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Ultrasound-Assisted Extraction of Flavonoids from Kiwi Peel: Process Optimization and Bioactivity Assessment

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Citation: Giordano, M.; Pinela, J.; Dias, M.I.; Calhelha, R.C.; Stojković, D.; Soković, M.; Tavares, D.; Cánepa, A.L.; Ferreira, I.C.F.R.; Caleja, C.; et al. Ultrasound-Assisted Extraction of Flavonoids from Kiwi Peel: Process Optimization and Bioactivity Assessment. *Appl. Sci.* **2021**, *11*, 6416. https://doi.org/10.3390/app11146416

Academic Editors: Gwiazdowska Daniela, Krzysztof Juś and Katarzyna Marchwińska

Received: 1 June 2021
Accepted: 8 July 2021
Published: 12 July 2021

Abstract: The nutritional quality of kiwifruit has been highlighted by several studies, while its peel is typically discarded as a by-product with no commercial value. This study was carried out to optimize the ultrasound-assisted extraction (UAE) of phenolic compounds from kiwi peel. Three independent variables (time (*t*), ultrasonic power (*P*) and ethanol concentration (EtOH)) were combined in a five-level central composite rotatable design coupled with the response surface methodology (RSM). The extraction yield determined gravimetrically and the content of phenolic compounds identified by HPLC-DAD-ESI/MS^n (namely two quercetin glycosides, one catechin isomer and one B-type (epi)catechin dimer) were the experimental responses used in the optimization. The polynomial models were successfully fitted to the experimental data and used to determine the optimal UAE conditions. The sonication of the sample at 94.4 W for 14.8 min, using 68.4% ethanol, resulted in a maximum of 1.51 ± 0.04 mg of flavonoids per g of extract, a result that allowed the experimental validation of the predictive model. The kiwi peel extract obtained under optimized conditions showed somehow promising bioactive properties, including antioxidant and antimicrobial effects, and no toxicity to Vero cells. Overall, this study contributes to the valorization of kiwi peel as a low-cost raw material for the development of natural ingredients (such as food preservatives) and also to the resource-use efficiency and circular bioeconomy.

Keywords: *Actinidia deliciosa*; by-product valorization; extraction optimization; bioactive properties; natural ingredients

1. Introduction

In the food industry, approximately 30% of the food produced each year is discarded worldwide. This represents 1300 million tons of food, one billion dollars in economic costs, 700 billion dollars in environmental costs and approximately 900 billion dollars in social costs [1]. Moreover, the peels, leaves, roots, tubers and seeds of fruits and vegetables that are discarded annually generate around 25% to 30% of the food industry waste [2]. These biowaste and by-products can cause environmental problems, such as aquatic life toxicity, surface and ground water contamination, changes in soil quality, greenhouse gases emissions, in addition to attracting disease vectors such as insects and rodents, among others [3]. Therefore, several alternatives have been studied to avoid

this environmental scenario and obtain economic benefits through the valorization and recycling of the generated by-products [2].

The attempt to valorize plant by-products has aroused great interest in their recycling as a low-cost material for the development of bio-based ingredients with a high content of phenolic compounds, dietary fibers, minerals, and other phytochemicals [2,4]. These high added-value compounds have been correlated with health-promoting effects due to their antioxidant, anti-inflammatory, anti-mutagenic, and anti-diabetic properties, among others [5]. Today there is a large amount and diversity of agri-food by-products with an extremely variable composition, but its potential as sources of bioactive molecules is still little explored, being a niche of opportunities for obtaining high added-value molecules that can be introduced in the food cycle as natural food ingredients [5,6]. This approach contributes to the resource-use efficacy and circularity, as well as to the growing consumer' demand for food products formulated with alternative natural additives.

According to the literature, kiwi was originally a wild fruit native to China, but its commercial exploitation began in New Zealand with the species *Actinidia deliciosa*, of which there are around 75 varieties, "Hayward" being the best known [7]. Regarding nutritional value, this fruit is defined as a good source of nutrients, mainly fibers and minerals, as also of bioactive compounds such as polyphenols, vitamin C, and carotenoids, which display antioxidant properties [8]. Due to these compositional features, this fruit has been extensively studied, while the non-edible parts such as stems, leaves, peels, and seeds have been poorly investigated despite being a potential source of valuable compounds [9]. Thus, the sustainable use of these underused plant parts, especially its peel that is discarded as an industrial by-product with no commercial value, can contribute to its valorization and be achieved through its recycling into bioactive natural ingredients for exploitation by the agri-food sector [8,9].

Today, there are different non-conventional method that can be used to recover bioactive compounds from plant materials, such as the ultrasound-assisted extraction (UAE). During UAE, the physical forces developed by the acoustic cavitation caused by ultrasound waves promote the rupture of the plant tissues and the release of extractable compounds into the solvent in much less time than the conventional methods [2,4,10]. UAE is pointed out as a time-saving non-thermal method, presenting advantages over conventional maceration techniques involving temperature as intensification factor [11]. Therefore, this study was carried out to optimize the UAE of phenolic compounds from kiwi peel using the response surface methodology (RSM), and evaluate the in vitro bioactive properties of the extract obtained under optimized conditions in order to validate its potential to be used as a natural ingredient in the food industry.

2. Materials and Methods

2.1. Samples Preparation

Kiwi (*Actinidia deliciosa* cv. "Hayward") fruit samples were provided by KiwiCoop®, an organization of kiwi producers based in Oliveira do Bairro, Portugal. The fruit peel was separated from the pulp, lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) and stored under vacuum at −20 °C until further analysis.

2.2. Experimental Design for Extraction Optimization

A central composite rotatable design (CCRD) combining five-levels of the independent variables X_1 (time, t, 1–45 min), X_2 (ultrasonic power, P, 5–500 W) and X_3 (ethanol concentration, EtOH, 0–100%, *v/v*) was implemented to optimize the extraction of flavonoids from kiwi peel using RSM (Supplementary Material Table S1). These variables and the respective range of values were selected based on previous optimization studies [11,12]. Design-Expert software, Version 11 (Stat-Ease, Inc., Minneapolis, MN, USA) was used to generate the 20 experimental points of the CCRD design, which included eight factorial points, six axial or star points chosen to allow rotatability, and six replicated center points. The 20 runs were randomized to minimize the effects of unexpected variability.

2.3. Ultrasound-Assisted Extractions (UAE)

The UAE was performed using an ultrasonic system (QSonica sonicators, model CL-334, Newtown, CT, USA) equipped with a titanium probe. A known sample weight (1.5 g) was mixed with 50 mL of solvent (0–100%) and sonicated at 5–500 W (at 20 kHz frequency) for 1–45 min. The temperature (~25 °C) and solid/liquid ratio (30 g/L) were kept constant. After processing, the mixtures were centrifuged at 450 rpm for 5 min and filtered through Whatman no. 4 filter paper. An aliquot of each filtrate was filtered through syringe filter discs for high-performance liquid chromatography (HPLC) analysis of flavonoids, another was used for determination of the extraction yield (extract weight or extracted solids, %, *w/w*) by gravimetry, and the remaining filtrate was lyophilized for further evaluation of bioactive properties.

2.4. Identification and Quantification of Phenolic Compounds

For analysis of phenolic compounds, a UPLC Dionex Ultimate 3000 system was used (Thermo Scientific, San Jose, CA, USA) following the analytical procedure previously described by Bessada et al. [13]. Detection was performed with a diode array detector (DAD) and a Linear Ion Trap (LTQ XL) mass spectrometer (Thermo Finnigan, San Jose, CA, USA) working in negative mode and equipped with an electrospray ionization (ESI) source. Chromatographic separation was made on a Waters Spherisorb S3 ODS-2 column (3 μm, 4.6 mm × 150 mm; Waters, Milford, MA, USA). The identification was achieved as previously described [13]. For quantitative analysis, 7-level calibration curves were constructed based on the UV-Vis signal of the quercetin-3-*O*-glucoside ($y = 21719x + 88805$; $r^2 = 0.9994$) and (-)-catechin ($y = 8387.1x + 71124$; $r^2 = 0.9964$) standards. The analysis was performed in triplicate and the results were expressed in mg per g of dry weight (dw).

2.5. Extraction Process Modelling and Statistical Analysis

The dependent variables Y_1 (extraction yield, %, *w/w*), Y_2 (B-type (epi)catechin dimer content, mg/g), Y_3 (epicatechin content, mg/g), Y_4 (quercetin-3-*O*-glucoside content, mg/g), Y_5 (quercetin-3-*O*-rhamnoside content, mg/g), and Y_6 (total flavonoids content, mg/g) were used in the extraction process optimization. The response surface models were fitted by means of least squares calculation using the following second-order polynomial equation:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{\substack{i=1 \\ j>i}}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} {X_i}^2 \quad (1)$$

where Y corresponds to the dependent variable to be modelled, X_i and X_j define the independent variables, b_0 is the constant coefficient, b_i is the coefficient of the linear effect, b_{ij} is the coefficient of the interaction effect, b_{ii} is the coefficient of the quadratic effect, and n is the number of variables ($n = 3$).

Fitting procedures, coefficient estimates, and statistical analysis were performed using the Design-Expert software. Analysis of variance (ANOVA) was used to assess the significance of the models and of all the terms that make up the models, as well as the lack-of-fit. Only the statistically significant terms ($p < 0.05$) were used in the models' construction (except those required to ensure hierarchy). Coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}), and adequate precision were used to estimate the adequacy of the polynomial equation to the response. The lack-of-fit measures the quality of the model's fit to the experimental data; thus, it must be non-significant ($p > 0.05$).

2.6. Models Validation and Evaluation of the Bioactivity of the Extract Produced under Optimized Extraction Conditions

The optimized global UAE conditions that maximize the recovery of flavonoids from kiwi peel were applied to obtain a flavonoid-rich extract, following the procedure

described above. This extract was used for experimental validation of the theoretical models, performed through the analysis of the experimental responses (extraction yield and flavonoid content) of this new extract and comparison with the model-predicted values. The in vitro bioactivity of this extract was also evaluated as described below.

2.7. Bioactivities Evaluation

2.7.1. Antioxidant Activity

To evaluate the extract capacity to inhibit the formation of thiobarbituric acid reactive substances (TBARS), an in vitro assay based on the monitoring of malondialdehyde (MDA)-TBA complexes was implemented as previously reported [14]. Porcine brain cells were used as biological substrates. The results were expressed as IC_{50} values (mg/mL).

The extract capacity to protect sheep erythrocytes from oxidative haemolysis was tested by the OxHLIA assay [14]. Briefly, an erythrocyte solution (2.8%, *v/v*) prepared in phosphate-buffered saline (PBS, pH 7.4) was mixed with either: (i) extract solution in PBS, (ii) Trolox in PBS, (iii) PBS (control), or (iv) water (100% haemolysis). After pre-incubation at 37 °C for 10 min with shaking, 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) (160 mM in PBS) was added, and the optical density was measured at 690 nm over time until complete haemolysis. The results were given as IC_{50} values (mg/mL) at a 60 min Δt.

2.7.2. Anti-Inflammatory Activity

The anti-inflammatory activity was evaluated by the extract capacity to inhibit the nitric oxide (NO) production by lipopolysaccharide (LPS)-stimulated RAW 264.7 cells, following a protocol previously described by Barros et al. [15]. Dexamethasone was used as positive control. The results were expressed as IC_{50} values (μg/mL).

2.7.3. Cytotoxicity to Tumor Cell Lines and Hepatotoxic Activity

The extract capacity to inhibit the cell growth was screened against human tumor cell lines, namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), AGS (gastric adenocarcinoma), and CaCo-2 (colorectal adenocarcinoma), and the normal African Green Monkey kidney epithelial Vero cell line. The Sulforhodamine B assay was followed as previously described [16]. Ellipticine was used as positive control. The results were expressed as GI_{50} values (μg/mL).

2.7.4. Antimicrobial Activity

The antibacterial activity was evaluated following the methodology previously described by Soković et al. [17] against the Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 35030), and *Salmonella* Typhimurium (ATCC 13311), and the Gram-positive bacteria *Listeria monocytogenes* (NCTC 7973), *Staphylococcus aureus* (ATCC 11632), and *Bacillus cereus* (food isolate). The results were given as minimum inhibitory and bactericidal concentrations (MIC and MBC, respectively).

For antifungal activity, the methodology described by Soković and van Griensven [18] was implemented and the microfungi *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *Penicillium aurantiogriseum* (food isolate), and *Trichoderma viride* (IAM 5061) were tested. MIC and minimum fungicidal concentration (MFC) were determined.

All microorganisms were obtained from the Mycological Laboratory, Institute for Biological Research "Sinisa Stanković", University of Belgrade (Belgrade, Serbia). Sodium benzoate (E211) and potassium metabisulfite (E224) were used as positive controls.

3. Results and Discussion

The extraction of polyphenols from plant materials is affected by different factors related to the compositional and structural nature of the plant material (which must be reduced to a small particle size to increase the sample-to-solvent contact area) and to the factors applied during the extraction process, such as solvent type, temperature, ultrasonic

power, solid/liquid ratio, and processing time. The selection of extraction methods and the determination of processing conditions that maximize the recovery of bioactive compounds has gained particular interest in recent years due to the current trend to valorize and recycle agri-food by-products [2,4]. In this sense, efforts have been made to develop more efficient and sustainable extraction processes, capable of improving extraction yield and selectivity [11,19,20]. However, the extrapolation of results obtained with different natural matrices or using different extraction techniques can be a difficult or wrong task. Therefore, in this study, the suitability of UAE to recover flavonoids from kiwi peel was investigated and optimized by RSM.

3.1. Phenolic Profile of the Kiwi Peel Extract and Experimental Data for UAE Optimization

Kiwi is rich in polyphenols with antioxidant activity and often described as a "super-fruit" due to its low caloric value and high amount of water, dietary fiber, and vitamin C, among other nutrients [21,22]. The HPLC-DAD-ESI/MS^n (Table 1) analysis allowed the tentative identification of two quercetin glycosides, namely quercetin-3-*O*-glucoside (identified based on the pseudomolecular ion $[M-H]^-$ at *m/z* 463 and the fragment ion at *m/z* 301 and by comparison with the available standard compound) and quercetin-3-*O*-ramnoside (pseudomolecular ion $[M-H]^-$ at *m/z* at 477 and fragment ion at *m/z* 301), as also two flavan-3-ols, namely epicatechin (identified based on the pseudomolecular ion $[M-H]^-$ at *m/z* 289 and fragments at *m/z* 245, 205, and 179), and a B-type (epi)catechin dimer (pseudomolecular ion $[M-H]^-$ at *m/z* 577 and fragments at *m/z* 451, 425, 407, and 289). A representative HPLC phenolic profile of the kiwi peel extracts is present in Supplementary Materials Figure S1.

Table 1. Flavonoids composition of the kiwi peel extract. The retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), and mass spectral data are presented.

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative Identification
1 [A]	5.85	281	577	451(27), 425(100), 407(32), 289(10)	B-type (epi)catechin dimer
2 [A]	7.63	280	289	245(100), 205(41), 179(17)	Epicatechin
3 [B]	18.71	352	463	301(100)	Quercetin-3-*O*-glucoside
4 [B]	20.28	351	477	301(100)	Quercetin-3-*O*-rhamnoside

Calibration curves used in the quantification: (A) catechin ($y = 8387.1x + 71{,}124$, $r^2 = 0.9964$) and (B) quercetin-3-*O*-glucoside ($y = 21719x + 88{,}805$, $r^2 = 0.9994$).

The results of the 20 experimental runs of the CCRD design applied to optimize the UAE of flavonoids from the kiwi peel are presented in Table 2. The extraction yield ranged from approximately 37% to 59% with runs 14, involving 100% ethanol and medium levels of the other two independent variables, and 12 and 4, which combined 500 W power with medium levels (0) of the other variables and medium-high levels of time and ultrasonic power with a medium-low ethanol concentration, respectively. In turn, the total content of flavonoids ranged from 1.00 to 1.75 mg/g of extract and the lowest values were obtained with runs 9 and 10, corresponding to the use of the lowest and highest levels (α values) of the variable time, mainly due to the low levels of epicatechin achieved with these runs. In general, the recovery of the B-type (epi)catechin dimer appeared to have been promoted by the two axial points corresponding to the extraction solvent (runs 13 and 14), while both quercetin glycosides were better extracted with the run 10.

Table 2. Experimental responses obtained under the extraction conditions defined by the CCRD design matrix for the extraction yield and flavonoids content.

Runs	Experimental Domain			Experimental Responses *					
	t (min)	*P* (W)	EtOH (%)	Y_1 (%, w/w)	Y_2 (mg/g dw)	Y_3 (mg/g dw)	Y_4 (mg/g dw)	Y_5 (mg/g dw)	Y_6 (mg/g dw)
1	10 (−1)	106 (−1)	20 (−1)	47.90	0.5389	0.6237	0.1411	0.1380	1.4416
2	36 (+1)	106 (−1)	20 (−1)	50.63	0.4629	0.8580	0.1440	0.1448	1.6097
3	10 (−1)	400 (+1)	20 (−1)	54.09	0.4954	0.4684	0.0601	0.1385	1.1624
4	36 (+1)	400 (+1)	20 (−1)	59.14	0.4873	0.7263	0.0601	0.1388	1.4126
5	10 (−1)	106 (−1)	80 (+1)	45.09	0.5013	0.4366	0.1415	0.1421	1.2214
6	36 (+1)	106 (−1)	80 (+1)	45.66	0.4892	0.4393	0.1430	0.1423	1.2138
7	10 (−1)	400 (+1)	80 (+1)	47.35	0.5249	0.4374	0.1442	0.1409	1.2475
8	36 (+1)	400 (+1)	80 (+1)	51.10	0.5126	0.3645	0.1451	0.1429	1.1651
9	1 (−1.68)	253 (0)	50 (0)	50.44	0.4215	0.2826	0.1498	0.1462	1.0001
10	45 (+1.68)	253 (0)	50 (0)	51.96	0.3686	0.3884	0.1622	0.1493	1.0685
11	23 (0)	5 (−1.68)	50 (0)	50.43	0.4304	1.0289	0.1446	0.1464	1.7502
12	23 (0)	500 (+1.68)	50 (0)	59.02	0.4258	1.2575	0.0833	0.1487	1.6300
13	23 (0)	253 (0)	0 (−1.68)	48.35	0.5983	0.6216	0.0851	0.1329	1.4379
14	23 (0)	253 (0)	100 (+1.68)	36.87	0.6038	0.3010	0.1378	0.1361	1.1787
15	23 (0)	253 (0)	50 (0)	52.02	0.3067	1.1225	0.1565	0.1471	1.7328
16	23 (0)	253 (0)	50 (0)	46.13	0.2932	0.9786	0.1518	0.1449	1.5685
17	23 (0)	253 (0)	50 (0)	49.73	0.3074	1.0002	0.1535	0.1459	1.6070
18	23 (0)	253 (0)	50 (0)	50.58	0.3172	1.0508	0.1547	0.1468	1.6695
19	23 (0)	253 (0)	50 (0)	50.02	0.3068	1.0231	0.1536	0.1455	1.6290
20	23 (0)	253 (0)	50 (0)	51.38	0.3189	1.0933	0.1592	0.1474	1.7189

* It is presented the mean value of three determinations. Y_1: extraction yield; Y_2: B-type (epi)catechin dimer; Y_3: epicatechin; Y_4: quercetin-3-*O*-glucoside; Y_5: quercetin-3-*O*-rhamnoside; Y_6: total content of flavonoids.

3.2. Models Fitting and Statistical Verification

The response values in Table 2 were fitted to the second-order polynomial Equation (1) using the Design-Expert software, but just the significant parameters (assessed at a 95% confidence level) were used in the development of the theoretical models. The results of ANOVA and regression analyses are presented in Supplementary Materials Table S2. The developed polynomial models, expressed in coded values, are presented in Equations (2)–(7):

$$Y_1 = 50.4 + 1.1t + 2.7P - 3.1S + 1.8P^2 - 2.5S^2 \quad (2)$$

$$Y_2 = 0.308 - 0.014t + 0.002P + 0.002S + 0.034t^2 + 0.046P^2 + 0.107S^2 \quad (3)$$

$$Y_3 = 1.04 + 0.04t - 0.11S - 0.07tS - 0.26t^2 - 0.22S^2 \quad (4)$$

$$Y_4 = 0.155 - 0.019P + 0.002S + 0.021PS - 0.015P^2 - 0.016S^2 \quad (5)$$

$$Y_5 = 0.1464 + 0.0011t + 0.001S - 0.0046S^2 \quad (6)$$

$$Y_6 = 1.66 + 0.03t - 0.05P - 0.09S - 0.06tS + 0.06PS - 0.22t^2 - 0.13S^2 \quad (7)$$

In the mathematical models developed for each response variable, the coefficients of the terms *t*, *P*, and *S* illustrate the effect of the independent variables time, ultrasonic power, and ethanol concentration, respectively, and their interactions. Since the expected effects on the response are denoted by the parametric values, the higher the parametric value, the more significant the weight of the respective variable will be, regardless of its sign. For interactions, synergistic effects are denoted by a positive sign, while an antagonistic interaction between variables is translated by a negative sign [23]. In each model Equations (2)–(7), the intercept corresponds to the overall average response of the 20 experimental runs of the CCRD design in Table 2. Based on the intercept values, epicatechin stands out as the main phenolic compound (corresponding to almost 63% of the total content) identified in the kiwi peel by-product, followed by B-type (epi)catechin dimer (18.6%), and the two quercetin glycosides (18.2%) (Supplementary Materials Table S2).

All Equations (2)–(7) presented a non-significant lack-of-fit ($p > 0.05$) and an adequate precision greater than 22.4, which indicates that the theoretical models adequately describe the effects of the independent variables on the target responses [24]. The coefficients R^2 and R^2_{adj} were higher than 0.888 and 0.864 in all cases, respectively (Supplementary Material Table S2), indicating that the variability of each response can be explained by

the independent variables involved in the extraction. In addition, values $\geq$ 22.49 were obtained for adequate precision, which is a measure of 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. Thus, the developed theoretical models were statistically validated and used in the following steps to predict the optimal UAE conditions for recovery of polyphenols from kiwi peel.

Certain peculiarities regarding the overall effects of the independent variables on the UAE of flavonoids from kiwi peel can be inferred from the complexity of the model equations. Based on Equation (2) and Supplementary Material Table S2, it can be settled that the extraction yield was significantly affected by the three independent variables involved in the extraction. The ethanol concentration was the most relevant process variable, effecting the extraction through negative linear and quadratic effects. The ultrasonic power ranked second and caused positive linear and quadratic effects, while the extraction time only induced a linear effect. No integrations between variables were observed for the extraction yield. In turn, Equation (7) translates the complexity of the extraction trend over the total flavonoid content, where it is interesting to highlight the negative quadratic effects of the variables time and ethanol concentration, as well as the negative and positive interactions of ethanol concentration with time and ultrasonic power, respectively. This result supports the use of RSM as optimization tool, since the one-factor-at-a-time approaches do not assess interactive effects. The individual flavonoid compounds appeared to have been somewhat differently affected, mainly B-type (epi)catechin dimer.

3.3. Effect of the Extraction Parameters on the Responses

The 3D response surface graphs constructed to illustrate the effect of the independent variables involved in the UAE of flavonoids from kiwi peel are presented in Figure 1. In each graph, the excluded variable was positioned at its individual optimal value shown in Table 3. The B-type (epi)catechin dimer showed an opposite extraction tendency compared to that of the other flavonoids.

As observed in Figure 1, the increase in the value of the three variables up to intermediate levels decreased the extraction of this proanthocyanidin, but the consequent increase induced a new improvement in its recovery rate. The extraction trend thus followed quadratic effects, which were more pronounced for EtOH > P > t. In this particular case, the central projections of the surfaces at the base of each 3D graph illustrate the variable ranges where the extraction of B-type (epi)catechin dimer was lower. Regarding epicatechin, its extraction was promoted by the increase in processing time and ethanol concentration up to around 25 min and 42% EtOH, respectively, but the consequent increased caused a reduction in the recovery yield, probably due to its degradation. Actually, the red colored response surface area of the 3D graph of the combined effects of these two variables illustrates very well the optimal response (Figure 1). Contrariwise, the ultrasonic power did not significantly affect ($p > 0.05$) the extraction of this antioxidant flavonoid (Supplementary Material Table S2). Based on these observations and the reverse response surfaces of B-type (epi)catechin dimer and epicatechin (Figure 1), it may be suggested that the UAE process may have promoted the breakdown of this B-type proanthocyanidin into its flavanol subunits.

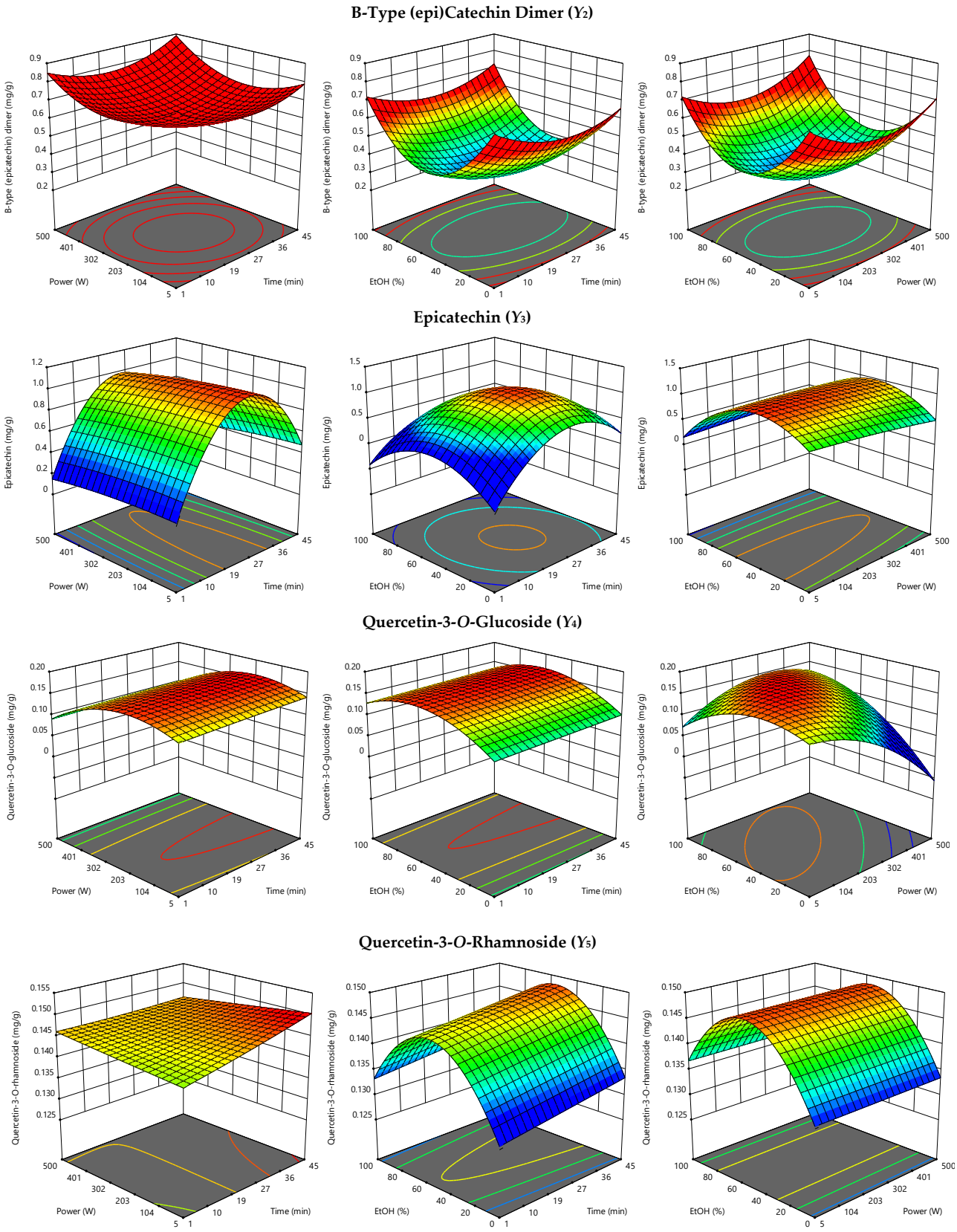

Figure 1. Response surface graphs for the combined effects of the independent variables on the content (mg/g) of individual flavonoids (Y_2–Y_5) recovered from kiwi peel. In each graph, the excluded variable was fixed at its optimal value.

Table 3. Optimal processing conditions that maximize the extraction of flavonoids from kiwi peel and model-predicted and experimental response optimum.

	Optimal Processing Conditions			Response Optimum	
	Time (min)	**Power (W)**	**EtOH (%)**	**Model-Predicted Values**	**Experimental Values**
Individual conditions for each response variable					
Extraction yield (extract)	34.4	483.0	34.1	61 ± 1% (*w/w*)	-
B-type (epi)catechin dimer	11.2	393.0	94.8	0.64 ± 0.01 mg/g dw	-
Epicatechin	24.6	222.6	41.6	1.06 ± 0.02 mg/g dw	-
Quercetin-3-*O*-glucoside	39.2	191.4	59.2	0.164 ± 0.002 mg/g dw	-
Quercetin-3-*O*-rhamnoside	45.0	257.6	53.2	0.148 ± 0.001 mg/g dw	-
Total flavonoids	24.9	5.0	34.1	1.82 ± 0.03 mg/g dw	-
Global conditions considering all response variables					
Extraction yield (extract)	14.8	94.4	68.4	46 ± 1% (*w/w*)	46 ± 2% (*w/w*)
B-type (epi)catechin dimer				0.426 ± 0.008 mg/g dw	0.432 ± 0.006 mg/g dw
Epicatechin				0.78 ± 0.02 mg/g dw	0.78 ± 0.04 mg/g dw
Quercetin-3-*O*-glucoside				0.148 ± 0.002 mg/g dw	0.150 ± 0.003 mg/g dw
Quercetin-3-*O*-rhamnoside				0.145 ± 0.001 mg/g dw	0.1468 ± 0.0002 mg/g dw
Total flavonoids				1.49 ± 0.03 mg/g dw	1.51 ± 0.04 mg/g dw

For the two quercetin glycosides, the 20 experimental runs of the CCRD design yielded comparable mean responses, as shown by the intercession values in Equations (5)–(6) and Supplementary Material Table S2. The longer the processing time, the greater the linear recovery of these quercetin glycosides, but this variable was significant ($p < 0.05$) only for quercetin-3-*O*-rhamnoside. In turn, the ultrasonic power merely impacted the extraction of quercetin-3-*O*-glucoside, whose yield increased with the application of up to ~190 W and then decreased for higher powers. Both compounds were significantly affected by the extraction solvent ($p < 0.05$); while the recovery of quercetin-3-*O*-glucoside mainly followed a linear effect, quadratic effects marked the trend of quercetin-3-*O*-rhamnoside extraction. Ethanol/water ranging from 50 to 60% appeared to be preferable mixtures. In the particular case of quercetin-3-*O*-glucoside, it is also interesting to note the strong positive interaction between ultrasonic power and ethanol concentration.

Figure 2 illustrates the response surface graphs for the effects of the independent variables on the total content of flavonoids and extraction yield obtained from kiwi peel. The excluded variable in each 3D graph was fixed at its optimal value. In general, the extraction of the total flavonoids content follows a trend comparable to that discussed above for epicatechin, with additional interactive effects between the solvent and the other two variables. So, the lower the ethanol concentration and ultrasonic power (EtOH × *P*), the greater the recovery of flavonoids, while lower ethanol concentrations combined with longer processing times (EtOH × *t*) seem to improve the UAE process efficiency.

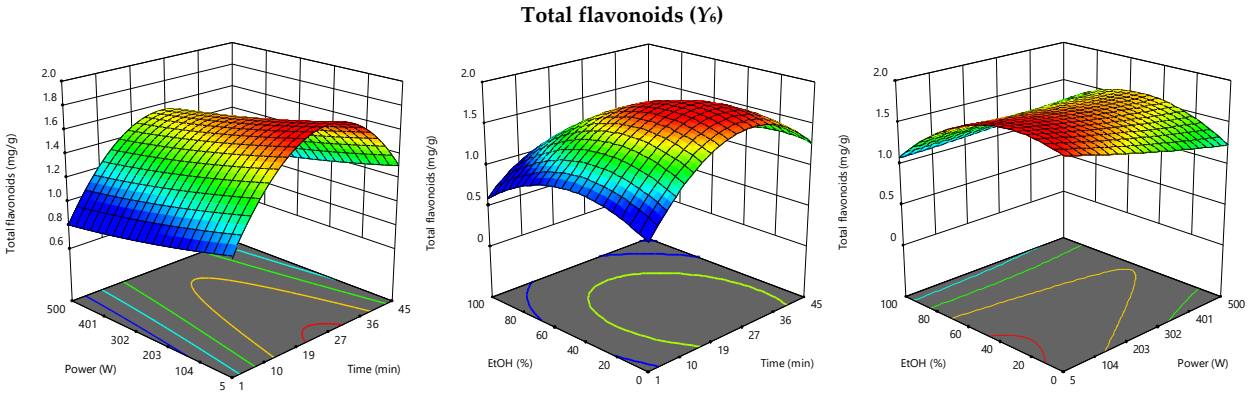

Figure 2. *Cont.*

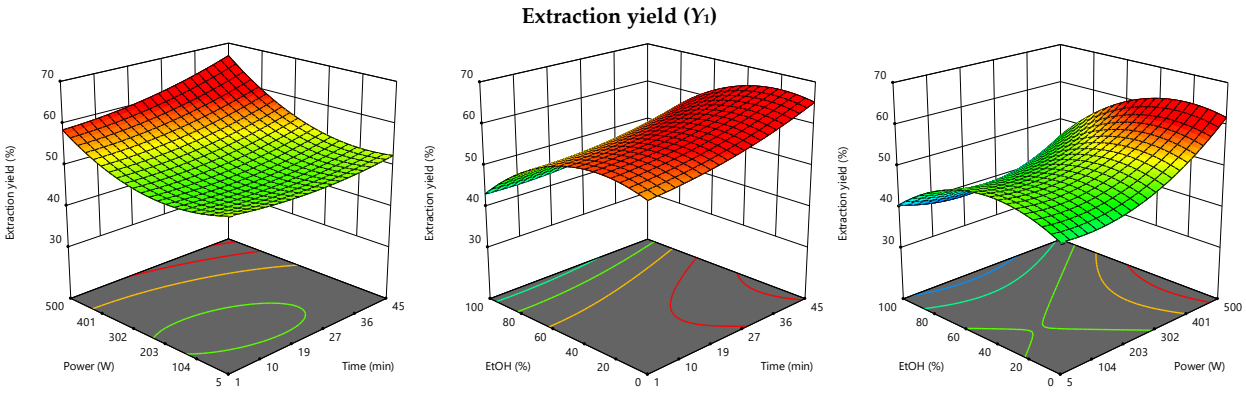

Figure 2. Response surface graphs for the combined effects of the independent variables on the total content of flavonoids (Y_6) and extraction yield (Y_1) obtained from kiwi peel. In each graph, the excluded variable was fixed at its optimal value.

The extraction solvent was the variables that most affected the extraction yield and the highest extract weights were obtained when sonicating the kiwi peel sample with lower ethanol concentrations (Figure 2). A higher ultrasonic power and longer extraction times also favored the recovery rate. However, it should be noted that the increase in the extract weight does not necessarily resulted in a greater extraction of flavonoids. As seen in Figures 2 and 3, although the more intense conditions of time and ultrasonic power have led to a greater extract weight, these have negatively affected the total flavonoids content. Therefore, compounds other than flavonoids were being extracted (possibly fibers and other carbohydrates, given the intrinsic nature of the kiwi peel) and represented the major fraction of the obtained extract. The optimal points of these two responses are also shown in the cube plots in Supplementary Materials Figure S2A,B, respectively, which shows how the three variables combine to affect the response. A flavonoid-rich extract can thus be obtained more selectively through the control of these two process variables (t and P). Interestingly, the ethanol percentage that led to a greater amount of from kiwi peel extract also favored the recovery of flavonoids (Figures 2 and 3 and Supplementary Materials Figure S2).

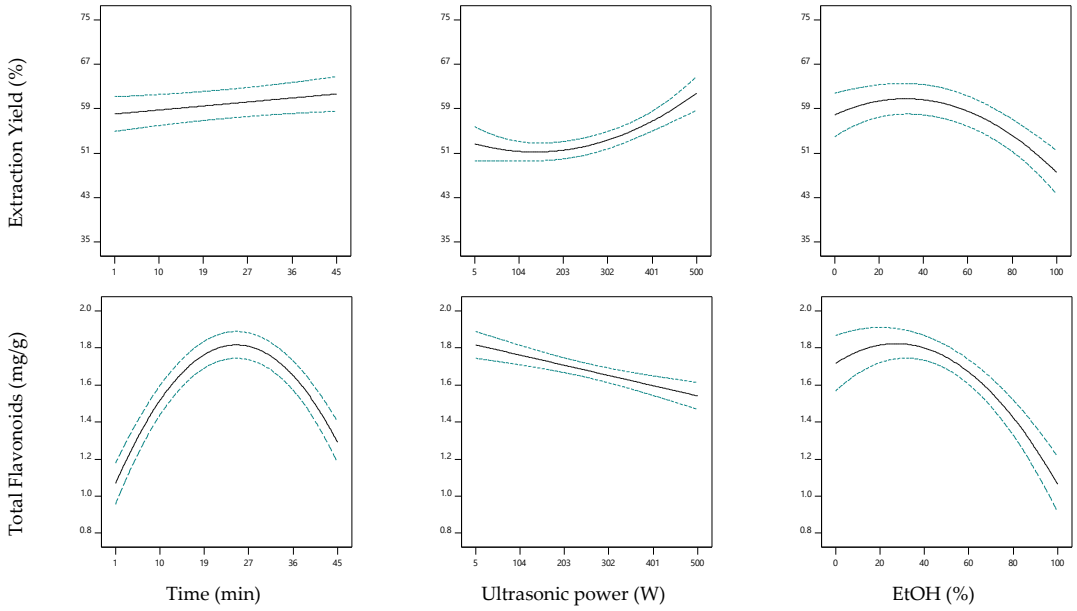

Figure 3. 2D response graphs for the effects of the independent variables on the total content of flavonoids (Y_6) and extraction yield (Y_1) obtained from kiwi peel. In each graph, the excluded variables were fixed at their optimal value.

3.4. Optimal UAE Conditions

For numerical optimization of the UAE process, the Design-Expert software used searches for a combination of factor levels that simultaneously met the requirements placed on each dependent and independent variable. The optimization required that goals be defined for both types of variables, in order to combine all goals into a desirability function. To find a good set of conditions that met the desired goals, the three independent variables were set within the experimental range, while the response or dependent variable was set at maximum. In addition, equal "importance" of goals was given to the variables. The model-predicted UAE conditions that maximize each individual response to optimal values are presented in Table 3. The maximum extraction yield of 61% was obtained by processing the kiwi peel sample at 483 W for 34.4 min with 34% ethanol. In turn, 1.82 mg/g dw of total flavonoids can be reached by sonicating the powdered sample at a power of only 5 W for 25 min, using 69% ethanol. While B-type (epi)catechin dimer was better extracted with a mere 11 min processing with a high power of 393 W and 95% ethanol, epicatechin required 24 min sonication at 223 W, using 42% ethanol. The quercetin glycosides required a longer sonication time of 39–45 min at 191–258 W using 53–59% ethanol, yielding 0.312 mg/g dw of both compounds.

Since for the industrial sector interested in bioactive plant extracts it is important to obtain a high amount of both extract and bioactive compounds through sustainable processes, global UAE conditions that simultaneously maximize all responses were also determined (Table 3 and Supplementary Materials Figure S2C). Based on this second optimization step, 15 min sonication at 94.4 W, using 68.4% ethanol as solvent, were found to be the optimal conditions to simultaneously maximize the response variables as much as possible (46 ± 1% extraction yield and 1.49 ± 0.03 mg/g dw of flavonoids).

3.5. Experimental Validation of the Predictive Model

The global UAE conditions that maximize both the extraction yield and the recovery of flavonoids from kiwi peel (Table 3) were experimentally tested in triplicate to evaluate the predictive accuracy of the model and to obtain a flavonoid-rich extract for evaluation of in vitro bioactive properties. As shown in Table 3, the experimental data were in good agreement with the model-predicted values, as confirmed by the post-analysis verification performed in Design-Expert software (α = 0.05). The UAE yielded 46 ± 2% of crude extract, a value that did not differ from the predicted 46 ± 1%, and 1.51 mg/g dw of total flavonoids. The predictive capacity of the model was thus experimentally validated.

3.6. Bioactivity of the Kiwi Peel Extract Obtained under Optimized UAE Conditions

3.6.1. Antioxidant Activity

The kiwi peel extract obtained under the global UAE conditions (Table 3) was tested for its capacity to inhibit the formation of thiobarbituric acid reactive substances (TBARS) and the oxidative hemolysis (OxHLIA). The results were expressed in IC_{50} values, which correspond to the extract concentration that provides 50% of antioxidant activity in the TBARS assay or required to protect 50% of the erythrocyte population from the free radicals generated by AAPH for a Δt of 60 min. Therefore, the lower the IC_{50} values, the greater the antioxidant activity [14]. The extract showed IC_{50} values of 0.37 ± 0.02 mg/mL and 1.01 ± 0.01 mg/mL for TBARS and OxHLIA, respectively, while the trolox presented a considerably higher activity (IC_{50} values of 5.4 ± 0.3 µg/mL and 21.8 ± 0.3 µg/mL, respectively). However, trolox is a pure antioxidant compound whereas the extract may contain other constituents without bioactivity, which justify the observed differences.

Kiwifruit has been described as having a high antioxidant capacity [8], but some studies on its peel have also been carried out. Dias et al. [25] compared the bioactive potential of the pulp and peel of two kiwi varieties and highlight the green kiwi peel as the sample with greater antioxidant activity than the others, including the pulps. These results are in agreement with those of Bernardes et al. [26] and Fiorentino et al. [27], who described higher values of antioxidant activity for kiwi peel than for pulp. The same conclusion

was reached by Soquetta [28], who compared flours obtained from by-products (epicarp and bagasse) of two kiwi varieties, which observed a greater antioxidant activity in the flours of the peel than in those of bagasse. The difference in the IC_{50} values described in these studies can be justified by the different kiwi varieties or by the different extraction methodologies and solvents applied, as well as by the treatment performed to obtain the plant sample. Considering that the extract evaluated in the present work was obtained under optimized conditions that aimed at maximizing the flavonoids content, a better performance was expected in the in vitro assays.

3.6.2. Cytotoxic and Anti-Inflammatory Activity

The cytotoxic activity of the kiwi peel extract against tumor (MCF-7, NCI-H460, AGS and CaCo-2) and non-tumor (Vero) cell lines was evaluated, as well as its anti-inflammatory activity via NO production inhibition. The extract showed activity against NCI-H460 (non-small cell lung cancer) cells, with an GI_{50} of 309 ± 16 µg/mL. However, no activity was observed against other cell lines (MCF-7, AGS and CaCo-2) at the tested concentrations (GI_{50} > 400 µg/mL). Despite these results, Moita [29] attributed cytotoxic effects to kiwi peel and pulp extracts against HepG2 (hepatocellular carcinoma) and CaCo-2 cells. Dias et al. [25] also demonstrated that kiwi peel hydroethanolic extracts have cytotoxicity to MCF-7, NCI-H460, HepG2 and HeLa (cervical carcinoma) cells. In turn, Lim et al. [30] investigated the anti-proliferative effects of kiwi extracts against HepG2, HT29 (colon carcinoma) and LoVo (colon carcinoma) tumor cells and observed that the extracts did not effectively inhibit cell proliferation. The absence of toxicity to non-tumor cells was herein demonstrated with the Vero cells (GI_{50} > 400 µg/mL). The kiwi peel extract also did not show anti-inflammatory activity, contrary to what has been described in the studies by An et al. [31] and Dias et al. [25].

The difference observed between the activities presented by our sample and the literature data may be explained by the fact that the data were obtained from different plant parts, by differences in the adopted analytical and extraction methodologies and in the used proportions of solvents, but also because they are from different varieties or species of the same genus. Therefore, further studies using different human tumor cell lines and purified compounds will be needed to confirm the bioactivity of the kiwi peel compounds.

3.6.3. Antimicrobial Activity

The antimicrobial activity of the kiwi peel extract was tested against some bacteria, namely *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Enterobacter cloacae* and *Salmonella* Typhimurium, and some fungi, namely *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium funiculosum*, *Penicillium aurantiogriseum* and *Trichoderma viride*. To compare the effectiveness of the kiwi peel extract, two synthetic food preservatives, sodium benzoate (E211) and potassium metabisulfite (E224), were used as positive controls. Table 4 shows the obtained values of minimum inhibitory and bactericidal or fungicidal concentration (MIC, MBC and MFC, respectively). As shown in Table 4, *Staphylococcus aureus* and *Escherichia coli* were the most sensitive bacteria to the tested extract and *Aspergillus ochraceus*, *Aspergillus versicolor*, *Penicillium funiculosum* and *Trichoderma viride* were the most sensitive fungi.

For antimicrobial activity, the MIC and MBC values show that the kiwi peel extract behaves very similar to the synthetic preservative E224, when it was used against Gram-positive bacteria, such as *Bacillus cereus* and *Staphylococcus aureus*, but adopts a behavior similar to the preservative synthetic E211, when tested against Gram-negative bacteria, such as *Escherichia coli* and *Enterobacter cloacae*. Regarding the bacteria *Listeria monocytogenes* and *Salmonella* Typhimurium, the extract was less effective compared to the respective controls. In general, the kiwi peel extract appears to have a greater effect against fungi compared to bacteria, with lower MIC and MFC values. In addition, the extracts showed, for most of the microorganisms, better or equal activity than both synthetic preservatives.

Table 4. Antimicrobial activity of the kiwi peel extract obtained under optimized UAE conditions.

	Kiwi Peel Extract		**E211**		**E224**	
Antibacterial activity	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	1	2	4	4	1	1
Bacillus cereus	2	4	0.5	0.5	2	4
Listeria monocytogenes	2	4	1	2	0.5	1
Escherichia coli	1	2	1	2	0.5	1
Salmonella Typhimurium	2	4	1	2	1	1
Enterobacter cloacae	2	4	2	4	0.5	0.5
Antifungal activity	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus ochraceus	0.5	1	1	2	1	1
Aspergillus niger	1	2	1	2	1	1
Aspergillus versicolor	0.5	1	2	2	1	1
Penicillium funiculosum	0.5	1	1	2	0.5	0.5
Penicillium aurantiogriseum	1	2	2	4	1	1
Trichoderma viride	0.5	0.5	1	2	0.5	0.5

E211: sodium benzoate; E224: potassium metabisulfite; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MFC: minimum fungicidal concentration.

These results are in agreement with studies that describe the antimicrobial potential of *Actinide delicious* extracts against bacterial and fungal growth, which may be useful to help prevent food deterioration processes and the occurrence of possible contamination [25]. Kichaoi et al. [32] studied the antimicrobial potential of kiwi, pomegranate and grapefruit peels, and found that, although all fruit peels have great antimicrobial power, in most cases the kiwi peel stood out from the other samples, regardless of the type of extraction and solvent used. Soquetta [28] reported the antimicrobial activity of kiwi peel and bagasse flours against *Salmonella* sp., *Bacillus cereus* and *Staphylococcus aureus*. In a study on the isolation and characterization of an allergenic kiwi protein *in natura*, Gavrovic-Jankulovic et al. [33] verified its antifungal activity against *Saccharomyces carlsbergensis* and *Candida albicans*. Furthermore, Xia and Ng [34] verified the antifungal activity of fresh kiwi against *Fusarium oxysporum*. Therefore, the results presented in this study are somewhat in agreement with the results previously reported in the literature. It can also be concluded that the observed effects could be attributed not only to phenolic compounds, but also to other bioactive constituents of this plant by-product.

4. Conclusions

The chromatographic analysis allowed identifying four flavonoids in kiwi peel extracts, namely B-type (epi)catechin dimer, epicatechin, quercetin-3-*O*-glucoside, and quercetin-3-*O*-rhamnoside. In order to valorize this by-product as a source of bioactive compounds, a five-level CCRD design coupled to RSM was successfully implemented. The UAE was significantly affected by the independent variables time, ultrasonic power and ethanol concentration, and the theoretical models, fitted to the experimental data by means of least squares calculation using a second-order polynomial equation, were validated based on different statistical criteria. Under the optimized overall UAE condition, it was possible to obtain 46% extract weight and 1.51 mg/g dw of flavonoids. After experimental validation of the predictive model, the bioactivity of the kiwi peel extract obtained under the optimal UAE conditions was evaluated in vitro, showing a somehow promising bioactive potential. Thus, this study contributed to the valorization of kiwi peel through its recycling into a bioactive extract with potential for application in the food industry as a natural preservative (given the antioxidant and antimicrobial properties). The reuse of kiwi peel could also contribute to a circular bioeconomy and resource-use efficiency.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11146416/s1, Figure S1: HPLC-chromatographic profile of phenolic compounds in kiwi peel extract and chemical structure of (–)-epicatechin and quercetin-3-*O*-glucoside. The compounds

identification is presented in Table 1. Figure S2: Cube plots illustrating the optimal values as a function of the three independent variables for extraction yield (A), total content of flavonoids (B), and all responses simultaneously (C). The values at each vertex of the first two cubes are response values. The model-predicted values represented in each cube plot are shown in Table 3; Table S1: Natural and codded values of the independent variables used in the five-level central composite rotatable design (CCRD) used to optimize the extraction of flavonoids from kiwi peel.; Table S2. Parametric values estimated with the polynomial Equation (1) and statistical information of the model fitting procedure. Parametric superscripted 1, 2, and 3 stand for the variables time, ultrasonic power, and ethanol concentration, respectively.

Author Contributions: Conceptualization, L.B., I.C.F.R.F., and C.C.; methodology, M.G., C.C., J.P., M.I.D., R.C.C. and D.S.; software, J.P.; validation, M.S., A.L.C., I.C.F.R.F. and L.B.; formal analysis, M.G., C.C., J.P., M.I.D., R.C.C. and D.S.; writing—original draft preparation, M.G., C.C., J.P. and M.I.D.; writing—review and editing, J.P., R.C.C., D.S., M.S., D.T., A.L.C., I.C.F.R.F. and L.B.; supervision, C.C., A.L.C., I.C.F.R.F. and L.B.; funding acquisition, L.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research conducted under the project "BIOMA—Bioeconomy integrated solutions for the mobilization of the Agro-food market" (POCI-01-0247-FEDER-046112), by "BIOMA" Consortium, and financed by European Regional Development Fund (ERDF), through the Incentive System to Research and Technological development, within the Portugal2020 Competitiveness and Internationalization Operational Program. This work has been supported by the Ministry of Education, Science and Technological Development of Republic of Serbia (451-03-9/2021-14/200007).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020). M.I. Dias, R.C. Calhelha, and L. Barros thank the national funding by FCT, P.I., through the institutional scientific employment program-contract, and J. Pinela through the individual scientific employment program-contract (CEECIND/01011/2018). C. Caleja thanks her contract through the project Healthy-PETFOOD (POCI-01-0247-FEDER-047073). The authors also thank the company KiwiCoop (Oliveira do Bairro, Portugal) for providing the kiwi samples.

Conflicts of Interest: The authors declare no conflict of interest.

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