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Optimization of Carotenoids and Other Antioxidant Compounds Extraction from Carrot Peels Using Response Surface Methodology

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Abstract: Carrots, scientifically known as *Daucus carota* L., are among the most popular and widely consumed vegetables. They are used for cooking and juice production, both industrially and in households, resulting in large amounts of waste each year, mainly from the peel. The peels are rich in antioxidant compounds that can be used either as cosmetics or as food and feed additives. Therefore, in this work, the extraction of these compounds was optimized using green techniques (pulsed electric field and/or ultrasonication) and solvents. Response surface methodology was applied to achieve the optimization. Under optimum conditions, the total polyphenol yield was 8.26 mg gallic acid equivalents per g dry weight (dw) and the total carotenoid content was 137.44 μg β-carotene equivalents per g dw. The optimum extract reportedly showed an antioxidant capacity of 76.57 μmol ascorbic acid equivalents (AAE) per g dw by FRAP assay and 63.48 μmol AAE per g dw by DPPH assay, while the total ascorbic acid content was 2.55 mg per g dw. Furthermore, chromatographic quantification of individual bioactive compounds through a diode array detector was performed, wherein catechin yielded the highest proportion (18.6%) of the total 6.88 mg/g dw. This study addressed inquiries regarding the valorization of bioactive compounds from carrot peels, as well as several strategies for recovering their diverse bioactive components using green procedures and solvents.

Keywords: *Daucus carota*; catechin; chlorogenic acid; polyphenols; flavonoids; ascorbic acid; antioxidant activity; pulsed electric field; ultrasonication; HPLC-DAD

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1. Introduction

Food consumption causes a great amount of plant waste, which might be regarded as a significant source of high-added-value biomolecules. The Food and Agriculture Organization of the United Nations (FAO) has reckoned that ~33% of the edible food cultivated for human consumption is thrown annually, amounting to nearly 1.3 billion metric tons [1]. Fruit wastes present significant environmental challenges, including the greenhouse effect, water and soil pollution, global warming, eutrophication, and various health issues if not managed effectively, owing to their high biodegradability and fermentability [2]. Food waste is typically redirected to economically advantageous non-food applications, including animal feed, compost, or bioenergy sources [3]. This turns waste into value-added products while reducing environmental impact. Natural bioactive compounds in fruits, including carotenoids, quercetin derivatives, phenolic acids, and saponins, are primarily located in the peels, with concentrations decreasing towards the flesh [4]. The peel is reported to contain elevated levels of bioactive compounds that serve to protect the inner

components from degradation caused by insects and microorganisms [5,6]. These bioactive compounds can be utilized as functional additives in medical care. Furthermore, it can serve as an effective method for waste reduction management [7].

Carrots (*Daucus carota* L.) are a globally cultivated crop of the Apiaceae family, produced annually for consumption [8]. Carrots contain a significant amount of polyphenols, flavonoids, and other antioxidant compounds, such as ascorbic acid and carotenoids [9]. Carotenoids and flavonoids are pigments that also contribute to the color of carrots along with their antioxidant properties [10]. Carotenes have been extensively studied for their role in pro-vitamin A, and it remains evident that vitamin A deficiency is the primary cause of early mortality in children [8,11]. Carrots serve as a significant source of vitamin A due to their content of β-carotene, which the human body readily converts into vitamin A. Given the dietary and health advantages, the marketing and development of various products are crucial in meeting individuals' nutrient needs, especially as an economical source of vitamin A [12]. Moreover, β-carotene is extensively employed across the food additive, cosmetics, health care, and pharmaceutical sectors, due to its several advantages, including enhanced human immunity, antioxidant properties, protection against various cancers, and a decreased risk of cardiovascular diseases through its capacity to regulate cholesterol levels [2,13].

In recent years, industry and researchers have endeavored to implement green extraction techniques on food waste and by-products due to sustainable environmental practices [14]. For that reason, multiple green extraction technologies, such as Supercritical Fluid Extraction, High Hydrostatic Pressure, Microwave-Assisted Extraction, Pulsed Electric Field (PEF), and Ultrasound-Assisted Extraction (US) have been employed to extract molecules with high biological activity from fruit and vegetable waste [15–17]. US treatment improves the mass transfer of the extractant and facilitates cell rupture by acoustic cavitation generated by ultrasonic impact in a liquid media [18]. PEF treatment is an environmentally friendly, non-thermal method for food preservation that employs short electrical pulses to inactivate microorganisms, thereby minimizing adverse effects on food quality [19]. Recently, PEF treatment has gained traction for its applications in the diffusion, pressing, osmosis, and drying of plant material, including by-products. Additionally, it mitigates the detrimental effects associated with traditional heating methods and can shockwave cell membranes. Consequently, it could serve as a pretreating technique to improve the extraction of bioactive molecules, including carotenoids and polyphenols [20].

Such green and sustainable extraction techniques could either be deployed as a standalone extraction method to sustainably recover diverse bioactive chemicals from plant sources or employed as a pretreatment, depending on the plant material [21]. Furthermore, green procedures may improve the extraction efficiency relative to traditional methods [14]. The primary object of this research was to identify the optimal combination of eco-friendly pretreatment techniques for generating extracts rich in antioxidant components, such as carotenoids, ascorbic acid, polyphenols, and flavonoids. These extracts could be exploited as feed additives, however, high-added-value extracts could be generated and employed by the food and pharmaceutical industries. Despite substantial research on the isolation of bioactive molecules from carrot peels, insufficient focus has been placed on integrating green pretreatment approaches to optimize yield in certain by-products. Furthermore, it is crucial to highlight that green solvents were employed to uphold an ecologically conscious profile, devoid of hazardous organic solvents. The extraction optimization was achieved by response surface methodology (RSM). The research examined the influence of green binary mixtures of ethanol and water, along with the impact of extraction duration and temperature of the extraction process. Alongside traditional extraction via stirring (ST), environmentally friendly sample pretreatment methods, such as PEF and US, were utilized

to enhance this process. Additionally, the most favorable conditions were ensured through partial least squares (PLS) model analysis.

2. Materials and Methods

2.1. Solvents and Reagents

Anhydrous sodium carbonate was from Penta (Prague, Czech Republic), whereas iron (III) chloride was bought from Merck (Darmstadt, Germany). Methanol, 2,2-diphenyl-1-picrylhydrazyl synthetic radical (DPPH$^{\bullet}$), hydrochloric acid, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), *L*-ascorbic acid, trichloroacetic acid, including β-carotene analytical standard, were obtained from Sigma-Aldrich (Darmstadt, Germany). Ethanol, gallic acid, and Folin–Ciocalteu reagent were obtained from Panreac Co. (Barcelona, Spain). All corresponding analytical standards for the HPLC determination of phenolic acids (β-resorcylic acid, chlorogenic acid, pyrocatechuic acid, caffeic acid, *p*-coumaric acid, ferulic acid) and flavonoids ((+)-catechin hydrate, rutin, quercetin-3-*O*-galactoside, apigenin-7-*O*-glucoside, 4′-hydroxychalcone, chrysin), were obtained from MetaSci (Toronto, ON, Canada), and were at least of 97% purity or higher. The deionized water that was employed in the performed experiments was generated through a deionizing column.

2.2. Instrumentation

The lyophilization process of carrot peels was done through a Biobase BK-FD10 (Jinan, China) freeze-dryer. After being grounded through an electric mill, a sieving process was conducted to isolate only fine powder (<400 μm diameter) by an Analysette 3 PRO sieving apparatus (Fritsch GmbH, Oberstein, Germany). For the stirring (ST) extraction procedure, a magnetic stirring hotplate from Heidolph Instruments GmbH & Co. KG (Schwabach, Germany) was used. For pretreatment procedures, a UPG100 mode/arbitrary waveform generator from ELV Elektronik AG (Leer, Germany), a Leybold high-voltage power generator from LD Didactic, GmbH (Huerth, Germany), a Rigol DS1052E digital oscilloscope (Beaverton, OR, USA), and two custom stainless-steel chambers from Val-Electronic (Athens, Greece) were employed for pulsed electric field (PEF) procedure. An Elmasonic P70H ultrasonication (US) bath purchased from Elma Schmidbauer, GmbH (Singen, Germany) was employed to conduct ultrasonication pretreatment. After extraction procedures, the supernatant was isolated from the extract through centrifugation with a NEYA 16R centrifuge from Remi Elektrotechnik Ltd. (Palghar, India). For spectrophotometric analyses, a Shimadzu UV-1900i double-beam PharmaSpec Spectrophotometer (Kyoto, Japan) was deployed. Finally, a liquid chromatograph, Shimadzu model CBM-20A was utilized for all chromatographic determinations, which was connected with a Shimadzu SPD-M20A diode array detector (DAD) (Shimadzu Europa GmbH, Duisburg, Germany). Molecules separation was done at 40 °C through a column from Phenomenex Inc. (Torrance, CA, USA), model Phenomenex Luna C18(2) (100 Å, 5 μm, 4.6 mm × 250 mm).

2.3. Plant Pretreatment and Extraction Procedure

Carrots were bought from a local grocery store in Karditsa, Greece. The carrots were rinsed and dried thoroughly. Their skin was peeled by hand using a steel knife. After an overnight lyophilization, the moisture was determined at ~88%. The grounded and sieved carrot peels were measured to have a mean particle diameter of 387 μm. Diverse extraction methods with various pre-treatment approaches were employed to obtain the most preferable conditions for the isolation of antioxidant molecules from carrot peels. In every case, 20 mL/g was the solvent-to-solid ratio. The solvents included binary water and ethanol mixtures from 0 to 100% v/v. Pretreatment techniques (i.e., US and PEF) were utilized to augment the traditional stirring extraction process. Prior to the application of

these approaches, the carrot peel powder was left to hydrate for 10 min by adding the suitable solvent. Each sample received treatment for 20 min with the use of PEF or US, separately. If both procedures were employed concurrently, the sample underwent PEF for 20 min treatment succeeded by US treatment for 20 min. Finally, all mixtures underwent an extraction process through stirring (ST). The electric field intensity chosen for the PEF procedure of the samples was constant at 1.0 kV/cm by utilizing a frequency of 1 kHz, within a pulse duration of 10 μs, and with a pulse period of 1 ms. To operate US treatment, the US bath functioned at 37 kHz of frequency and a constant temperature (30 °C).

The conventional stirring process demanded the solvent-powder mixtures to be heated at temperatures between 20 and 80 °C for durations ranging from 30 to 150 min, within 500 rpm of continuous magnetic stirring. Following the extraction process, the sample extracts were subjected to centrifugation for 10 min at 10,000× *g*. Right after, the supernatants were collected and preserved at −40 °C for further examination. Several combinations of the examined factors were required for that process, the coded levels of which are presented in Table 1.

Table 1. The coded and actual levels of the utilized independent variables for the optimization process.

Independent Variables	Code Units	Coded Variable Level				
		1	**2**	**3**	**4**	**5**
Technique	X_1	ST	PEF + ST	US + ST	PEF + US + ST	–
C (%, *v*/*v*)	X_2	0	25	50	75	100
t (min)	X_3	30	60	90	120	150
T (°C)	X_4	20	35	50	65	80

2.4. Experiment Design and Response Surface Methodology (RSM) Optimization

To evaluate the antioxidant properties and quantity of bioactive molecules in carrot peel extracts, the RSM methodology was utilized. The optimization process of bioactive molecules, especially carotenoids, ascorbic acid, and polyphenols through the RSM technique was the primary step. Such an increase could indeed enhance the antioxidant properties of the extracts. This goal was achieved by improving the extraction technique (i.e., ST with or without PEF and US pretreatment techniques) and adjusting the ethanol to water concentration (*C*, % *v*/*v*), extraction duration (*t*, min), and temperature (*T*, °C). The optimization was founded on an experiment utilizing a Main Effect Screening Design with 20 design points. Five levels were used for the process variables as per the experimental design. A minimum threshold of 95% using analysis of variance (ANOVA) and summary-of-fit tests was employed to evaluate the overall model significance (R^2, *p*-value), along with the model coefficients significance. The prediction of response variables of the analyzed independent factors was done by a second-order polynomial model, as represented by Equation (1) below:

$$Y_k = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (1)$$

The independent variables X_i and X_j are shown; the expected response variable is determined as Y_k. The intercept, along with regression coefficients of the linear, quadratic, and interaction terms of the model are denoted as β_0, β_i, β_{ii}, and β_{ij}, respectively.

The model equation acquired from the surface-response equation was visually represented by the 3D plot graphs. The highest peak area was calculated among the effect of a significant independent variable on the response.

2.5. Quantification of Bioactive Compounds

2.5.1. Determination of Total Polyphenol Content (TPC)

The TPC was evaluated spectrophotometrically at 740 nm and expressed as mg gallic acid equivalents (GAE) per g of dry weight (dw) as indicated in Equation (2), based on a prior established method [22]. Prior to this analysis, a calibration curve of 10–100 mg GAE/L water was conducted, in which the total polyphenol concentration, the exact volume of the extraction solvent, and the sample's dried weight were denoted with C_{TP} (in mg/L), V (in L), and w (in g), respectively.

$$\text{TPC (mg GAE/g dw)} = \frac{C_{TP} \times V}{w} \quad (2)$$

2.5.2. Chromatographic Polyphenol Quantification

A High-Performance Liquid Chromatography (HPLC) method was utilized to detect and quantify individual polyphenolic molecules in the carrot extracts. The mobile phase included a binary mixture of 0.5% v/v aqueous formic acid (A) and 0.5% v/v formic acid in acetonitrile (B), the flow rate of which was kept at a constant level of 1 mL/min. Flavonoid and non-flavonoid compounds require different programs in order to be successfully separated and quantified. To that end, the gradient program utilized for the identification of flavonoids included a gradual increase from 5% to 30% B in a 3-min span, followed by 68% B in 34 min, 100% B in 1 min, and a constant value for 3 min and then 5% B in 40 min. On the other hand, the gradient program for the identification of non-flavonoids involved gradual increase from 5% to 12% B in a 15-min span, with subsequent 55% of B in 35 min, and finally 100% B in 1 min, with a constant value for 3 min and then 5% B in 40 min. The identification of the total polyphenolic compounds was done through an absorbance spectrum and retention time comparison to those of pure standards quantified. Their quantification was eligible through calibration curves (0–500 mg/L) of excellent linearity (<0.99). Table S1 provides the equation of calibration curves of each identified compound. The quantification of each compound was conducted at the wavelength where each peak shows a maximum, in accordance with the standards. Throughout the analysis, the separation of the compounds was conducted at a constant temperature of 40 °C.

2.5.3. Determination of Ascorbic Acid Content (AAC)

A previously described protocol by Kalompatsios et al. [22] was used to conduct ascorbic acid spectrophotometric determination at 760 nm. A calibration curve (50–500 mg/L of ascorbic acid in 10% w/v trichloroacetic acid) was used to calculate the specific compound in mg/g dw.

2.5.4. Determination of Total Carotenoids Content (TCC)

The TCC was evaluated using the protocol outlined by Athanasiadis et al. [23], in which the extracts were properly diluted with an appropriate solvent prior to analysis. The TCC was calculated after recording the absorbance at 450 nm in micrograms of β-carotene equivalents per gram of dry weight (μg CtE/g dw), based on a β-carotene calibration curve (0.5–50 μg/mL).

2.6. Antioxidant Assays

2.6.1. Ferric-Reducing Antioxidant Power (FRAP)

An electron transfer-based method developed by Shehata et al. [24] was employed for the assessment of antioxidant activity via FRAP, in which reduced iron cations from $FeCl_3$ with TPTZ solution to form a stable blueish coordination complex (Fe^{+2}–TPTZ). Prior to analysis, the concentration of the potent antioxidant ascorbic acid (C_{AA}) was determined

with a 50–500 μM calibration dissolved in 0.05 M HCl. The ferric-reducing power (P_R) was determined by using Equation (3) as μmoles of ascorbic acid equivalents (AAE) per g of dw, taking into consideration the exact volume of the extraction solvent (in L) and the dried mass of carrot powder (in g).

$$P_R\ (\mu\text{mol AAE/g dw}) = \frac{C_{AA} \times V}{w} \quad (3)$$

2.6.2. DPPH• Scavenging Activity

A radical-scavenging assay was also employed to further assess the antioxidant capacity of carrot peel extracts. Each carrot peel extract was mixed with a purplish DPPH• methanolic solution, as established by Shehata et al. [24]. Subsequently, the depigmentation of DPPH• was determined at 515 nm, in which the initial ($A_{515(i)}$) and final ($A_{515(f)}$) absorbance after 30-min storage in the dark were involved in the calculations shown in Equation (4). To measure the antiradical activity (A_{AR}) of each extract, a widely known potent antioxidant with scavenging potential was employed (i.e., ascorbic acid). The results were calculated as μmol AAE per g of dw, as indicated in Equation (5), by also calculating the extraction solvent volume (in L), and the dried mass of carrot powder (in g).

$$\text{Inhibition}\ (\%) = \frac{A_{515(i)} - A_{515(f)}}{A_{515(i)}} \times 100 \quad (4)$$

$$A_{AR}(\mu\text{mol AAE/g dw}) = \frac{C_{AA} \times V}{w} \quad (5)$$

2.7. Statistical Data Processing

The quantitative analysis of each assay was conducted at least in triplicate, as well as the extraction processes were repeated twice for each batch of carrot peel extracts. JMP® Pro 16 software (SAS, Cary, NC, USA) was used to conduct the statistical process involving RSM and distribution analysis. The data normality was evaluated using Kolmogorov–Smirnov test. Statistically significant differences within the significance level of 95% were evaluated using one-way analysis of variance (ANOVA). The results are reported as means, while also taking into account the standard deviations. Correlation and partial least squares (PLS) analyses, along with Pareto chart were all performed through the same software.

3. Results and Discussion

3.1. Optimization of Extraction Parameters

In recent years, a major shift towards products with potent antioxidant properties has been observed, including in both the food and cosmetics sectors. Food fortification is frequently accomplished by employing food by-products, which are typically abundant in polyphenols and antioxidants. The peels of carrots are a common by-product of vegetation, whether on an industrial or household scale. Consequently, conducting an extraction optimization study to isolate bioactive compounds from carrot peels is essential. For optimization, critical parameters such as the combination of techniques (X_1), the solvent composition (X_2), the effect of extraction time (X_3), and the temperature (X_4) were studied. Previous studies proved that the integration of US and PEF into the extraction procedure can augment its efficiency. PEF is an efficient method with numerous advantages, as it inactivates microorganisms, enhances mass transfer in food products, and facilitates the recovery of valuable bioactive compounds from food waste [16]. PEF has become increasingly popular across various food industries, providing additional incentives for businesses to reduce waste and mitigate environmental impact [25]. Ultrasonication is

also a sustainable technique that offers multiple advantages, such as decreased extraction time, lower energy and power consumption, reduced thermal degradation of bioactive compounds, and the generation of high-quality extracts [14]. Moreover, the composition of the solvent is crucial for enhancing recovery, as the solubility and polarity of polyphenols, carotenoids, and antioxidant compounds are linked to their efficacy [26]. In that context, ethanol is an ideal solvent as it can be combined with ethanol to produce an extraction solvent that is also well-suited for use in the food processing sector [27]. The extraction temperature is another critical factor to consider, as polyphenols, like the majority of bioactive and antioxidant compounds, are thermolabile compounds. Their optimal recovery temperature is between 50 and 80 °C [28]. Similarly, the effect of extraction time needs to be investigated, as both short and long extraction times have been shown to have a positive effect on yield [29].

3.2. Determination of Bioactive Molecules and Antioxidant Capacity

The results of the bioactive compound assays showed substantial variance, suggesting the potential benefits of the extraction optimization process. The TPC range was measured from 0.82 to 9.14 mg/g dw, with design point 12 displaying the highest value. Conventional extraction using 25% v/v ethanol at elevated temperatures and extended durations (i.e., 80 °C for 150 min) were the most effective conditions for polyphenol recovery. This finding also influenced the elevated antioxidant capacity of the extract, as the correlation between total polyphenols and antioxidant activity is well-established [30]. This sample exhibited the highest value in FRAP (3.34–84.84 μmol AAE/g dw) and DPPH (1.13–64.08 μmol AAE/g dw) methods. However, design point 15 revealed the highest values for both ascorbic acid (0.81–4.48 mg/g dw) and total carotenoids (22.42–441.35 μg/g dw). This sample illustrates the significance of both PEF as a pretreatment method and the solvent, specifically the application of 100% v/v ethanol. Nevertheless, the more critical parameters will be described below (*vide infra*).

Molecules such as ascorbic acid, carotenoids, and polyphenols have significant biological activity and enhance the value of our extracts; hence, it was vital to identify them spectrophotometrically to obtain an initial understanding of the composition of each extract. In addition, all mentioned molecules are known to have strong antioxidant capacity. To assess this capacity, two different antioxidant assays were conducted, wherein reducing power and radical scavenging activity (i.e., FRAP and DPPH, respectively) were undertaken. Detailed information about all the conducted assays in each of the 20 design points is shown in Table 2.

The statistical parameters, coefficients for each model, and second-order polynomial equations (models) are all displayed in Table 3 and reveal a strong fit for the resulting models. Figures S1–S5 in the Supplementary Material contains corresponding plots of the predicted versus the actual response for each examined parameter and their desirability functions. Detailed TPC-related 3D response plots are presented in Figure 1 while corresponding 3D plots for the other responses can be found in Figures S6–S9. Figures S1–S3 show that for the TPC, FRAP, and DPPH assays the model has satisfactory desirability, hence the model fits the data perfectly, while in Figures S4 and S5, which refer to the ascorbic acid and carotenoid assays, desirability indicates a good fit. Figures 1 and S6–S9 describe how each parameter affects the extraction results.

Table 2. Findings from the experiment involving the four independent variables with corresponding responses of each dependent variable.

Design Point	Independent Variables				Responses (Actual)				
	X_1	X_2	X_3	X_4	TPC [a]	FRAP [b]	DPPH [b]	AAC [c]	TCC [d]
1	3	1	3	4	3.66	50.63	31.32	0.81	62.14
2	3	2	1	3	7.64	81.27	58.41	1.52	64.92
3	2	3	4	3	7.42	57.81	41.76	1.15	38.10
4	2	4	5	4	6.84	48.26	47.70	2.03	48.17
5	3	5	4	2	0.82	9.93	1.13	2.61	177.67
6	4	1	4	5	5.96	35.56	43.37	1.31	55.27
7	4	2	3	1	2.90	14.28	9.03	1.01	166.61
8	1	3	3	2	5.04	45.31	51.36	1.04	53.40
9	1	4	4	1	4.49	33.29	48.90	1.76	69.44
10	1	5	1	4	1.18	3.34	6.74	2.92	232.97
11	1	1	2	3	3.24	41.53	5.89	1.05	136.91
12	1	2	5	5	9.14	84.85	64.08	1.43	66.21
13	4	3	2	4	6.01	62.48	35.95	1.09	34.86
14	3	4	2	5	6.54	70.23	41.01	1.98	43.29
15	2	5	3	5	3.31	25.30	10.16	4.48	441.35
16	2	1	1	1	4.16	26.51	3.63	1.22	166.62
17	2	2	2	2	3.14	26.60	5.59	0.82	135.39
18	3	3	5	1	3.57	33.09	15.99	0.82	46.84
19	4	4	1	2	4.65	45.03	27.64	1.75	22.42
20	4	5	5	3	1.50	13.87	3.10	2.36	195.09

[a] Values calculated in mg GAE/g dw; [b] values calculated in μmol AAE/g dw; [c] values calculated in mg/g dw; [d] values calculated in μg CtE/g dw.

Table 3. Mathematical models utilizing RSM were applied to optimize the extraction process from the carrot peels. The models present only important terms.

Second-Order Polynomial Equations (Models)	R^2 Predicted	R^2 Adjusted	*p*-Value	Eq.
$TPC = 1.51 + 0.25X_1 + 3.56X_2 - 1.15X_3 - 0.41X_4 - 0.67{X_2}^2 + 0.32{X_3}^2 - 0.43X_1X_3 + 0.29X_1X_4 + 0.15X_3X_4$	0.9075	0.8243	0.0004	(6)
$FRAP = -33.67 + 17.85X_1 + 19.84X_2 - 8.29X_3 + 25.81X_4 - 5.63{X_2}^2 + 3.04{X_3}^2 - 6.71X_1X_3 + 4.31X_2X_3 - 1.83X_2X_4 - 2.98X_3X_4$	0.9355	0.8639	0.0003	(7)
$DPPH = -24.03 + 10.39X_1 + 29.08X_2 + 0.37X_3 - 5.36X_4 - 4.22{X_2}^2 + 2.96{X_3}^2 - 2.57X_1X_2 - 6.12X_1X_3 + 4.33X_1X_4$	0.8442	0.7039	0.0048	(8)
$AAC = 2.57 - 1.01X_2 - 0.58X_4 + 0.20{X_2}^2 + 0.08{X_4}^2 + 0.09X_2X_4$	0.8803	0.8376	<0.0001	(9)
$TCC = 505.58 - 256.56X_2 - 71.29X_4 + 34.01{X_2}^2 + 23.69X_2X_4$	0.7242	0.6507	0.0004	(10)

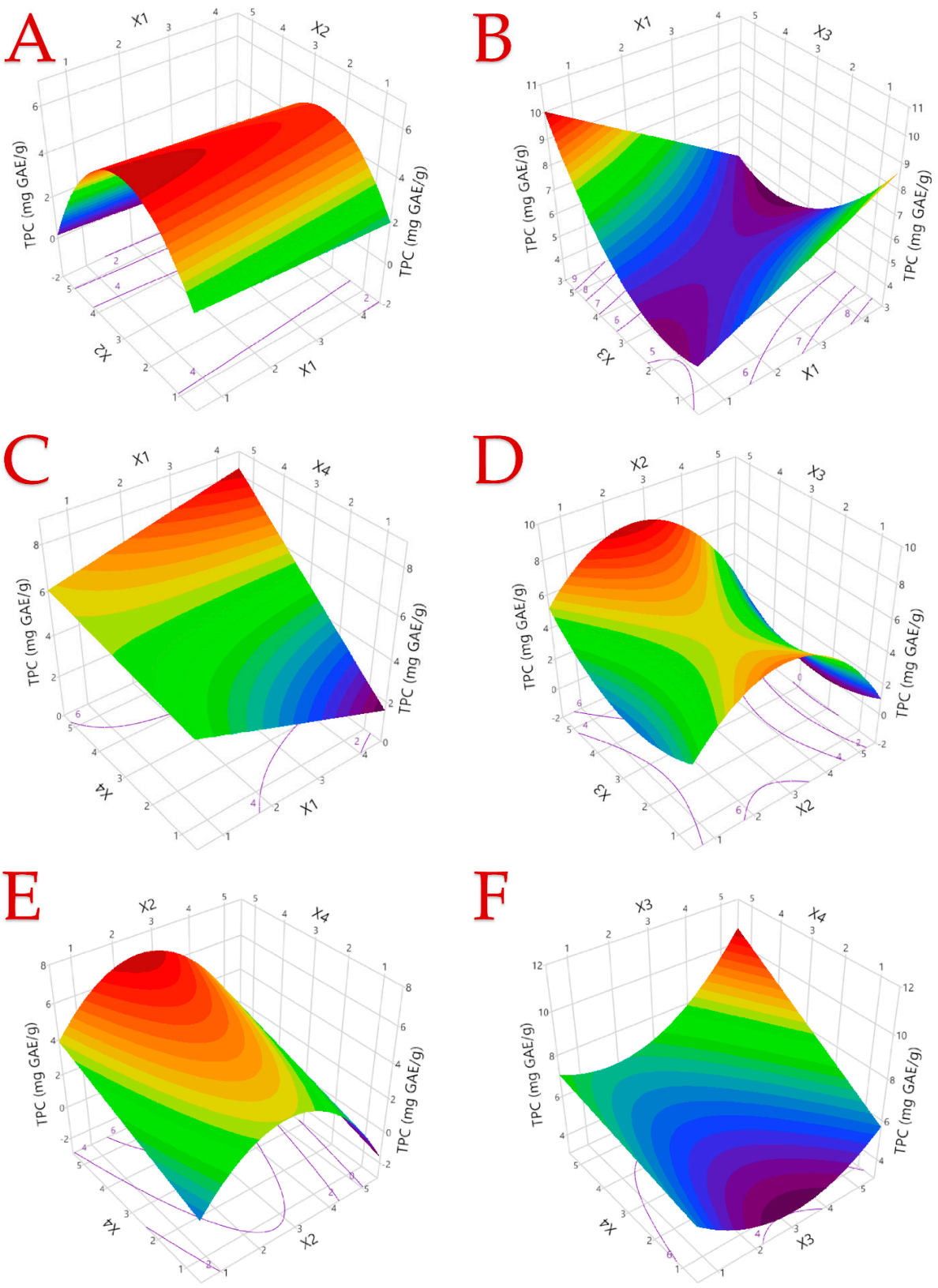

Figure 1. The optimal extraction of the carrot peels is depicted in 3D graphs, illustrating the effects of the process variables on the response, specifically the TPC. Plot (**A**) displays the covariation of X_1 and X_2; plot (**B**) displays the covariation of X_1 and X_3; plot (**C**) represents the covariation of X_1 and X_4; plot (**D**) demonstrates the covariation of X_2 and X_3; plot (**E**), exhibits the covariation of X_2 and X_4; plot (**F**) reveals the covariation of X_3 and X_4.

3.3. Extraction Parameters Main Effects Through Standardized Pareto Plots

The evaluation of the Main Effects and their interactions with each extraction parameter was done using Pareto plots, with a significance level of $p < 0.05$ for statistical analysis. Figure 2 shows the effect of the independent factors on bioactive molecules and antioxidant activity, which include technique (X_1), solvent concentration (X_2), extraction duration (X_3), and extraction temperature (X_4). The orthogonality-applying technique also yields the orthogonal coded estimates, which are also displayed. Based on the results of the Pareto plot, one could deduce that among the extraction parameters, X_2 was deemed undesirable for TPC and antioxidant assays. An answer to this could be a strong correlation between the isolated polyphenols and the antioxidant capacity. Specifically, the $X_2 \times X_2$ combination showed that these variables negatively affected elevated ethanol concentration in water, indicating that TPC recovery is quite sensitive to these changes. However, this independent factor had a high positive impact on the recovery of ascorbic acid and total carotenoids. This trend could be a matter of bioactive compounds polarity, wherein polyphenols could demand a more polar solvent compared to ascorbic acid and carotenoids. A negative effect of the X_3 and X_4 factors in combination was also noted in these tests. Bioactive chemicals may degrade when exposed to high temperatures and long periods. Figure 2A further showed that the temperature of extraction, when considered independently, did not significantly impact. In comparison to the other two independent factors, X_3 had a negligible effect on the recovery of bioactive compounds, but it did have a slight effect on the recovery of polyphenols, as previously mentioned. Nevertheless, More et al. [31] stated that elevated temperature value can solubilize bioactive molecules and improve their recovery, as evidenced by the substantial positive impact observed in all assays concerning factor X_4.

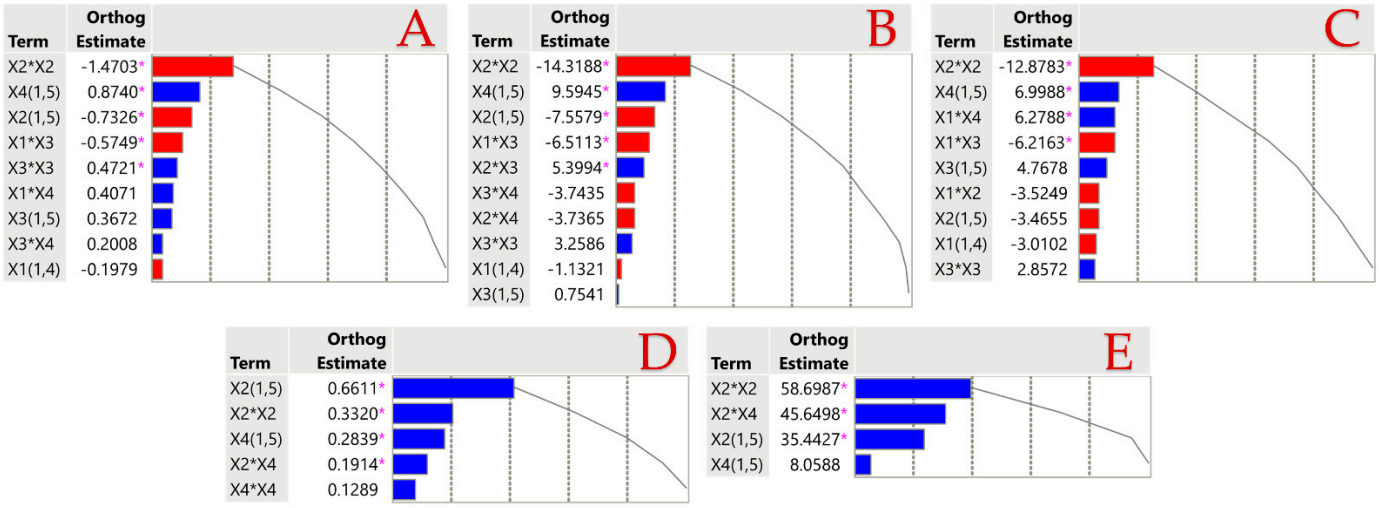

Figure 2. Transformed estimates for TPC (**A**), FRAP (**B**), DPPH (**C**), AAC (**D**), and TCC (**E**) assays are represented by Pareto plots. A pink asterisk was included in each plot to denote the significance level ($p < 0.05$). Blue bars indicate positive effects, while red bars represent negative effects.

3.4. Investigating Optimal Extraction Conditions

Polyphenolic compounds extraction from carrots was the subject of many research studies. To start with, carrot flour from three different types (i.e., orange, purple, and red) was extracted using water, 50% v/v of aqueous ethanolic mixture, and 50% v/v of aqueous methanolic mixture in a similar study from Purewal et al. [32]. The authors yielded 13.16–18.57 mg GAE/g dw with the findings revealing that aqueous extract from purple carrot achieved the highest yield and indicating water as a preferable solvent to recover

polyphenols. The drying process is essential for achieving a high-quality dry product, maintaining both organoleptic properties and bioactive compounds integrity. To that end, Nguyen et al. [33] explored the impact of hot-air drying, freeze-drying, microwave drying, and vacuum drying in bioactive compounds extraction from *Scarlet Nantes* carrot peels. The results revealed a wide range of recovered polyphenols, as the TPC range was from 2.74 to 23.49 mg GAE/g dw. The authors stated that the drying method through a microwave apparatus performed at 1200 W was the most preferable for polyphenol recovery.

Regarding carotenoid recovery from carrots, Sabahi et al. [34] optimized ultrasound-assisted extraction conditions in carrot pomace. Despite accomplishing a high TPC value at 85 mg GAE/g dw, the authors yielded 12.20 μg/g dw of TCC during pilot experiments. Kaur et al. [15] examined several organic solvents for the isolation of carotenoids from carrot waste, including ethanol, ethyl acetate, acetone, hexane, and their mixtures. The results ranged from 980 to 1728 μg/g dw, in which hexane-ethanol mixture (50% *v/v*) was the most preferable. Another study in which different drying treatments were explored, including hot-air drying, vacuum drying, freeze-drying, dehumidification drying, and microwave drying, was conducted by Lau et al. [35]. The results in TCC determination using a 2:1:1 *v/v/v* hexane:acetone:ethanol mixture ranged from 2060 to 2900 μg/g dw, wherein the freeze-drying process was the most preferable option, validating our choice of the specific drying method. Regarding the significantly high carotenoid content in the specific study compared to ours, the authors suggest that the carrot variety is likely a contributing factor, however, the impact of extraction solvents may be more significant since we employed ethanol as a food-compatible solvent for carotenoid extraction. Finally, two other "Nantes" carrot cultivars (i.e., *Dordogne* and *Maestro*) were extracted using hexane:acetone:ethanol (2:1:2 *v*:*v*:*v*) in the study from Aubert et al. [36]. The authors studied the top, middle, and bottom carrot peels. It was revealed that the highest TCC was obtained from the top of carrot peels, yielding 108 and 116 μg/g fresh weight of carotenoids, respectively.

Molecules that are among the most extensively recognized classes for their health-promoting capabilities found in nature are polyphenols. These molecules could be implemented in a variety of sectors, such as the pharmaceutical and food industries [37]. The significant reduction in cancer, cardiovascular diseases, and age-related macular degeneration is facilitated by carotenoids, which are the primary precursor of vitamin A [38]. Consequently, it is imperative to extract carotenoids from carrots. Additionally, since humans are incapable of synthesizing L-ascorbic acid, they must obtain the vitamin from other sources, such as fruits and vegetables [39]. To that end, the specific bioactive compounds have been determined spectrophotometrically. Table 4 contains the measured responses, along with antioxidant assays (FRAP and DPPH) for each prepared extract. It should be noted that different extraction conditions were required to maximize the values of each assay. TPC and DPPH necessitate similar conditions for optimal performance, differing solely in temperature, with the optimum temperature being highest for TPC and lowest for DPPH. FRAP necessitates the integration of all techniques (PEF, US, and ST), and the elevated ethanol concentration appears to adversely affect this assay. A significant distinction is the optimal duration, as FRAP results in greater yield within a reduced timeframe. The elevated temperature generally enhances all responses, except for DPPH, whereas the solvent composition exhibits significant variability.

Table 4. Predicted optimal extraction conditions along with maximum predicted responses for the dependent variables.

Responses	Optimal Conditions for Individual Assays				
	Maximum Predicted Response	**Technique (X_1)**	***C* (%, *v*/*v*) (X_2)**	***t* (min) (X_3)**	***T* (°C) (X_4)**
TPC [a]	9.59 ± 1.83	ST (1)	50 (3)	150 (5)	80 (5)
FRAP [b]	94.9 ± 17.41	PEF + US + ST (4)	25 (2)	60 (2)	80 (5)
DPPH [b]	71.15 ± 21.81	ST (1)	50 (3)	150 (5)	35 (2)
AAC [c]	3.75 ± 0.55	–	100 (5)	–	80 (5)
TCC [d]	308.79 ± 83.13	–	100 (5)	–	80 (5)

[a] Values calculated in mg GAE/g dw; [b] Values calculated in μmol AAE/g dw; [c] Values calculated in mg/g dw; [d] Values calculated in μg CtE/g dw.

3.5. Correlation Analyses on Independent Factors and Assays

3.5.1. Principal Component Analysis (PCA)

To provide a clearer picture of the interactions between assays and extraction conditions, correlation analyses were implemented, including PCA and MCA, as graphically illustrated in Figure 3, and descriptive in Table 5, respectively. The correlation analyses were conducted to ascertain whether there was a relationship between the variables and TPC, AAC, TCC, DPPH, and FRAP in the context of PCA. The chart explained 92.9% of the variance, with PC1 explaining 73.9% and PC2 explaining 19%. The independent variables were also deemed to have a substantial impact on the analysis. The graph showed that TPC and both antioxidant capacity variables were positively positioned in both Components and were depicted in proximity. The high ethanol concentration highly positively influenced these analyses, which is the rationale for their high correlation. Their shared effect on extraction parameters was analogous. Contrastingly, the positive positioning of AAC in PC2 and at a substantial distance from the other variables could imply a weakened connection between them. Previous results demonstrated a positive correlation between AAC recovery and an increase in ethanol concentration.

3.5.2. Multivariate Correlation Analysis (MCA)

The MCA provides supplementary information regarding the correlation between variables. This method's primary advantage is its ability to identify the degree of positive or negative correlation among the variables under investigation. The results of this analysis are presented in Table 5. Strong positive correlations (>0.9) were observed between antioxidant assays and TPC, a highly anticipated pattern that has been previously supported [40]. Moreover, it is observed that AAC has a significant correlation with TCC (>0.7), presumably due to the polarity of the respective molecules. Finally, the negative correlation between TPC with AAC and TPC is highlighted again, however, it is of high interest that molecules with significant antioxidant activity have a negative correlation with antioxidant assays.

3.6. Partial Least Squares (PLS) Analysis

The PLS model was used to estimate the impact of the extraction condition parameters (X_1, X_2, X_3, and X_4). As such, Figure 4 illustrates a correlation loading plot of this model. Such research on carrot peels revealed the impact of extraction conditions, wherein the authors expected to maximize extraction efficiency. Conditions that significantly affect the extraction of molecules with biological activity include temperature, solvent composition, and extraction period [41], in addition to the extraction technique [42]. Regarding variable

X_2 which was the matter of most bioactive compound recovery, maximum responses in most assays were seen at level 4 (i.e., 75% v/v ethanol), indicating that the specific variable played a significant role. In terms of extraction methods, it was found that bioactive chemicals could be recovered satisfactorily with the sole ST technique, where a different pattern from that of our previous work [14] on apple peels, valorization was found. A thorough examination using partial least squares was necessary to guarantee the effect of extraction duration with respect to the extraction duration parameter (X_3), as AAC and TCC recovery seemed independent of this parameter. This led to the selection of a high extraction duration (i.e., 150 min) since the other variables (i.e., TPC, FRAP, and DPPH) required so. Lastly, the widely recognized behavior of molecules that are readily solubilized and extracted at high temperatures was once again demonstrated, despite the negative correlation of the DPPH assay. As a result, the optimal temperature variable (X_4) was determined to be 80 °C.

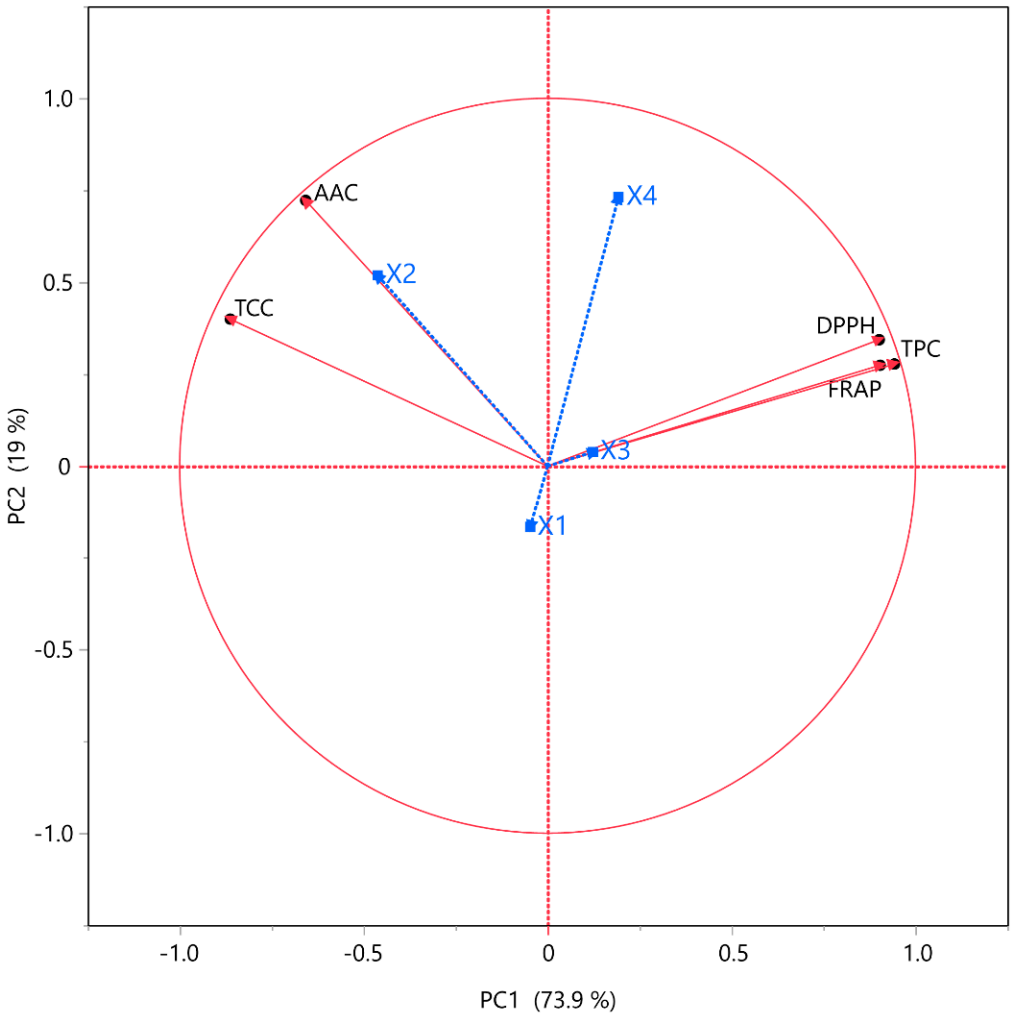

Figure 3. Principal component analysis (PCA) was applied to the measured variables, with each X variable represented in blue.

Table 5. Multivariate correlation analysis of the five measured variables.

	TPC	FRAP	DPPH	AAC	TCC
TPC	–	0.9205	0.9311	−0.4373	−0.6757
FRAP		–	0.8367	−0.4273	−0.6197
DPPH			–	−0.3249	−0.6751
AAC				–	0.7897
TCC					–

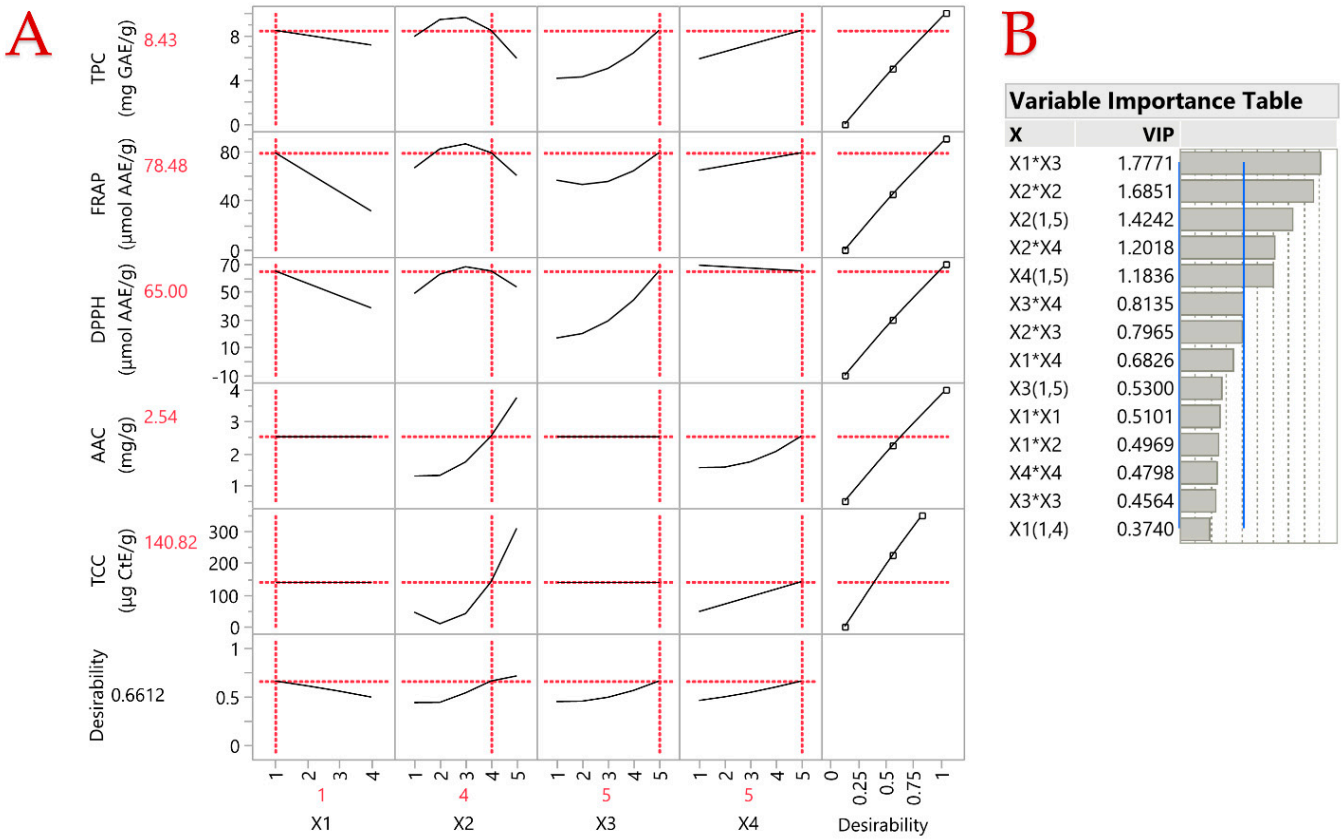

Figure 4. The desirability function illustrated with extrapolation control and partial least squares (PLS) prediction profiler for optimizing the carrot peel extracts is depicted in Plot (**A**). Plot (**B**) displays the variable importance plot (VIP) option values for each predictor variable. A blue dashed line at the 0.8 mark on the VIT indicates each variable's significance level.

3.7. Optimal Extraction Conditions

The high determination coefficient (R^2) of 0.9946 and the strong correlation coefficient of 0.9973 shows that the experimental data and the predictions from the PLS model accord very well. Additionally, no statistically significant difference in the variations between the experimental and PLS model (predicted) values was assured since the through *p*-value ($p < 0.0001$). With the obtained results from the PLS model, the optimal extraction conditions for all assays, along with the predicted and actual values are shown in Table 6. The experimental values acquired for all assays closely align with those anticipated by the statistical model, thereby highlighting an exceptional fit. The individual phenolic compounds as identified through HPLC-DAD are provided in Table 7, whereas Figure 5 illustrates two representative chromatograms. Additionally, Figure S10 depicts the characteristic absorption peaks for each compound, facilitating their identification and analysis.

Furthermore, Figure S11 presents a two-dimensional (2D) contour plot of chromatographs for phenolic acids and flavonoids. This figure visually demonstrates the separation and identification of these compounds, highlighting their retention times and absorption characteristics. Table S1 provides the linear equations, correlation coefficients, retention times, and UVmax, ensuring accurate quantification of each compound. The optimal extract seems to consist mainly of phenolic acids and flavonoids. Catechin was seen as the most abundant phenolic compound, followed by rutin and chlorogenic acid. The results of the present study follow those published by El-Sawi et al. [7], who also determined catechin, chlorogenic acid, ferulic acid, caffeic acid, and *p*-coumaric acid on carrot peels. Moreover, Balkrishna et al. [43] identified low quantities of chlorogenic acid (0.23 mg/g), ferulic acid, and caffeic acid (equal to or less than 0.01 mg/g in both cases) in carrot roots.

Table 6. Maximum desirability under optimized extraction conditions (X_1: 1, X_2: 4, X_3: 5, and X_4: 5) for all variables using partial least squares (PLS) prediction profiler.

Variables	PLS Model Values	Experimental Values
TPC [a]	8.43	8.26 ± 0.32
FRAP [b]	78.48	76.57 ± 4.90
DPPH [b]	65.00	63.48 ± 2.41
AAC [c]	2.54	2.55 ± 0.11
TCC [d]	140.82	137.44 ± 8.38

[a] Values calculated in mg GAE/g dw; [b] Values calculated in μmol AAE/g dw; [c] Values calculated in mg/g dw; [d] Values calculated in μg CtE/g dw.

Table 7. Phenolic compounds under optimized extraction conditions (X_1: 1, X_2: 4, X_3: 5, and X_4: 5).

A/A	Phenolic Compounds	Optimal Extract (mg/g)
	Phenolic acids	
1	β-Resorcylic acid	0.63 ± 0.02
2	Chlorogenic acid	0.97 ± 0.05
3	Pyrocatechuic acid	0.28 ± 0.02
4	Caffeic acid	0.19 ± 0.01
5	*p*-Coumaric acid	0.23 ± 0.01
6	Ferulic acid	0.76 ± 0.05
	SUM of Phenolic acids	3.06 ± 0.15
	Flavonoids	
7	(+)-Catechin hydrate	1.28 ± 0.08
8	Rutin	1.07 ± 0.07
9	Quercetin-3-*O*-galactoside	0.45 ± 0.03
10	Apigenin-7-*O*-glucoside	0.64 ± 0.02
11	4′-Hydroxychalcone	0.17 ± 0.01
12	Chrysin	0.22 ± 0.01
	SUM of Flavonoids	3.82 ± 0.21
	Total Identified	6.88 ± 0.36

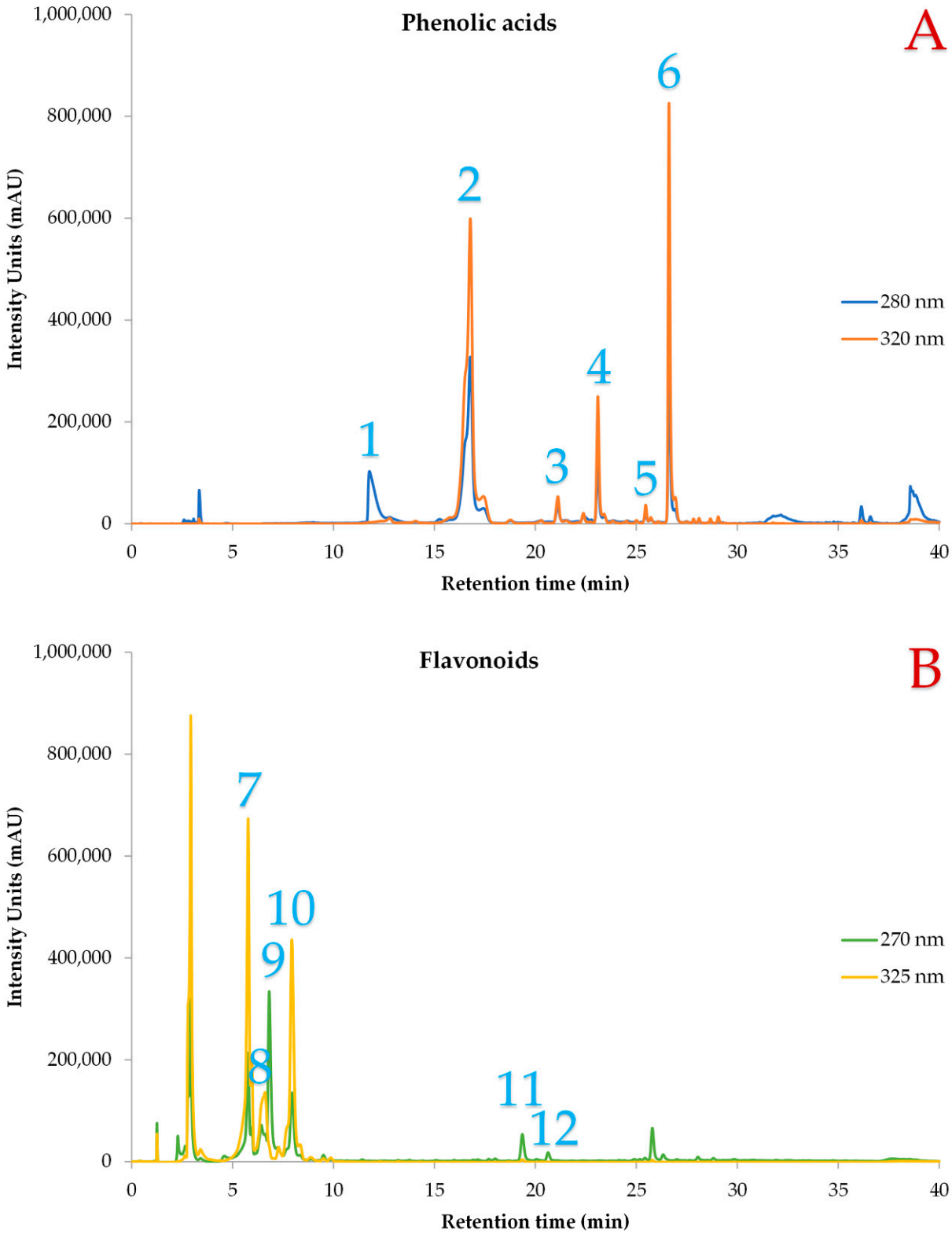

Figure 5. Exemplary HPLC chromatograms of the optimal carrot peel extract in the UV spectra reveal the identified phenolic compounds. Plot (**A**) displays the phenolic compounds, while plot (**B**) presents the flavonoids. 1: β-Resorcylic acid; 2: Chlorogenic acid; 3: Pyrocatechuic acid; 4: Caffeic acid; 5: *p*-Coumaric acid; 6: Ferulic acid; 7: (+)-Catechin hydrate; 8: Rutin; 9: Quercetin-3-*O*-galactoside; 10: Apigenin-7-*O*-glucoside; 11: 4′-Hydroxychalcone; 12: Chrysin.

4. Conclusions

This research aimed to explore sustainable methods to reduce industrial and household food waste by utilizing carrot peel, either obtained through cooking or juicing, as a valuable source of bioactive compounds. The findings highlight the significant potential of carrot peel for recovering important nutrients, particularly carotenoids. The application of vitamin A, derived from carotenoids, is already well-established in the cosmetic industry, and carrot waste can serve as a promising raw material for its production. The optimal extract achieved a high total carotenoid content of 137 μg CtE/g dw, indicating its potential utility in both the pharmaceutical and cosmetic sectors. Additionally, the presence of other bioactive compounds in carrot peel, such as chlorogenic acid, catechin, and rutin, offers a rich source for fortifying foods, beverages, animal feeds, and even in cosmetic or medicinal formulations. These compounds not only enhance nutritional value but also contribute high antioxidant capacity to the products they fortify. Future research could involve further in vitro or in vivo testing to provide a more detailed understanding of these findings and to explore additional applications of carrot peel extracts.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomass5010003/s1: a detailed comparison between the actual and predicted responses for each examined parameter is depicted in Figures S1–S5, which also includes the desirability functions. Further 3D response plots are illustrated in Figures S6–S9 for the remaining responses. The UV spectrum index for phenolic acids and flavonoids is illustrated in Figure S10. Two-dimensional (2D) contour of chromatographs for phenolic acids and flavonoids are displayed in Figure S11. Table S1 presents the equations of calibration curves for the phenolic compounds identified through HPLC-DAD.

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