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Dynamic Maceration of Acerola (*Malpighia emarginata* DC.) Fruit Waste: An Optimization Study to Recover Anthocyanins

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Abstract: Acerola (*Malpighia emarginata* DC.) is a tropical fruit with a vibrant red color attributed to anthocyanins, natural pigments, with several applications in the food, nutraceutical, and cosmetic industries. Therefore, the suitability of acerola fruit waste for producing anthocyanin colorants by dynamic maceration was investigated. The extraction process was optimized by combining the factors time (2–90 min), temperature (20–90 °C), and ethanol percentage (0–100%) in a central composite rotatable design (CCRD) coupled with response surface methodology (RSM). The extraction yield determined by a gravimetric method and the levels of cyanidin-*O*-deoxyhexoside and pelargonidin-*O*-deoxyhexoside anthocyanins quantified in the 20 run extracts by HPLC-DAD were used as dependent variables. After fitting the experimental data to a quadratic equation, the obtained statistically valid predictive models were used to determine optimal macerating conditions. Under global settings (25 min processing at 41 °C with 12% ethanol), the extraction yielded 57.1% (*w/w*) and each gram of extract contained 2.54 mg of anthocyanins. Overall, this study highlights the renewable potential of acerola fruit waste for obtaining natural anthocyanin extracts that could represent a sustainable alternative to artificial colorants used in food and other products.

Keywords: *Malpighia emarginata* DC.; agricultural waste; anthocyanins; natural colorants; extraction methods; process optimization; response surface methodology; biowaste valorization

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1. Introduction

Nowadays, the considerable increase in the world's population associated with climate change and consumers' urban lifestyles has promoted the development and marketing of alternative and more sustainable food ingredients and products [1,2]. Thus, it has become important to develop safer and more efficient food production and distribution systems, in harmony with the environment and the growing scarcity of natural resources. In the agri-food sector, fruit and vegetable processing operations generate a large amount of waste worldwide, whose upcycling must be seen as a future-oriented strategy to ensure resource-use efficiency, circularity, and food security [3]. In fact, food waste can be a good source of pigments, phenolic compounds, organic acids, vitamins, and other valuable compounds, with technological and bioactive effects when added to foods and other consumer goods [4,5].

The growing demand for food products with better organoleptic characteristics and a longer shelf-life has promoted the development and application of new food additives and ingredients, leading both researchers and industry professionals to reconsider the

formulation of processed foods [6]. Among the various sensory attributes, the visual appearance of food plays a crucial role in consumers' choices, as it significantly influences their expectations regarding its palatability and overall quality. Consequently, the food's appearance is often improved or modified by the incorporation of natural and mainly artificial colorants to promote their acceptance and marketing [7,8].

Several studies have been conducted with the aim of producing new food ingredients with coloring capacity from plant matrices, including agri-food waste. These studies have focused mainly on the extraction, purification, and stability of natural pigments, such as anthocyanins, one of the most studied classes of plant pigments [7,9]. These flavonoid group compounds provide red, purple, violet, and blue colors to many plants, flowers, and fruits depending on the pH and their structural composition [10]. They exhibit a wide range of biological and health-promoting effects, with them being involved in the prevention of coronary heart disease, cancer, and diabetes [11–13]. Acerola (*Malpighia emarginata* DC., Malpighiaceae family) is a functional food rich in bioactive constituents, including anthocyanins, which provide the characteristic red color to the fruit [14,15]. Therefore, it stands out as a potential raw material for the development of natural food colorants, mainly the waste of its industrial processing, which, if not properly managed, may lead to negative consequences at economic, environmental, and social levels.

Extraction is the most critical step in order to obtain natural colorants from plant materials. It depends on the intrinsic characteristics of the plant material and various process-influencing factors, such as the processing temperature and time and the type of solvent [16–18]. Among the techniques currently used to extract anthocyanins, dynamic maceration stands out as a simple, easy-to-perform, and relatively inexpensive method [18–20]. It involves placing the plant material in continuous contact with a solvent while being agitated or stirred at a controlled temperature. Solvents such as water, ethanol, methanol, or mixtures thereof are commonly used [18]. Glycosylated anthocyanins are highly soluble in water, but their polyphenolic structure imparts a hydrophobic characteristic [21]. In turn, solvent acidification (pH~3) with food-grade weak acids such as citric acid and acetic acid helps to stabilize the flavylium cation and increase the intensity of the red hue of anthocyanins [18,21], making them good candidates as colorants.

Regardless of the selected extraction procedure, its optimization is an important step towards maximizing extraction yields while minimizing time, resource input (i.e., energy and solvents), and associated costs. For optimization, response surface methodology (RSM) allows for the evaluation of the influence of several factors or independent variables (e.g., time, temperature, solvent percentage, pH, and solid/liquid ratio) on the extraction yield of one or more target compounds. This statistical tool allows for the assessment of the existence of possible interactions between the process factors and the conditions that maximize (or minimize) the selected dependent variables [16,22]. However, the intrinsic characteristics of plant materials, such as chemical composition, degree of polymerization, and solubility, create challenges when attempting to extrapolate the extraction conditions determined from one matrix to another. Therefore, plant material-oriented optimization studies are necessary.

Motivated by the rationale described above, this work was carried out to optimize the dynamic maceration of powdered acerola fruit waste to obtain an anthocyanin-enriched extract for further application as a natural colorant. For that, the effects of the ethanol percentage, temperature, and time were investigated in a central composite rotatable design (CCRD) coupled with RSM for process optimization. The extraction yield and the anthocyanin content (monitored by HPLC-DAD-ESI/MS) in the 20 run extracts were the selected dependent (or response) variables.

2. Materials and Methods

2.1. Plant Material

Acerola fruits without the required standards for commercialization (quality, caliber, appearance, etc.) and therefore considered biowaste were kindly provided by a Brazilian

company in September 2020. The fresh waste sample (≈89.7% moisture) was freeze-dried (FreeZone 4.5, Labconco, Kansas City, MO, USA) to complete dryness and further reduced to a 20-mesh particle size using a domestic grinder. The resulting powdered acerola fruit waste was vacuum-sealed to prevent moisture absorption and stored at −20 °C until use.

2.2. Experimental Design

An RSM-CCRD combining the effects of the factors X_1 (time, t, 2–90 min), X_2 (temperature, T, 20–90 °C), and X_3 (ethanol percentage, E, 0–100% *v/v*) was implemented to optimize the extraction of anthocyanins from acerola fruit waste. These three factors and the respective range of values were selected based on previous studies of the research group [17,23]. Ethanol was chosen because it is a generally recognized as safe (GRAS) solvent permitted for the production of foodstuffs and food ingredients. Design-Expert software Version 11 (Stat-Ease, Inc., Minneapolis, MN, USA) was used to generate the CCRD matrix, which included 8 factorial points, 6 axial points, and 1 center point replicated six times. The 20 runs were randomized to minimize the effects of unexpected variability.

2.3. Dynamic Maceration

Solid–liquid extractions were performed in glass vials in a water bath. Approximately 1 g of sample was mixed with 20 mL of solvent (0, 20, 50, 80, or 100% ethanol, *v/v*) acidified to pH ≈ 3 with 0.05% citric acid and stirred at 5000 rpm (Cimarec™ i Micro Stirrers, Thermo Scientific) at 20, 34, 55, 76 or 90 °C for 2, 20, 46, 72, or 90 min, according to the 20-run CCRD matrix. The mixtures were then centrifuged at 5000 rpm for 10 min at 10 °C, and the collected supernatants were filtered through Whatman No. 4 filter paper. The obtained filtrate solutions were divided into two fractions, one for determining the extraction yield and another for the chromatographic analysis of anthocyanins. Macerations were performed in duplicate, and each dependent variable was measured three times.

2.4. Analysis of Dependent Variables

2.4.1. Extraction Yield

The extraction yield (%, *w/w*) was determined gravimetrically by oven-drying 5 mL of each filtrate solution in pre-calcined porcelain crucibles at 105 °C for 48 h (Figure 1).

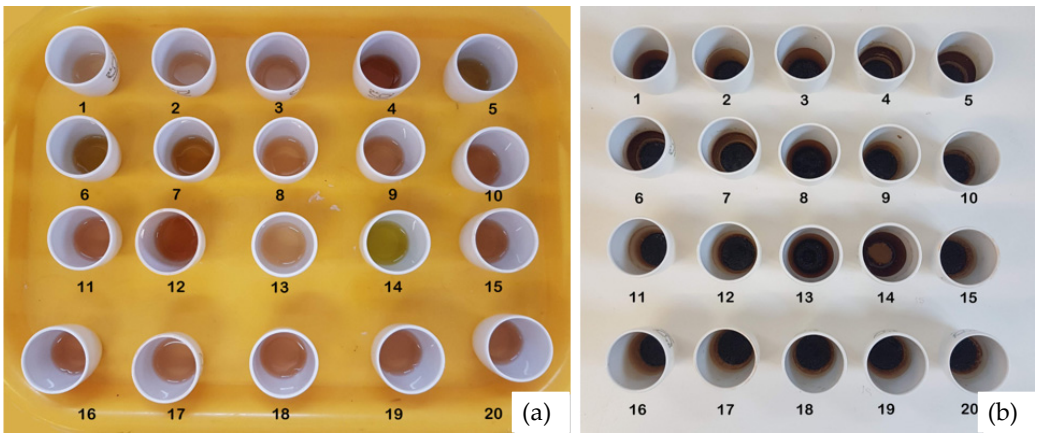

Figure 1. Acerola fruit waste extract solutions obtained with the 20 runs of the experimental design before (**a**) and after (**b**) the determination of the extraction yield. The different color of the liquid extracts is also noticed in the first image.

2.4.2. Anthocyanins Content

Each filtrate solution was filtered through 0.22 µm syringe filter disks and injected in a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA) equipped with a quaternary pump, an automatic injector at 5 °C, a degasser, and a thermostated column compartment. Compound separation was carried out on an AQUA® (Phenomenex,

Torrance, CA) reverse phase column (150 mm × 4.6 mm, 5 µm, C18), following analytical specifications previously described [24]. Detection was performed with a diode array detector (DAD) at 280, 330, and 370 nm and an electrospray ionization mass spectrometry (MS) detector. Compound identification was achieved by comparing the samples' retention time, fragmentation pattern, and UV–Vis spectrum with those of cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-glucoside standards (purity ≥ 96%) acquired from Extrasynthèse (Genay, France). These commercial anthocyanins (0.25–50 µg/mL) were used to construct the seven-level calibration curves $y = 134{,}578x - 3 \times 10^6$ ($r^2 = 0.9986$) and $y = 61{,}493x - 628{,}875$ ($r^2 = 0.9957$), respectively, used to quantify (mg/g extract) the detected compounds.

2.5. Extraction Process Modelling and Statistical Analysis

The dependent (or response) variables Y_1 (extraction yield, %, *w/w*), Y_2 (cyanidin-*O*-deoxyhexoside content—COD, mg/g extract), Y_3 (pelargonidin-*O*-deoxyhexoside content—POD, mg/g extract), and Y_4 (total anthocyanins content—TA, mg/g) were used in the optimization of the dynamic maceration of acerola fruit waste. The response surface models were fitted to the experimental data using the following quadratic equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

where Y corresponds to the dependent variable to be modeled, X defines the factors, b_0 is the constant coefficient, b_1, b_2, and b_3 are linear term coefficients, b_{11}, b_{22}, and b_{33} are quadratic term coefficients, and b_{12}, b_{13}, and b_{23} are interaction term coefficients. Subscripts 1, 2, and 3 in each term stand for *t*, *T*, and *E*, respectively.

The steps of model fitting, coefficient estimation, and statistical analysis were performed using Design-Expert software. The significance of the models and their terms was assessed using an analysis of variance (ANOVA) at a 95% confidence level, and only the significant terms or those necessary for the hierarchy were considered in the fitting procedure. The model F-value, lack-of-fit, R^2 and adjusted R^2 coefficients, adequate precision, and coefficient of variation were the statistical criteria considered to assess the model-fitting adequacy. Design-Expert was also used to generate 2D and 3D plots.

3. Results and Discussion

In this study, the dynamic maceration of powdered acerola fruit waste was optimized by RSM in order to recover anthocyanins [25]. The process combined the effects of processing time, temperature, and ethanol percentage as relevant factors. Using HPLC-DAD-ESI/MS, two anthocyanins were tentatively identified in the obtained extracts, namely cyanidin-*O*-deoxyhexoside (COD), with $[M\text{-}H]^+$ at *m/z* 433, an MS^2 fragment at *m/z* 287(100), and maximum absorption in the UV-Vis range at 330 nm, and pelargonidin-*O*-deoxyhexoside (POD), with $[M\text{-}H]^+$ at *m/z* 417, an MS^2 fragment ion at *m/z* 271(100), and maximum absorption at 330 nm. This result agrees with previous studies reporting cyanidin and pelargonidin anthocyanins in acerola fruit extracts [14,15].

3.1. Experimental Data for Extraction Process Optimization

The results of the 20 experimental runs of the CCRD used to optimize the extraction of anthocyanins from acerola fruit waste by dynamic maceration are shown in Table 1. The extraction yield ranged from 48% to 63% with runs 5 and 8, which combined medium–low time and temperature levels with medium–high ethanol percentage levels or medium–high levels of the three factors, respectively. In turn, the levels of COD and POD ranged from 0.32–1.22 mg/g extract and 0.41–1.10 mg/g extract, respectively, and the total anthocyanins (TA) content varied from 0.73–2.32 mg/g extract. In both cases, the lowest contents originated from run 5, the same run that led to the lower extraction yield, while the highest concentrations were reached with run 1, combining the medium–low levels of the three factors.

Table 1. Extraction yield and anthocyanins content experimentally obtained under the dynamic maceration conditions defined by the CCRD matrix.

Runs	Experimental Domain			Experimental Responses			
	***t* (min)**	***T* (°C)**	***E* (%)**	**Yield (%, *w*/*w*)**	**COD (mg/g Extract)**	**POD (mg/g Extract)**	**TA (mg/g Extract)**
1	20 (−1)	34 (−1)	20 (−1)	54.53	1.413	1.115	2.528
2	72 (+1)	34 (−1)	20 (−1)	53.57	1.110	0.963	2.073
3	20 (−1)	76 (+1)	20 (−1)	55.44	0.946	0.882	1.828
4	72 (+1)	76 (+1)	20 (−1)	53.30	0.723	0.740	1.341
5	20 (−1)	34 (−1)	80 (+1)	48.03	0.320	0.408	0.728
6	72 (+1)	34 (−1)	80 (+1)	58.88	0.727	0.721	1.448
7	20 (−1)	76 (+1)	80 (+1)	52.81	0.666	0.720	1.385
8	72 (+1)	76 (+1)	80 (+1)	63.21	1.220	1.100	2.180
9	2 (−1.68)	55 (0)	50 (0)	50.09	0.956	0.765	1.721
10	90 (−1.68)	55 (0)	50 (0)	58.69	1.010	0.966	1.976
11	46 (0)	20 (−1.68)	50 (0)	50.85	0.946	0.880	1.826
12	46 (0)	90 (−1.68)	50 (0)	52.45	0.741	0.751	1.492
13	46 (0)	55 (0)	0 (−1.68)	58.19	0.857	1.030	1.887
14	46 (0)	55 (0)	100 (−1.68)	58.19	0.396	0.681	1.077
15	46 (0)	55 (0)	50 (0)	51.35	0.836	0.784	1.620
16	46 (0)	55 (0)	50 (0)	52.11	0.924	0.839	1.764
17	46 (0)	55 (0)	50 (0)	50.47	0.802	0.754	1.556
18	46 (0)	55 (0)	50 (0)	53.74	0.903	0.851	1.754
19	46 (0)	55 (0)	50 (0)	50.25	0.812	0.773	1.585
20	46 (0)	55 (0)	50 (0)	52.32	0.843	0.780	1.623

t: time; *T*: temperature; *E*: ethanol percentage; Yield: extraction yield; COD: cyanidin-*O*-deoxyhexoside; POD: pelargonidin-*O*-deoxyhexoside; TA: total anthocyanins.

3.2. Models' Fitting and Statistical Verification

To construct the predictive model equations, the experimental data in Table 1 were fitted to the second-order polynomial Equation (1) using Design-Expert software, but just the significant terms ($p < 0.05$) or those necessary for the hierarchy were considered. The models, expressed in coded values, are presented in Equations (2)–(5). The results of ANOVA and regression analyses are shown in Table 2.

$$Y_{Yield} = 51.7 + 2.39t + 0.91T + 3.04tE + 1.06TE + 0.96t^2 + 2.30E^2 \quad (2)$$

$$Y_{COD} = 0.86 + 0.05t - 0.15E + 0.19tE + 0.21TE + 0.07t^2 - 0.07E^2 \quad (3)$$

$$Y_{POD} = 0.83 + 0.05t - 0.10E + 0.12tE + 0.14TE \quad (4)$$

$$Y_{Total} = 1.66 + 0.07t - 0.25E + 0.31tE + 0.35TE + 0.07t^2 - 0.05E^2 \quad (5)$$

In each model equation, the coefficients represent the linear, quadratic, and/or interaction effects of each process factor on the target responses. Consequently, the magnitude of the coefficient (regardless of its sign) indicates the significance of the corresponding effect. In the case of interaction terms, positive coefficients signify synergistic effects, whereas negative coefficients indicate antagonistic interactions between factors [23]. In Equations (2)–(5), the intercept represents the anticipated mean value resulting from the dynamic macerations conducted with the three factors positioned at the center of the CCRD as shown in Table 1 (i.e., $X = 0$). Under these "mild" conditions, the extraction yield was found to be 51.7% (*w*/*w*), while the dependent variables COD, POD, and TA had values of 0.86 mg/g, 0.86 mg/g, and 1.66 mg/g extract, respectively.

Table 2. Regression coefficients of Equations (2)–(5) estimated by fitting the experimental data in Table 1 to Equation (1) and statistical information of the fitting analysis. Parametric subscripts b_1, b_2, and b_3 stand for time (t), temperature (T), and ethanol percentage (E), respectively.

Regression Coefficients		**Yield**	**COD**	**POD**	**TA**
Intercept	b_0	51.7 ± 0.4	0.86 ± 0.02	0.83 ± 0.01	1.66 ± 0.03
Linear terms	b_1	2.4 ± 0.3	0.05 ± 0.02	0.05 ± 0.01	0.07 ± 0.02
	b_2	0.9 ± 0.3	ns	ns	ns
	b_3	ns	-0.15 ± 0.02	-0.10 ± 0.01	-0.25 ± 0.02
Quadratic terms	b_{11}	0.96 ± 0.3	0.07 ± 0.02	ns	0.07 ± 0.02
	b_{22}	ns	ns	ns	ns
	b_{33}	2.3 ± 0.3	-0.07 ± 0.02	ns	-0.05 ± 0.02
Interactive terms	b_{12}	ns	ns	ns	ns
	b_{13}	3.0 ± 0.4	0.19 ± 0.02	0.12 ± 0.02	0.31 ± 0.03
	b_{23}	1.1 ± 0.4	0.21 ± 0.02	0.14 ± 0.02	0.35 ± 0.03
Modeling Statistics					
Model F-value		34.67	43.45	36.43	66.38
Lack of Fit		ns	ns	ns	ns
R^2		0.9529	0.9620	0.9286	0.9748
Adjusted R^2		0.9254	0.9399	0.9031	0.9601
Adequate precision		22.60	28.20	26.58	36.81
CV (%)		1.92	7.11	6.07	4.67

Yield: extraction yield; COD: cyanidin-*O*-deoxyhexoside; POD: pelargonidin-*O*-deoxyhexoside; TA: total anthocyanins; R^2: coefficient of determination; Adjusted R^2: adjusted coefficient of determination; CV: coefficient of variation or relative standard deviation; ns: not significant.

Equations (2)–(5) presented a non-significant lack-of-fit ($p > 0.05$), which indicated that the theoretical models adequately describe the effects of the factors t, T, and E on the target responses [26]. In all cases, the R^2 and adjusted R^2 coefficients were higher than 0.928 and 0.903, respectively (Table 2), indicating that the variability of each response variable can be explained by the factors involved in the extraction process. In addition, values ≥ 22.6 were obtained for adequate precision, which is a measure of the signal-to-noise ratio that compares the range of the predicted values at the design points to the average prediction error. High accuracy was also demonstrated by the low values of the coefficient of variance (CV). Thus, the developed theoretical models were statistically validated and used in the following steps to predict the optimal conditions for the extraction of anthocyanins from acerola fruit waste through dynamic maceration.

Certain peculiarities regarding the overall effects of the process factors on the extraction of anthocyanins can be inferred from the complexity of the model equations. Based on Equation (1) and Table 2, it can be concluded that the extraction yield was significantly affected by the three factors involved in the process. The extraction time was the most relevant process variable, affecting the extraction through positive linear (b_1 = 2.39) and quadratic (b_{11} = 0.96) effects, and strongly interacted with the solvent (b_{13} = 3.04). The ethanol percentage ranked second and caused positive quadratic effects (b_{33} = 2.30) and interacted with the temperature (b_{23} = 1.06), which, in turn, caused a linear effect on the response (b_3 = 0.91). On the other hand, Equation (5) reflects the complexity of the extraction trend regarding total anthocyanins (TAs), in which it is interesting to note the negative linear (b_3 = −0.25) and quadratic (b_{33} = −0.05) effects of ethanol percentage and its positive interactions with the other two process variables (b_{13} = 0.31 and b_{23} = 0.35). Equations (3) and (4) showed extraction trends somewhat similar to those in model Equation (5), with parametric values more marked in Equation (3). Overall, these results support the use of RSM, since one-factor-at-a-time approaches do not assess interaction effects.

3.3. Effect of the Process Factors and Optimal Dynamic Maceration Conditions

The response surface graphs built to visually illustrate the effect of the process factors in the extraction of acerola fruit waste anthocyanins are presented in Figure 2. In each graph, the unplotted factor (*t*, *T*, or *E*) was kept constant at its individual optimal value shown in Table 3. The extraction yield (extract weight) was promoted by longer processing times at high temperatures and using higher ethanol percentages. In fact, as evidenced by the parametric values of the interaction effects (Table 2), the increase in ethanol percentage induced a synergism in the extraction when combined with longer times and, mainly, with higher temperatures. The effect of each factor on the extraction yield is visually demonstrated in Figure 3, depicting 2D individual responses. The factors not plotted in each graph were fixed at their optimum value.

Table 3. Processing conditions that maximize the recovery of anthocyanins from acerola fruit waste by dynamic maceration.

	Optimal Processing Conditions			**Response Optimum**
	***t* (min)**	***T* (°C)**	***E* (%)**	
Individual Conditions for Each Response Variable				
Extraction yield	73.9	62.7	85.8	63.9 ± 0.8% (*w/w*)
Cyanidin-*O*-deoxyhexoside	20.0	33.5	21.3	1.38 ± 0.04 mg/g extract
Pelargonidin-*O*-deoxyhexoside	36.3	42.9	0.0	1.19 ± 0.03 mg/g extract
Total anthocyanins	23.7	34.4	18.1	2.55 ± 0.06 mg/g extract
Global Conditions Considering All Response Variables				
Extraction yield	24.8	40.5	11.8	57.1 ± 0.8% (*w/w*)
Cyanidin-*O*-deoxyhexoside				1.35 ± 0.05 mg/g extract
Pelargonidin-*O*-deoxyhexoside				1.16 ± 0.04 mg/g extract
Total anthocyanins				2.54 ± 0.06 mg/g extract

t: time; *T*: temperature; *E*: ethanol percentage.

The individual projections in Figure 3 show the opposite extraction trend observed for anthocyanins compared to the extraction yield. The lower the variable ranges, the higher the recovery rate of anthocyanins. Moreover, the two anthocyanins presented similar response surfaces (Figure 2). In addition to low ethanol percentages being more favorable for the recovery of these water-soluble vacuolar pigments, they seemed to be negatively affected by longer extraction times and higher temperatures, which may have caused their degradation. Therefore, it can be concluded that, although the 73.9 min processing at 62.7 °C with 85.8% ethanol maximizes the extraction yield to 63.9% (*w/w*) (Table 3), other compounds in addition to anthocyanins are being extracted. Interestingly, the conditions that favored the extraction of anthocyanins were more sustainable, requiring a lower processing time (23.7 min), temperature (34.4 °C), and ethanol percentage (18.1%, *v/v*) to reach 2.55 mg/g extract. Thus, selectivity for anthocyanins can be achieved by applying these time-saving and eco-efficient conditions of dynamic maceration. It should be noted that, during numerical optimization in Design-Expert software, the factors were set within the experimental range, while the response variables were set at "maximize". In addition, equal "importance" of goals was also given to the variables.

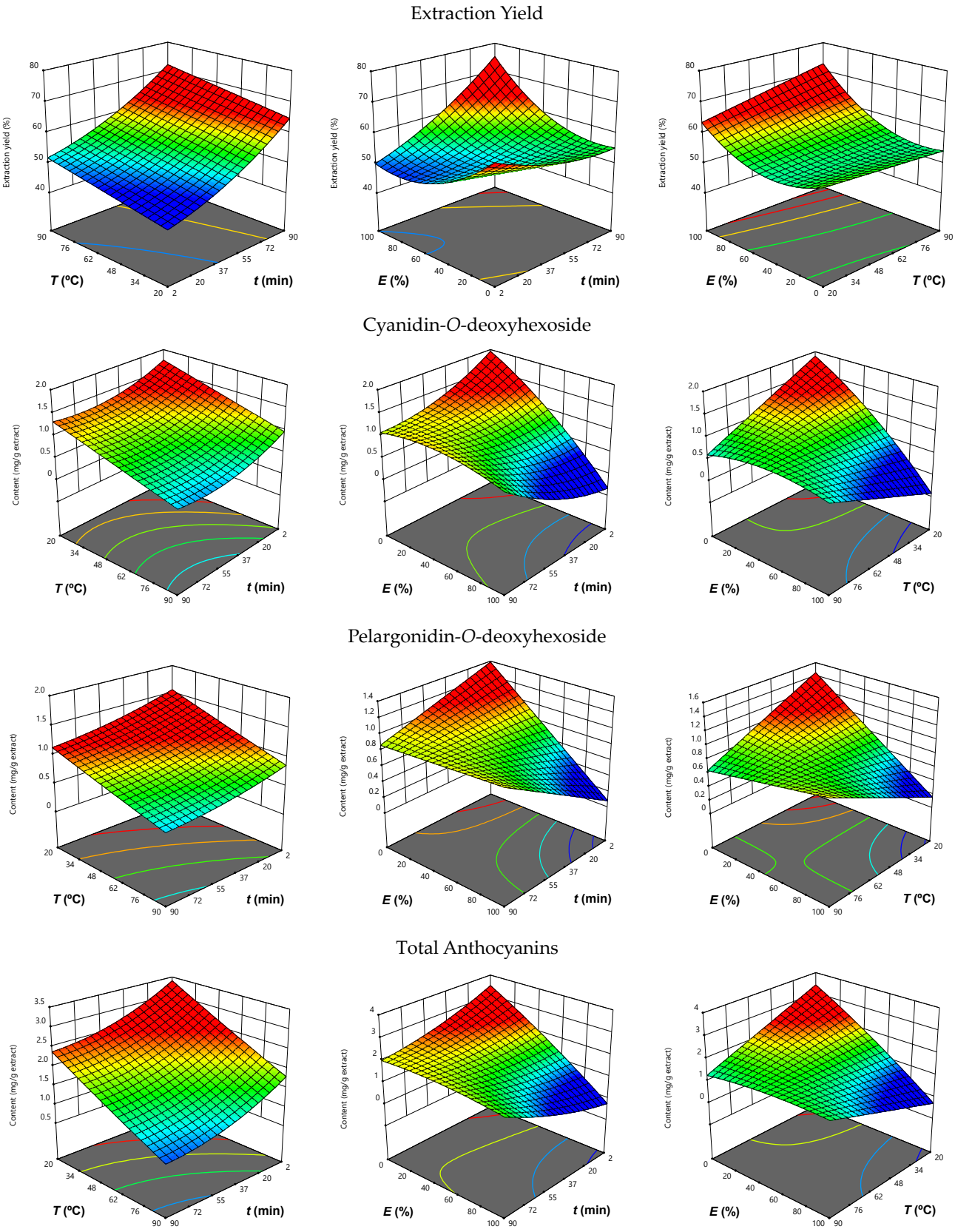

Figure 2. Response surface graphs illustrating the combined effects of the factors time (*t*), temperature (*T*), and ethanol percentage (*E*) on the extraction yield and anthocyanins content obtained from acerola fruit waste. In each graph, the unplotted factor was kept constant at its optimum value shown in Table 3.

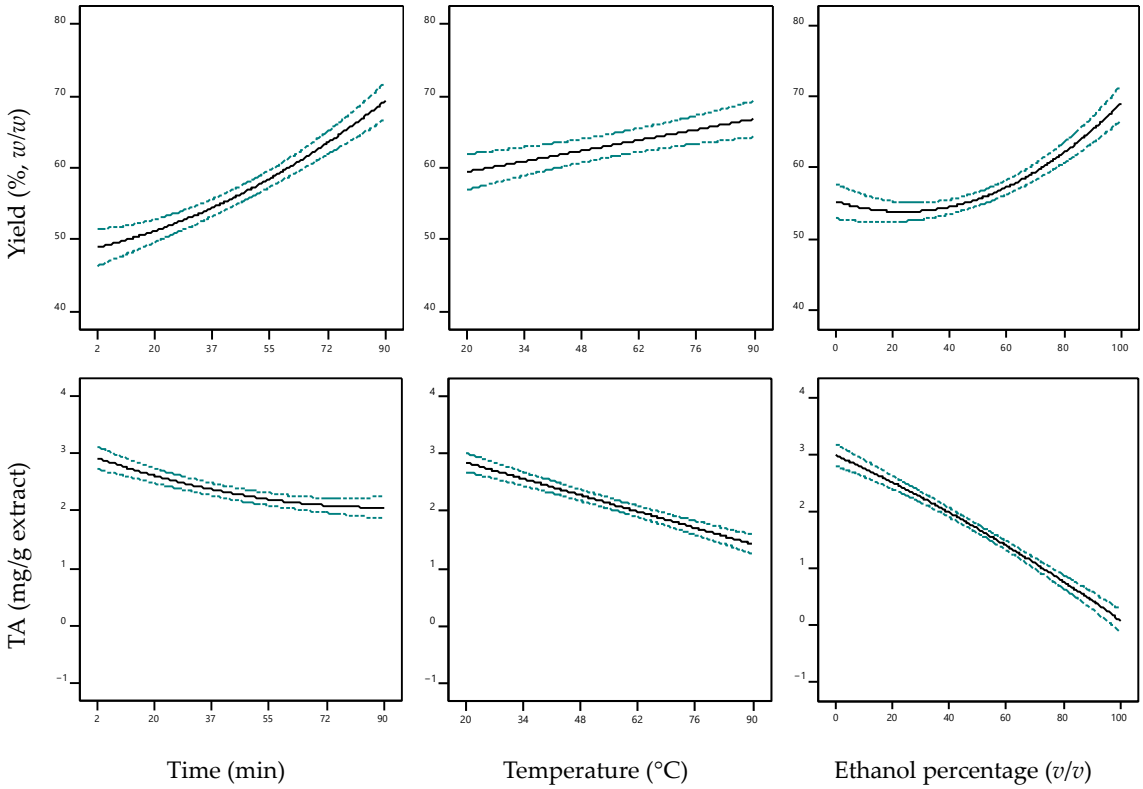

Figure 3. Individual 2D response graphs for the effects of the factors on the extraction yield and total anthocyanins content obtained from acerola fruit waste. In each graph, 95% confidence interval bands are represented in green and the unplotted factors were kept constant at their optimum value shown in Table 3. Yield: extraction yield; TA: total anthocyanins.

For industries interested in anthocyanin-rich extracts to be used as natural colorants, it is important to obtain a large amount of both extract weight and natural pigment through sustainable processes. Therefore, processing conditions that simultaneously maximize all responses were also determined in this work (Table 3). Based on this second optimization step, 24.8 min extraction at 40.5 °C with 11.8% ethanol was found to be the optimal setting to simultaneously maximize the extraction yield (57.1%, *w/w*) and anthocyanins content (2.54 mg/g extract) as much as possible. Compared to the individual dynamic maceration conditions (Table 3), these global settings were associated with less than 0.5% loss of total anthocyanins and about 10% extraction yield. These processing conditions found for acerola fruit waste are comparable to those previously reported for the recovery of anthocyanins from jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg.) epicarp (namely cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside; 21.8 min processing at 47.1 °C with 9.1% ethanol) [27] and roselle (*Hibiscus sabdariffa* L.) calyces (cyanidin-3-*O*-sambubioside and delphinidin-3-*O*-sambubioside; 30 min processing at 30 °C with 0% ethanol) [28] and more sustainable than those reported for the extraction of red raspberry (*Rubus idaeus* L.) anthocyanins (cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside; 75.7 min processing at 38.1 °C with 21.4% ethanol) [23].

In previous studies with acerola, Rezende et al. [29] optimized the extraction of bioactive compounds from acerola pulp and its residue from juice extraction through a 17-run CCRD, combining the effects of time (10–60 min), ethanol percentage (0–99.5%, acidified to pH 2 with hydrochloric acid (2 M)), and liquid/solid ratio (1–10 mL/g). For total anthocyanins, whose contents were measured by a spectrophotometric method, the process was significantly affected by the ethanol percentage and liquid/solid ratio and their interaction effects, which positively influenced the extraction. However, optimum processing conditions were determined only for the general set of response variables (total

anthocyanins, carotenoids, ascorbic acid, total phenolics and flavonoids, and antioxidant activity) and the use of data from colorimetric determinations may have influenced the outcome somewhat. The extraction of bioactive compounds from acerola waste was also optimized by Silva et al. [30]. The combined effects of processing time, temperature, liquid/solid ratio, and ethanol percentage were assessed in a central composite design, and extraction patterns were evaluated considering the results of total phenolics and flavonoids and antioxidant activity as dependent variables. As mentioned above, the use of experimental data from colorimetric methods may not be the most appropriate approach to follow in optimization studies due to the possible selectivity and sensitivity limitations of these analytical determinations that may affect the quantitative data. Furthermore, although the extract obtained at the optimum point was characterized by HPLC-UV, no anthocyanins were described. Therefore, to the best of the authors' knowledge, the present work is the first optimization study focused on individual anthocyanins rather than total compounds, thus bringing novel insights on the impact of the tested extraction factors.

4. Conclusions

The dynamic maceration process of powdered acerola fruit waste for pigment recovery was optimized by RSM in order to obtain an anthocyanin-rich extract with coloring properties. The applied RSM-CCRD allowed for assessing the linear, quadratic, and interaction effects of the key factors, time, temperature, and ethanol proportion, which were assembled into statistically valid predictive quadratic equations. Anthocyanin recovery was favored by lower ethanol percentages, while longer processing and higher temperatures negatively impacted these pigments. Notably, the developed time-saving maceration process exhibited selectivity towards anthocyanins. In addition to the optimum processing conditions determined for each response variable, global settings were also established (24.8 min at 40.5 °C with 11.8% ethanol) and predicted an extraction yield of 57.1% (*w/w*) and recovery of 2.54 mg of anthocyanins (sum of cyanidin-*O*-deoxyhexoside and pelargonidin-*O*-deoxyhexoside) per gram of extract. Overall, this could be a sustainable valorization strategy for upcycling this agri-food waste into a valuable ingredient with the potential for industrial exploitation, which is aligned with resource-efficient circular economy concepts. The food, nutraceutical, and cosmetic industries could be the main interested parties in the developed natural ingredient, as well as in the large-scale exploitation of the extraction process. In future studies, it would be interesting to investigate the effects of the solid/liquid ratio and pH on the extraction rate, as well as the stability, bioactivity, and color quality of the anthocyanin-rich extract produced from acerola fruit waste.

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