolox equivalent (µmol TE/L)*
DPPH	W100	736.96 ± 300.50^{bA}	W100-A	588.71 ± 2.33^{bA}
	E50	1118.22 ± 10.30^{aA}	E50-A	1114.78 ± 17.17^{aA}
ABTS	W100	1194.24 ± 784.62^{bA}	W100-A	846.00 ± 8.35^{bA}
	E50	2551.29 ± 177.66^{aA}	E50-A	2531.91 ± 102.83^{aA}

Table 5: Antioxidant activity of acerola seed residue extracts determined by the DPPH and ABTS assays.

*Values are expressed as mean ± standard deviation. Statistical analyses were performed separately for each antioxidant assay. Different lowercase letters indicate significant differences within the same column according to Tukey's test ($p < 0.05$). Different uppercase letters indicate significant differences between non-acidified and acidified extracts within the same row according to Tukey's test ($p < 0.05$).

The antioxidant activity determined by both DPPH and ABTS assays showed the same extraction trend observed for total phenolic compounds. Extracts obtained with distilled water (W100) exhibited significantly lower antioxidant activity than those extracted with 50% ethanol (E50) ($p < 0.05$), with mean values of 736.96 and 1194.24 µmol TE/L for the DPPH and ABTS assays, respectively. Conversely, E50 exhibited the highest antioxidant

activity, reaching 1118.22 µmol TE/L (DPPH) and 2551.29 µmol TE/L (ABTS). Acidification did not significantly affect antioxidant activity in either extraction system.

The greater antioxidant activity observed in the E50 extracts closely paralleled their higher total phenolic content, suggesting that phenolic compounds were the main contributors to the antioxidant capacity of the extracts obtained under the evaluated extraction conditions [45].

A greater variability was observed among the aqueous extracts, as indicated by the higher standard deviations associated with W100. This variability may reflect differences in solvent–matrix interactions during extraction, resulting in less reproducible recovery of antioxidant compounds than that obtained with the hydroethanolic system.

The higher antioxidant values obtained by the ABTS assay compared with the DPPH assay can be explained by the different physicochemical properties of the radicals employed. The DPPH radical is soluble primarily in organic solvents and is more responsive to hydrophobic antioxidants, whereas ABTS is soluble in both aqueous and organic media, allowing the detection of both hydrophilic and lipophilic antioxidant compounds [46].

No significant differences in the antioxidant capacity of acerola extracts obtained using water or methanol have been reported [43]. Based on these findings, antioxidant activity was subsequently evaluated in different acerola products using water as the extraction solvent, yielding values ranging from 74.30 to 91.76 mmol/kg in fruit pulp and from 82.33 to 125.66 mmol/L in juice, as determined by the ABTS and DPPH assays, respectively [43]. Similarly, a 50% ethanol-water mixture was identified as the most effective solvent for the ultrasound-assisted extraction of bioactive compounds from *Alpinia officinarum*, corroborating the results obtained in the present study, in which 50% ethanol consistently produced extracts with the highest antioxidant activity [46]. Likewise, optimization of acerola pomace extraction using a central composite rotational design demonstrated that hydroethanolic extraction was the most efficient approach. Under optimized conditions employing 34% ethanol, antioxidant activity reached 3510.64 mg TE/100 g against the DPPH radical [47]. In that study, the authors attributed the antioxidant activity to the presence of phenolic acids and yellow flavonoids identified in their extracts.

The positive association between total phenolic content and antioxidant activity observed in the present study is consistent with previous reports for *Baccharis dracunculifolia* leaves [48], grape pomace [49], and acerola by-products extracted under optimized gas-expanded liquid conditions [44]. This relationship is attributed to the chemical structure of phenolic compounds, particularly the number and position of hydroxyl groups and the substitution pattern of their aromatic rings, which directly influence their free radical scavenging capacity [50].

Protein content

Differences among studies evaluating bioactive compounds from acerola residues may be attributed to variations in extraction methodologies, solvent systems, processing conditions, and the specific fruit tissues analyzed. These factors also influence extraction selectivity, promoting the co-extraction of other cellular macromolecules, including proteins. The protein concentrations recovered in the extracts are presented in Table 6.

Extracts	Non-acidified (mg/mL)	Acidified (mg/mL)
Ac100	0.16 ± 0.09	Not applicable
W100	0.05 ± 0.01	0.04 ± 0.02
E50	0.06 ± 0.00	0.07 ± 0.01
E70	0.08 ± 0.01	0.05 ± 0.05
E100	0.24 ± 0.05	0.24 ± 0.01

Table 6: Protein concentration in extracts obtained from the seed-rich acerola processing residue.

Values are expressed as mean ± standard deviation. Statistical comparisons were not performed because no significant differences were detected among treatments.

Overall, protein concentrations were very low regardless of the extraction solvent employed. Although absolute ethanol (E100) produced the highest protein concentration, the recovered amounts remained limited, suggesting that the extraction conditions evaluated were not suitable for efficient protein recovery from the seed-rich acerola processing residue.

Low protein recovery from ethanolic extracts of acerola residues using the Bradford assay has also been reported, with protein being detected only in the edible portion of the fruit (0.40 ± 0.01 g/100 g) and not in the processing residue [51]. In contrast, protein contents of 1.40 ± 0.33 g/100 g in fresh acerola pomace and 7.32 ± 0.21 g/100 g in pomace flour were reported using the Kjeldahl method [52]. The authors attributed this difference primarily to the marked reduction in moisture content during drying, from 88% in fresh pomace to 7.7% in pomace flour [52].

In the present study, the dried residue used for extraction exhibited an intermediate moisture content (21.06%), which may partially explain the differences relative to previous reports. Furthermore, methodological differences between the Bradford and Kjeldahl assays should also be considered, since the former quantifies soluble proteins whereas the latter determines total nitrogen, frequently resulting in higher estimated protein contents.

Although studies investigating protein recovery from acerola by-products are available, information regarding the influence of extraction solvents on protein recovery remains limited. Future studies should therefore optimize extraction parameters, including solvent composition and extraction conditions, to improve protein recovery from this agro-industrial residue.

Antimicrobial activity

To evaluate the antimicrobial potential of the extracts, all extraction systems, including acidified and non-acidified formulations, were tested against *L. lactis* ATCC 19435 using the agar well diffusion assay. None of the non-acidified extracts produced detectable antimicrobial activity beyond that observed for their corresponding extraction solvent controls. Likewise, the acidified water extract (W100-A) showed no inhibitory effect.

Statistical comparison between each extract and its corresponding extraction solvent control showed that only the acidified 70% ethanol extract (E70-A) produced an inhibition zone significantly larger than its respective control ($p < 0.05$). However, the increase was limited to 0.75 mm (8.75 ± 0.29 mm versus 8.00 ± 0.00 mm), indicating only marginal antimicrobial activity under the experimental conditions employed. Similarly, the acidified 100% and 50% ethanol extracts (E100-A and E50-A) produced inhibition zones only 0.50 mm and 0.63 mm larger than their respective solvent controls, and these differences were not statistically significant ($p > 0.05$).

Overall, these findings indicate that the extraction conditions evaluated in the present study were insufficient to produce extracts with relevant antibacterial activity against *L. lactis*.

These findings are consistent with previous reports showing that the antimicrobial activity of acerola-derived extracts depends strongly on extraction conditions and extract composition. For example, optimization of ethanol extraction from dehydrated acerola residue resulted in extracts that were inactive against *Staphylococcus aureus* and *E. coli*, while only moderate inhibition was observed against *Listeria monocytogenes* (7.85 mm) [47]. The authors attributed this selective activity to differences in phenolic composition and bacterial susceptibility. Notably, the optimized extract contained 903.64 mg GAE/100 g of total phenolic compounds, substantially higher than the concentrations obtained in the present study [47].

Overall, the present results indicate that the extraction conditions evaluated were effective for recovering antioxidant compounds but insufficient to obtain extracts with relevant antibacterial activity against *L. lactis*. Future studies should focus on increasing extract concentration, optimizing extraction parameters and solvent composition, and evaluating the antimicrobial activity against a broader range of foodborne pathogens. Although *L. lactis* is widely employed as a screening microorganism for antimicrobial susceptibility assays [22,23], further studies should evaluate the activity of the extracts against relevant foodborne pathogens, including *Listeria monocytogenes* and *Salmonella enterica*, to better define their potential for food applications.

A limitation of the present study is the relatively small number of independent biological replicates used for the extraction experiments, which may have limited the statistical power to detect subtle differences among treatments. Nevertheless, the

experimental design enabled a standardized comparison among the evaluated extraction systems, and consistent trends were observed, particularly regarding the superior performance of the hydroethanolic extracts. These findings provide a solid basis for future studies involving a larger number of biological replicates.

Conclusion

This study showed that hydroethanolic solvents, particularly 50% ethanol, were effective, economically viable, and sustainable extraction media for recovering antioxidant compounds from acerola seed residues. Among the evaluated extraction systems, 50% ethanol yielded the highest concentrations of total phenolic compounds and vitamin C, which were associated with greater antioxidant capacity.

Although the extracts exhibited considerable antioxidant potential, they showed negligible antibacterial activity against *L. lactis* under the experimental conditions employed, indicating that the extraction conditions evaluated were insufficient to obtain extracts with relevant antimicrobial activity.

These findings support the valorization of acerola seed residues primarily as a source of antioxidant-rich natural ingredients. Future studies should focus on optimizing extraction conditions, evaluating the antimicrobial potential of concentrated extracts against a broader range of foodborne microorganisms, and characterizing the individual phenolic compounds using chromatographic techniques such as HPLC, UHPLC, or LC-MS. Such analyses will help elucidate the compounds associated with the observed antioxidant activity and provide a more comprehensive understanding of the relationship between extract composition and biological activity, thereby supporting future food applications of acerola seed extracts.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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