applied sciences

MDPI

Article

Optimized Isolation Procedure for the Extraction of Bioactive Compounds from Spent Coffee Grounds

Theodoros Chatzimitakos, Vassilis Athanasiadis *, Konstantina Kotsou, Dimitrios Palaiogiannis, Eleni Bozinou and Stavros I. Lalas

Department of Food Science & Nutrition, University of Thessaly, Terma N. Temponera str., 43100 Karditsa, Greece
* Correspondence: vaathanasiadis@uth.gr; Tel.: +30-24410-64783

Abstract: Due to the worldwide consumption of coffee, many tons of spent coffee grounds (SCGs) are discarded each year, as a by-product of coffee preparation. Not only their disposal is costly, but also it may cause the release of compounds that can endanger the environment. However, there are valuable chemical compounds that can be extracted from SCGs and used in the food industry. The aim of this study was to investigate the main parameters affecting the extraction of caffeine and polyphenols (i.e., chlorogenic acid, neochlorogenic acid, and caffeic acid) and to evaluate the antioxidant properties of the extracts. To this end, extraction solvent, temperature, time, and liquid-to-solid ratio were studied. A response surface methodology was used to optimize the extraction process. According to the results, the caffeine content of the optimum extract was found to be 6.14 mg/g in dry SCGs, the total polyphenol content was 19.85 mg gallic acid equivalents/g, while the ferric reducing antioxidant power and DPPH scavenging values were 136.69 μmoL ascorbic acid equivalents/g and 230.41 μmoL DPPH/g, respectively. The experimental values were in close agreement with the predicted ones, highlighting the potential of SCGs to be used for the isolation of bioactive compounds with the proposed extraction procedure.

Keywords: spent coffee grounds; extraction; response surface methodology; caffeine; polyphenols; antioxidants; HPLC-DAD

Citation: Chatzimitakos, T.; Athanasiadis, V.; Kotsou, K.; Palaiogiannis, D.; Bozinou, E.; Lalas, S.I. Optimized Isolation Procedure for the Extraction of Bioactive Compounds from Spent Coffee Grounds. *Appl. Sci.* **2023**, *13*, 2819. https://doi.org/10.3390/app13052819

Academic Editor: Monica Gallo

Received: 8 February 2023
Revised: 20 February 2023
Accepted: 21 February 2023
Published: 22 February 2023

1. Introduction

One of the major global problems is food waste. Food waste currently totals 1.3 billion tons annually, and it is predicted that in less than 10 years it will increase to two billion tons annually. It is also important that huge amounts of water are consumed for the production of these foods. Given that in the European Union alone, 50% of irrigated water is used for food production, this ecological problem has also economic ramifications [1]. After the COVID-19 pandemic, consumers have acquired a completely different perspective on food choices, causing a total transformation in the food industry. More specifically, they have now turned their interest to organic products of local origin, while the bioavailability of bioactive compounds is also taken under consideration [2]. Due to the above reasons, more and more scientists are investigating food by-products to reuse them in the best possible way, ensuring a kind of circular economy. For instance, in this context, many researchers use various by-products for the re-production of food, in the pharmacological field, and in the livestock industry [3,4]. Alfano et al. [5] reported that hydroxytyrosol, a polyphenol of high added value, can be isolated from olive mill wastewater and used as a functional ingredient in bread.

Coffee is the food product that has the largest consumption worldwide and is the second largest commercial product after petroleum [6]. The global coffee consumption ranged between 9.1 and 9.4 million tons between 2015 and 2017. It may be made and served in a variety of ways, has an intense dark color, and is bitter and slightly acidic. All coffee plants belong to the *Rubiaceae* family. Today, coffee plants are grown in more than

70 countries, in America, Brazil, Southeast Asia, the Indian subcontinent, and Africa, with Brazil producing nearly 35% of the world production [7]. Today, the two most commercial species mainly cultivated are *Coffea canephora* (mainly a form known as "robusta") and *C. arabica*, and the less popular species are *C. liberica*, *C. stenophylla*, *C. mauritiana,* and *C. racemosa*. Millions of tons of waste are produced and discarded annually, with the roasting process as well as the brewing process resulting in vast amounts of spent coffee grounds (SCGs) [8]. One of the existing uses of these by-products is the creation of poultry feed and the manufacture of paper and biocomposites, using it as a raw material [9], but Getachew et al. [10] argued that this coffee waste, due to its rich content of tannins and caffeine, could pose a potential environmental hazard if disposed of incorrectly. This means that the further study of coffee by-products is imperative to develop new, alternative uses, so as to avoid an improper disposal and enhance the circular economy.

Coffee is widely consumed due to its cognitive effects, causing increased alertness and wakefulness among others [11]. This effect is caused by the caffeine present in the beverage. Due to its effects, caffeine-containing beverages and energy drinks are also widely consumed [12]. Such drinks contain plant extracts as a source of caffeine. However, in order to cover the increasing demand for caffeine, synthetic caffeine is being produced at an industrial scale and added to beverages. The synthesis of caffeine includes many laborious steps such as the methylation of theobromine under various conditions [13]. However, the cost of such synthesis may increase, and the extraction of caffeine from renewable plant sources could be a more economical option. SCGs are a good source of caffeine, as reported in previous studies [14,15], and as such, have the potential to be used as a renewable source of caffeine. Aside from caffeine, SCGs contain also other bioactive compounds such as polyphenols, which are well known for their antioxidant properties [16,17]. Caffeic acid, chlorogenic acid, and its isomer, neochlorogenic acid are phenolic compounds present in coffee and are well known for their anticancer, antilipidemic, antidiabetic, antiviral, and antipyretic activities, among others [18–20]. A few methods have been developed for the extraction of polyphenols and caffeine from spent coffee grounds, including solid–liquid extraction, supercritical extraction, and microwave-assisted extraction [21,22]. Of these methods, solid–liquid extraction is the most widely used and requires no specific apparatus. The extraction efficiency of polyphenols and caffeine from spent coffee grounds can be influenced by a number of factors, including the type of solvent used, the extraction time, and the temperature [15]. As such, the optimization of the above parameters can significantly increase the extraction yield.

The main objective of this study was to examine the recovery of bioactive compounds from SCGs, in order to assist the circular economy of coffee (reduce the cost for the preparation of value-added products, as well as that for transporting and handling the waste material, while at the same time, recovering the solvent), further lowering the environmental impact of SCGs and the overall cost of the production of bioactive compounds. In this context, the experimental parameters related to the extraction of bioactive compounds were studied and optimized. The obtained extracts were studied in terms of total polyphenol content and antioxidant activity, as well as of their content in caffeine, caffeic acid, chlorogenic acid, and neochlorogenic acid.

2. Materials and Methods

2.1. Chemicals and Reagents

Folin–Ciocalteu reagent, anhydrous sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri-2-pyridinyl-1,3,5-triazine (TPTZ), iron (II) chloride, and hydrochloric acid were at least of analytical grade and purchased from Penta (Prague, Czech Republic). All solvents used (HPLC grade) were purchased from Carlo Erba (Val de Reuil, France). Caffeine, caffeic acid, chlorogenic acid, and neochlorogenic acid standards were purchased from Sigma Aldrich (Steinheim, Germany). For all experiments, deionized water was used.

2.2. Sample Preparation and Extract Preparation

SCGs were collected from local coffee stores within 2 h of coffee preparation, transferred to the laboratory, and freeze-dried for 24 h in order to remove water in a Biobase BK-FD10P freeze-dryer (Jinan, China). The coffee grounds were 60% Arabica and 40% Robusta. Next, the dried SCGs were placed in sieves and were separated according to their size. For the extract preparation, SCGs with an average particle diameter of 490 μm were used. For the analysis of coffee grounds prior to brewing, the same process was followed. For the preparation of the extracts, the SCGs were placed in Duran bottles along with the solvent and then in an oil bath. The extraction was carried out according to the conditions described below (in Section 2.3), under constant stirring, using hydroethanolic mixtures. After extraction, the samples were centrifuged at 4000 rpm for 5 min. The supernatant was collected and further analyzed.

2.3. Design of the Experiments and Response Surface Methodology (RSM) Optimization

An RSM approach was employed in order to maximize the extraction yield of caffeine, as well as those of polyphenols (total polyphenol content; TPC) and other compounds with antioxidant activity (evaluated using the DPPH and FRAP assays). Thus, caffeine concentration and TPC, DPPH, and FRAP values were the objectives of the design. The composition of the solvent (C, % v/v ethanol in water; selected so as to examine solvents with various polarities), the extraction time (t, min; selection was based on preliminary experiments), the extraction temperature (T, °C; a maximum temperature of 80 °C was selected, so that the extracted compounds would be stable, and the extraction feasible, as the extraction could not be carried out with ethanol above that temperature), and the ratio of liquid to solid ($R_{L/S}$, mL/g; selection was based on preliminary experiments) were examined so as to achieve optimum results. The optimization was based on an experiment with a 20-design point main effects screening design. The process variables were set up in 5 levels, according to the experimental design: −2, −1, 0, 1, and 2. The coded and actual levels are listed in Table 1. The significance of the model (equations) coefficients and the overall model significance (R^2, p) were evaluated at a minimum level of 95% using analysis of variance (ANOVA) and summary-of-fit tests.

Table 1. The actual and coded levels of the independent variables were used to optimize the process.

Independent Variables	Code Units	Coded Variable Level				
		−2	**−1**	**0**	**1**	**2**
C (%, v/v)	X_1	0	25	50	75	100
t (min)	X_2	18	69	120	171	222
T (°C)	X_3	20	35	50	65	80
$R_{L/S}$ (mL/g)	X_4	5	13	20	28	35

The response variable was estimated using a second-order polynomial model, expressed as follows:

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

where Y_k represents the predicted response variable, while X_i and X_j represent the independent variables. The values β_0, β_i, β_{ii}, and β_{ij} stand for the intercept and the regression coefficients associated with the linear, quadratic, and interaction terms of the model, respectively.

The RSM was used to optimize the extraction yield of caffeine. The purpose was to find the highest peak area and to understand the impact of significant independent variables on the response. The results of the model are represented through 3D surface response graphs, which showed the relationship between the response and the independent variables.

2.4. Total Polyphenol Content (TPC) Determination

The TPC of the coffee extracts was determined using the Folin–Ciocalteu assay, with gallic acid as a reference [23]. In an Eppendorf tube, 100 µL of the coffee extract was mixed with 100 µL of the Folin–Ciocalteu reagent, and the reaction was allowed to proceed for 2 min. Afterward, 800 µL of a 5% w/v Na_2CO_3 solution was added, and the mixture was heated for 20 min at 40 °C. The absorbance was then measured with a spectrophotometer at 740 nm (Shimadzu UV-1700 PharmaSpec Spectrophotometer, Kyoto, Japan). By establishing a calibration curve with gallic acid, the total polyphenol content (C_{TP}) was determined. The results are expressed in milligrams of gallic acid equivalents per liter (mg GAE/L). The total polyphenols extraction yield (Y_{TP}) was calculated as milligrams of GAE per gram of dry weight (dw), using Equation (2):

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

where V is the volume of the extraction medium (in L), and w is the dry weight of the sample (in g).

2.5. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed using a previously published method [24]. The sample extracts was mixed thoroughly with 0.05 mL of $FeCl_3$ solution (4 mM in 0.05 M HCl) and incubated for 30 min at 37 °C. After adding 0.90 mL of TPTZ solution (1 mM in 0.05 M HCl), the absorbance at 620 nm was measured after 5 min. Using an ascorbic acid calibration curve (C_{AA}, 50–500 µmol/L in 0.05 M HCl) and the following Equation (3), the ferric reducing antioxidant power (P_R) was calculated as µmoL ascorbic acid equivalents (AAE) per g of dry weight (dw):

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

where V is the volume of the extraction medium (in L), and w is the dry weight of the sample (in g).

2.6. DPPH Radical Scavenging Assay

A previously described method was used to measure the DPPH radical scavenging activity [23]. In total, 25 µL of the sample was mixed with 975 µL of DPPH solution (100 µM) in an Eppendorf tube. At 515 nm, the solution's absorbance was measured both immediately upon mixing ($A_{515(i)}$) and 30 min later ($A_{515(f)}$). Equation (4) was used to calculate the antiradical activity (A_{AR}):

$$A_{AR}\ (\text{µmoL DPPH/g dw}) = \frac{\Delta A}{\varepsilon \times l \times C} \times Y_{TP} \quad (4)$$

where $\Delta A = A_{515(i)} - A_{515(f)}$; ε (DPPH) = 11,126 × 10^{-6} μM^{-1} cm^{-1}; $C = C_{TP} \times 0.025$; Y_{TP} is the total polyphenol yield of the extract (mg/g), and l is the path length (1 cm).

2.7. HPLC-Based Determination of Compounds

An HPLC system was utilized to analyze the sample extracts. The analysis was performed using a Shimadzu CBM-20A liquid chromatograph and a Shimadzu SPD-M20A diode array detector (both provided by Shimadzu Europa GmbH in Duisburg, Germany). The separation of the compounds was achieved using a Phenomenex Luna C18(2) column (100 Å, 5 µm, 4.6 × 250 mm) from Phenomenex Inc., Torrance, CA, USA, which was maintained at 40 °C. The mobile phase consisted of 0.5% aqueous formic acid (A) and a mixture of 0.5% formic acid in acetonitrile/water (6:4) (B). The gradient program used to elute the compounds was as follows: 0% B to 40% B, then to 50% B in 10 min, to 70% B in another 10 min and then held constant for 10 min. The flow rate of the mobile phase was

1 mL/min. The levels of caffeine, caffeic acid, chlorogenic acid, and neochlorogenic acid in the samples were measured. The retention time and absorbance spectrum were compared to those of pure chemical standards to identify the compounds and then quantified using calibration curves (0–50 μg/mL).

2.8. Statistical Analysis

JMP® Pro 16 (SAS, Cary, NC, USA) software was used to create the experimental design and perform the statistical analysis linked to the response surface methodology and the distribution analysis. The extraction procedures were carried out at least twice, and the quantitative analysis was performed in triplicate. The results are presented as medians with standard deviation.

3. Results and Discussion

Due to the importance of the compounds contained in SCGs, a response surface methodology was employed to finely tune the parameters that affect their extraction from SCGs and obtain extracts rich in these compounds that can be used for the production of beverages or the enrichment of other products. This is not the first study to examine the extraction of compounds from SGCs. Mitraka et al. [15] focused only on the extraction of caffeine and chlorogenic acid from SCGs using accelerated solvent extraction. Similarly, Gigliobianco et al. [25] optimized the extraction of caffeine, trigonelline, and nicotinic acid from SCGs. However, the parameters that they evaluated were the solvent extraction volume and the extraction temperature. Solomakou et al. [26] focused on the extraction of polyphenols from SGCs using three different techniques. However, for the conventional extraction method, the effect of extraction time was not studied. Our aim was to examine all important parameters for the extraction of caffeine, caffeic acid, chlorogenic acid, and neochlorogenic acid, as well as total polyphenols. As such, four extraction parameters were examined in a wide range of values. The parameters that were examined were (X_1), the solvent (water and ethanol were examined, as well as their mixtures), (X_2), the extraction time, ranging from 18 to 222 min, (X_3), the temperature (ranging between 20 and 80 °C), and (X_4), the ratio of liquid to solid (ranging between 5 and 35 mL/g).

In order to optimize the extraction of the compounds as well as examine the effect of each extraction factor, an RSM approach was employed. The responses were the caffeine content of the extracts, the TPC, as well as their FRAP and DPPH values. The content of caffeic acid, chlorogenic acid, and neochlorogenic acid was also examined in each extract. Their concentrations in the extracts followed the same trend as the TPC. Based on ANOVA and summary-of-fit tests, the adequacy of the response surface and model fitting was evaluated, considering how closely the measured and predicted values corresponded. The experimental conditions of the prepared extracts, as well as their responses, are reported in Table 2. Moreover, Table 2 includes data regarding the responses of the extract prepared using the lower examined levels (−2) for each parameter, for means of comparison. Table 3 shows also statistical information about the models used to describe the relationship between the independent variables and the response variables. This information includes the second-order polynomial equations, the coefficients of each term in the equations, and other statistical parameters that quantify the goodness of fit of the models to the data. It was discovered that all of the coefficients increased, indicating that the models fit the data well. The desirability function was used to determine the maximum predicted values for caffeine concentration, TPC, and antioxidants (FRAP and DPPH), as well as the optimal levels for each of the four variables taken into consideration (Figure S1–S4, respectively). Additionally, bivariate response fitting plots are shown in Figures S5–S7, and Figures 1–4 provide 3D response graphs for each response.

Table 2. Experimental values of the four independent variables investigated and responses of the dependent variable (Caffeine, TPC, FRAP, and DPPH).

Design Point	Independent Variables				Responses			
	X_1 (*C*, %)	X_2 (*t*, min)	X_3 (*T*, °C)	X_4 ($R_{L/S}$, mL/g)	Caffeine (mg/g)	TPC (mg GAE/g)	FRAP (μmoL AAE/g)	DPPH (μmoL DPPH/g)
1	0	171	65	20	4.23	11.58	72.60	82.03
2	25	222	65	13	3.66	13.69	83.46	108.40
3	50	222	50	35	5.68	16.78	102.14	230.41
4	75	69	50	20	3.55	12.59	76.09	150.58
5	100	222	35	20	3.16	5.45	39.24	39.23
6	0	18	50	5	3.89	6.98	50.02	53.86
7	25	69	20	5	3.52	7.78	53.61	88.76
8	50	120	35	5	3.36	13.57	93.82	114.70
9	75	222	80	5	3.46	14.14	93.13	84.51
10	100	120	80	13	4.16	8.61	53.34	72.33
11	0	69	35	13	4.26	6.99	51.08	49.39
12	25	18	35	28	5.54	11.18	76.51	124.40
13	50	18	80	20	4.57	15.54	102.92	186.64
14	75	171	20	13	2.50	10.83	75.22	113.51
15	100	18	20	35	1.70	5.49	26.17	45.61
16	0	120	20	28	6.14	9.36	66.11	106.66
17	25	171	80	35	5.61	17.29	112.19	181.99
18	50	69	65	28	4.37	17.79	120.53	182.47
19	75	120	65	35	4.20	17.00	117.46	157.84
20	100	171	50	28	2.86	9.43	60.86	89.57
Basic conditions	0	18	20	5	3.17	4.42	32.7	43.89

Table 3. Mathematical models created using the response surface methodology used to optimize the hydroethanolic solution extraction of used coffee grounds; the models contain only significant parameters.

Responses	Second-Order Polynomial Equations (Models)	R^2	*p*
Caffeine	$Y = 4.8269 - 0.03X_1 - 0.0171X_2 + 0.0247X_3 - 0.0635X_4 + 0.0003{X_1}^2 + 0.0001{X_2}^2 - 0.0008{X_3}^2 + 0.0034{X_4}^2 - 0.0001X_1X_2 + 0.0009X_1X_3 - 0.0022X_1X_4 - 0.0002X_2X_3 - 0.0001X_2X_4 + 0.0018X_3X_4$	0.9746	0.0046
TPC	$Y = -9.6576 + 0.2904X_1 + 0.1137X_2 + 0.2914X_3 + 0.2982X_4 - 0.0034{X_1}^2 - 0.0005{X_2}^2 + 0.0003{X_3}^2 + 0.0025{X_4}^2 + 0.0003X_1X_2 - 0.0011X_1X_3 + 0.0017X_1X_4 - 0.0003X_2X_3 + 0.0001X_2X_4 - 0.0073X_3X_4$	0.9879	0.0008
FRAP	$Y = -62.4571 + 1.464X_1 + 0.983X_2 + 2.0347X_3 + 0.997X_4 - 0.0208{X_1}^2 - 0.0031{X_2}^2 + 0.0006{X_3}^2 + 0.0474{X_4}^2 + 0.0033X_1X_2 - 0.006X_1X_3 + 0.0106X_1X_4 - 0.0042X_2X_3 - 0.0057X_2X_4 - 0.0372X_3X_4$	0.9880	0.0007
DPPH	$Y = -57.8049 + 5.2065X_1 + 0.0605X_2 + 0.8282X_3 + 7.9198X_4 - 0.0541{X_1}^2 - 0.0042{X_2}^2 + 0.0239{X_3}^2 - 0.1226{X_4}^2 + 0.0027X_1X_2 - 0.0117X_1X_3 + 0.0143X_1X_4 + 0.0012X_2X_3 + 0.0392X_2X_4 - 0.1216X_3X_4$	0.9905	0.0004

As regards the caffeine content of the extracts, the ethanol ratio of the solvent (X_1) and the ratio of liquid to solid (X_4) were statistically highly significant, as well as the interactions $X_1 \times X_3$ and $X_1 \times X_4$. In Figure 1, it can be seen that caffeine was more readily extracted when water was used. The increase in ethanol content in the solvent resulted in a decreased extraction of the compound. This can be justified by the hydrophilic nature of caffeine, due to which it can form hydrogen bonds with water molecules, while the number of hydrogen bonds formed with ethanol would be less [27]. Moreover, it can be seen that in order to increase the extraction yield, an increased liquid-to-solid ratio was needed. As can be seen in Table 4, the maximum predicted value for caffeine was 6.14 mg/g of dry SCGs. These results could be achieved by performing the extraction using 28 mL of water per g of SCGs and stirring the mixture for 120 min at 20 °C. This result is better than the optimized pressurized liquids extraction method followed by Shang et al. [28] and comparable to the results of Mitraka et al. [15]. Another study by Andrade et al. used a supercritical fluid extraction (SFE) method to extract caffeine from SCGs. They found that the highest extraction yield (6.45 mg/g) was obtained at a pressure of 300 bar, a temperature of 60 °C, and a CO_2 flow rate of 1.2 L/min. Although the extraction conditions were different from those used in the current study, the extraction yield was comparable to the maximum predicted value of caffeine (6.14 mg/g) obtained in the current study [29].

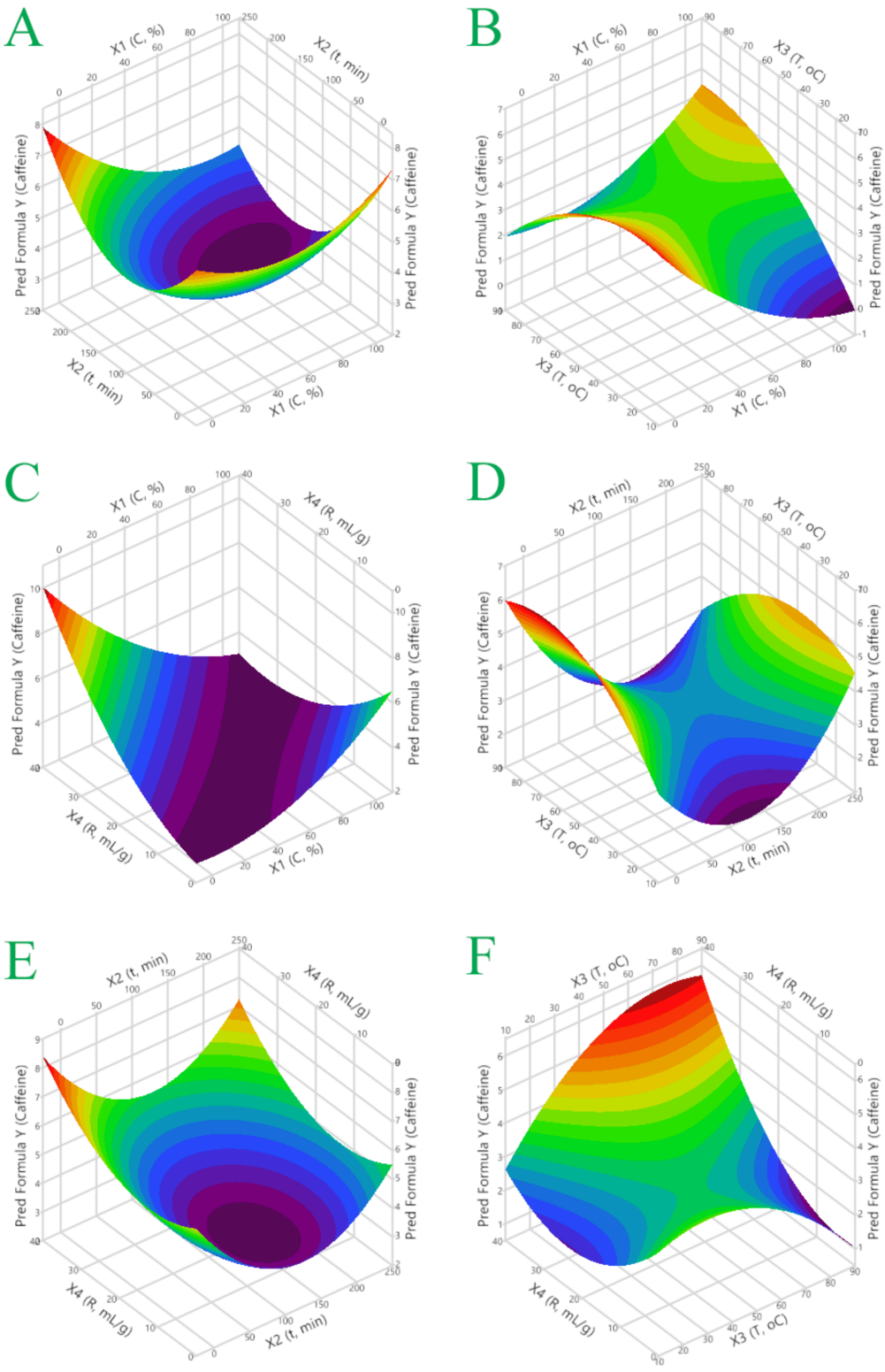

Figure 1. 3D graphs depicting the effect of the process variables considered on the response (caffeine, mg/g), for the optimization of spent coffee grounds extraction performed with hydroethanolic solutions. Plot (**A**), covariation of X_1 (*C*, %) and X_2 (*t*, min); plot (**B**), covariation of X_1 (*C*, %) and X_3 (*T*, °C); plot (**C**), covariation of X_1 (*C*, %) and X_4 (*R*, mL/g); plot (**D**), covariation of X_2 (*t*, min) and X_3 (*T*, °C); plot (**E**), covariation of X_2 (*t*, min) and X_4 (*R*, mL/g); plot (**F**), covariation of X_3 (*T*, °C) and X_4 (*R*, mL/g).

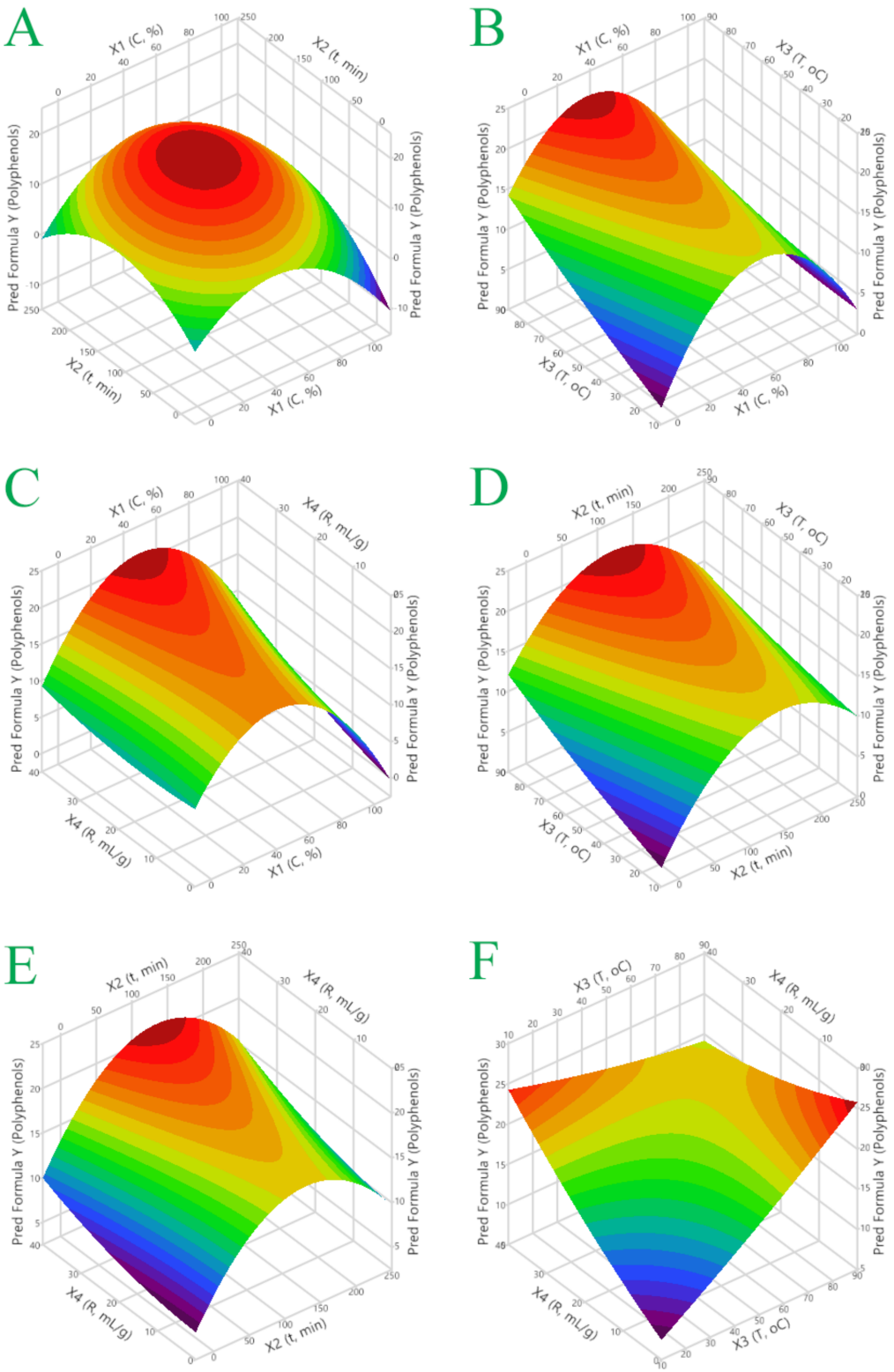

Figure 2. 3D graphs depicting the effect of the process variables considered on the response (polyphenols, mg GAE/g), for the optimization of spent coffee grounds extraction performed with hydroethanolic solutions. Plot (**A**), covariation of X_1 (C, %) and X_2 (t, min); plot (**B**), covariation of X_1 (C, %) and X_3 (T, °C); plot (**C**), covariation of X_1 (C, %) and X_4 (R, mL/g); plot (**D**), covariation of X_2 (t, min) and X_3 (T, °C); plot (**E**), covariation of X_2 (t, min) and X_4 (R, mL/g); plot (**F**), covariation of X_3 (T, °C) and X_4 (R, mL/g).

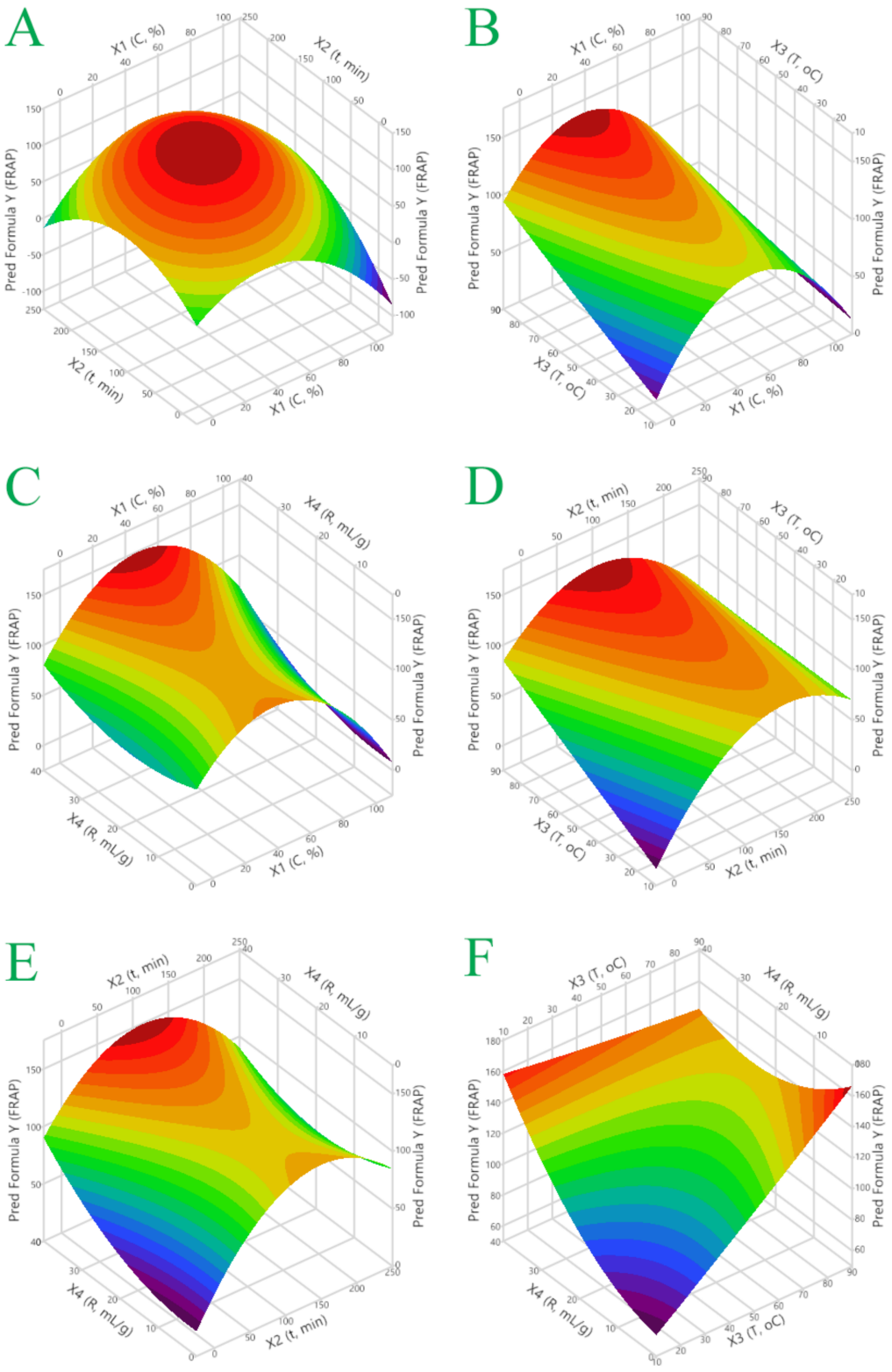

Figure 3. 3D graphs depicting the effect of the process variables considered on the response (FRAP, μmoL AAE/g), for the optimization of spent coffee grounds extraction performed with hydroethanolic solutions. Plot (**A**), covariation of X_1 (*C*, %) and X_2 (*t*, min); plot (**B**), covariation of X_1 (*C*, %) and X_3 (*T*, °C); plot (**C**), covariation of X_1 (*C*, %) and X_4 (*R*, mL/g); plot (**D**), covariation of X_2 (*t*, min) and X_3 (*T*, °C); plot (**E**), covariation of X_2 (*t*, min) and X_4 (*R*, mL/g); plot (**F**), covariation of X_3 (*T*, °C) and X_4 (*R*, mL/g).

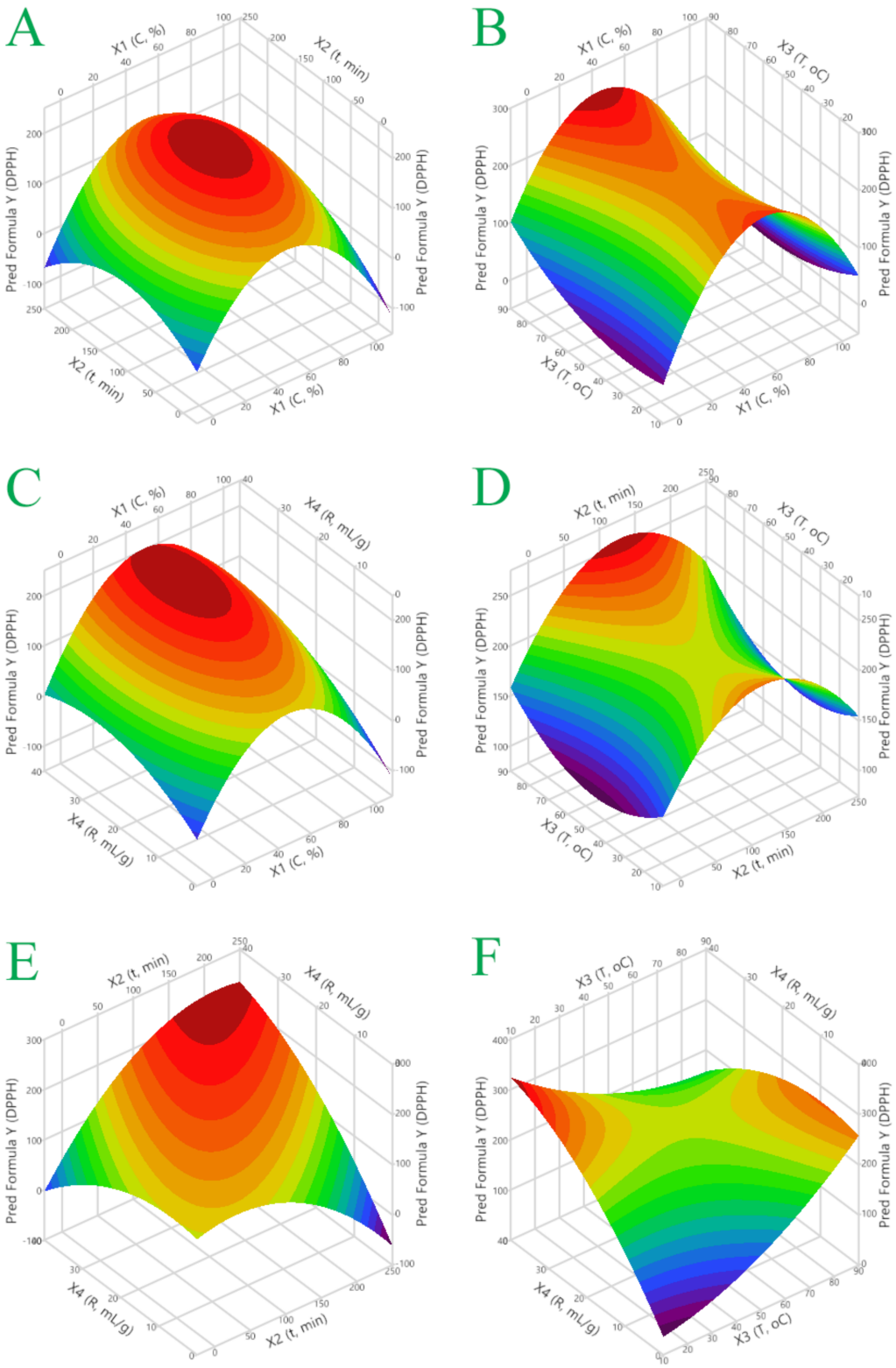

Figure 4. 3D graphs depicting the effect of the process variables considered on the response (DPPH, μmoL DPPH/g), for the optimization of spent coffee grounds extraction performed with hydroethanolic solutions. Plot (**A**), covariation of X_1 (*C*, %) and X_2 (*t*, min); plot (**B**), covariation of X_1 (*C*, %) and X_3 (*T*, °C); plot (**C**), covariation of X_1 (*C*, %) and X_4 (*R*, mL/g); plot (**D**), covariation of X_2 (*t*, min) and X_3 (*T*, °C); plot (**E**), covariation of X_2 (*t*, min) and X_4 (*R*, mL/g); plot (**F**), covariation of X_3 (*T*, °C) and X_4 (*R*, mL/g).

Table 4. Maximum predicted responses and optimum extraction conditions for caffeine, total polyphenol content (TPC), and antioxidants using hydroethanolic solutions.

Responses	Maximum Predicted Response	Optimal Conditions			
		C (%)	*t* (min)	*T* (°C)	$R_{L/S}$ (mL/g)
Caffeine	6.14 ± 0.84 mg/g dw	0	120	20	28
TPC	19.85 ± 1.71 mg GAE/g dw	50	120	65	35
FRAP	136.69 ± 13.70 μmoL AAE/g dw	50	120	80	35
DPPH	230.41 ± 24.42 μmoL DPPH/g dw	50	222	50	35

As regards the TPC content of the extracts, the effects of all the examined parameters (X_1, X_2, X_3, and X_4) were found to be statistically significant, as well as the quadratic terms ${X_1}^2$ and ${X_2}^2$. However, the effect of temperature on the extraction, as well as that of the liquid-to-solid ratio were less pronounced, compared to those of the two other factors. As can be seen in Figure 2, the extraction of TPC increased as the ethanol content of the solvent increased up to 50% *v*/*v*. A further increase in the ethanol content resulted in a decreased extraction of the polyphenols. This can be attributed to the change in the polarity of the solvent, as polyphenols are more readily extracted in less polar solvents than in water [15]. The maximum predicted value for TPC was 19.85 mg GAE/g of dry SCGs, which could be achieved by adding 35 mL of a 50% *v*/*v* water/ethanol solution to 1 g of SCGs and stirring the mixture for 120 min at 65 °C. The results obtained are comparable to those previous studies [26,28]. As regards neochlorogenic acid, chlorogenic acid, and caffeic acid, they followed the same trend as the TPC. Gigliobianco et al. [25] used a microwave-assisted extraction (MAE) method to extract TPC from SCGs. They found that the maximum TPC content was obtained at a solvent ratio of 50% ethanol and 50% water, an extraction time of 30 min, and a temperature of 80 °C, yielding 19.62 mg GAE/g. The optimal extraction conditions were different from those used in the current study, but the results were comparable in terms of the maximum predicted TPC content (19.85 mg GAE/g) obtained in the current study.

As regards the ferric-reducing antioxidant power of the extracts, the effects of all the examined parameters (X_1, X_2, X_3, and X_4) were found to be statistically significant, as well as those of the quadratic terms ${X_1}^2$ and ${X_2}^2$. The FRAP response was affected in a similar way as the TPC. This was somewhat expected, since the antioxidant properties of the extracts, stemmed, mainly, from the polyphenols. This was also the case with the DPPH response. The maximum response in the FRAP assay can be achieved by adding 35 mL of a 50% *v*/*v* water/ethanol solution to 1 g of SCGs and stirring the mixture for 120 min at 80 °C. Likewise, the maximum response in the DPPH assay was achieved by adding 35 mL of a 50% *v*/*v* water/ethanol solution to 1 g of SCGs and stirring the mixture for 222 min at 50 °C.

As can be seen in Table 4, the optimum extraction parameters for each response varied. However, according to the desirability plots (Figures S1–S4), the temperature variations did not cause significant changes in the predicted response. As such, in order to render the extraction method more environmentally friendly and lower its overall cost, 20 °C is suggested as the optimum extraction temperature for the TPC, FRAP, and DPPH responses.

In order to examine whether the predicted values could be achieved in the proposed conditions, two additional extractions were conducted. SCGs were extracted using the optimum conditions for caffeine extraction, and another extract was prepared using the conditions for TPC. In both cases, the extraction was carried out at 20 °C. According to the results, the caffeine content of the extract was found to be 6.6 mg/g of dry SCGs. The experiment values for TPC, FRAP, and DPPH of the other extract were found to be 19 mg GAE/g dw, 140 μmoL AAE/g dw, and 206 μmoL DPPH/g, respectively. Based on these results, it can be concluded that the experimental values were in close agreement with the predicted ones. Moreover, the content of the extract in neochlorogenic acid, chlorogenic acid,

and caffeic acid was assessed. According to the results, the extract contained 0.30 mg/g of neochlorogenic acid, 0.62 mg/g of chlorogenic acid, and 0.27 mg/g of caffeic acid.

Finally, coffee grounds not previously used for the preparation of coffee, as well as espresso coffee were also examined for the abovementioned parameters. The results are presented in Table 5. As can be seen, the extracts from spent coffee grounds contained significant amounts of the examined compounds. For instance, 33% of the total caffeine was not extracted during espresso making and was present in the SCGs. Moreover, it can be deduced that the conditions used for the preparation of the extract were highly suitable, since the extracted compounds (those responsible for the antioxidant activity) were almost quantitatively extracted (a second extraction was carried out from previously extracted SCGs, using the optimum conditions, and its content their bioactive compounds was very low (<4% in all cases).

Table 5. Content of extracts from SCGs, coffee grounds, and espresso coffee in caffeine and TPC and FRAP and DPPH values.

	Caffeine (mg/g)	TPC (mg GAE/g)	FRAP (μmoL AAE/g)	DPPH (μmoL DPPH/g)
Spent coffee grounds	6.6 ± 0.3	19 ± 1	140 ± 7	206 ± 10
Coffee grounds	20 ± 1	54 ± 3	495 ± 25	572 ± 29
Espresso coffee	12.7 ± 0.6	36 ± 2	312 ± 16	274 ± 14

4. Conclusions

Coffee will continue to be one of the favorite beverages in the world. Therefore, more and more waste is going to be produced, and the need for its valorization will continue to grow. Based on our study, SCGs are a good source of many bioactive components, which are usually disposed of, along with the SCGs. By performing an extraction procedure using the SCGs, the valorization and reintegration of the SCGs in the industry through a circular economy concept by providing new valuable bioactive compounds are plausible. By optimizing simple experimental conditions (i.e., composition and amount of the solvent as well as time and temperature of the extraction), the extraction yield of the compounds can be altered. Under optimum conditions, the concentration of caffeine and polyphenols can be maximized, yielding extracts that can be further used in the food industry either to enhance the properties of existing products or to develop new beverages with enhanced content in antioxidant compounds. The findings of the current study suggest that a low extraction temperature (20 °C) may be suitable for the extraction of bioactive compounds from SCGs, which could have implications for reducing the environmental impact and cost of the extraction process. Using food-compatible solvents and benign extraction conditions, extracts from SCGs can be easily prepared, in an environmentally friendly manner. This way, new products may be obtained with additive value, while at the same time, the circular economy is enhanced, benefiting both the food sector and the ecosystems. The limitations of this study include its focus on the extraction of caffeine and the three main polyphenols, while other, potentially valuable, compounds could also be extracted. Further research could explore the extraction of a broader range of bioactive compounds from spent coffee grounds, including those that may have applications in fields beyond the food industry (such as pharmaceuticals). Moreover, alternative extraction methods (e.g., ultrasound extraction and pulsed electric field) could be compared to the method used in this study to determine the most efficient and sustainable approach.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13052819/s1, Figure S1: Plot of actual vs. predicted response (caffeine, mg/g) (plot A) and desirability function (plot B) for the optimization of the extraction of spent coffee grounds performed with hydroethanolic solutions. The inset tables provide statistics related to the evaluation of the resulting model. Values with color and asterisk are statistically significant.; Figure S2: Plot of actual vs. predicted response (polyphenols, mg GAE/g) (plot A) and desirability function (plot B) for the optimization of the extraction of spent coffee grounds performed with hydroethanolic solutions. The inset tables provide statistics related to the evaluation of the resulting model. Values with color and asterisk are statistically significant.; Figure S3: Plot of actual vs. predicted response (FRAP, µmoL AAE/g) (plot A) and desirability function (plot B) for the optimization of the extraction of spent coffee grounds performed with hydroethanolic solutions. The inset tables provide statistics related to the evaluation of the resulting model. Values with color and asterisk are statistically significant.; Figure S4: Plot of actual vs. predicted response (DPPH, µmoL DPPH/g) (plot A) and desirability function (plot B) for the optimization of the extraction of spent coffee grounds performed with hydroethanolic solutions. The inset tables provide statistics related to the evaluation of the resulting model. Values with color and asterisk are statistically significant.; Figure S5: Bivariate response fitting plot (caffeine vs. polyphenols) for the optimization of spent coffee grounds extraction performed with hydroethanolic solutions. The inset tables provide statistics related to the evaluation of the resulting model. Values with color and asterisk are statistically significant.; Figure S6: Bivariate response fitting plot (FRAP vs. polyphenols) for the optimization of spent coffee grounds extraction performed with hydroethanolic solutions. The inset tables provide statistics related to the evaluation of the resulting model. Values with color and asterisk are statistically significant.; Figure S7: Bivariate response fitting plot (DPPH vs. polyphenols) for the optimization of spent coffee grounds extraction performed with hydroethanolic solutions. The inset tables provide statistics related to the evaluation of the resulting model. Values with color and asterisk are statistically significant.

Author Contributions: Conceptualization, T.C., V.A. and S.I.L.; methodology, T.C. and V.A.; software, V.A.; validation, V.A.; formal analysis, T.C. and V.A.; investigation, K.K., V.A., T.C., D.P. and E.B.; resources, S.I.L.; data curation, V.A.; writing—original draft preparation, K.K.; writing—review and editing, K.K., V.A., T.C., D.P., E.B. and S.I.L.; visualization, V.A.; supervision, S.I.L.; project administration, V.A. and T.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Jamaludin, H.; Elmaky, H.S.E.; Sulaiman, S. The future of food waste: Application of circular economy. *Energy Nexus* **2022**, *7*, 100098. [CrossRef]
2. Massari, S.; Principato, L.; Antonelli, M.; Pratesi, C.A. Learning from and designing after pandemics. CEASE: A design thinking approach to maintaining food consumer behaviour and achieving zero waste. *Socio-Economic Plan. Sci.* **2021**, *82*, 101143. [CrossRef]
3. De Oliveira, M.M.; Lago, A.; Magro, G.P.D. Food loss and waste in the context of the circular economy: A systematic review. *J. Clean. Prod.* **2021**, *294*, 126284. [CrossRef]
4. Kharola, S.; Ram, M.; Mangla, S.K.; Goyal, N.; Nautiyal, O.; Pant, D.; Kazancoglu, Y. Exploring the green waste management problem in food supply chains: A circular economy context. *J. Clean. Prod.* **2022**, *351*, 131355. [CrossRef]
5. Alfano, A.; Corsuto, L.; Finamore, R.; Savarese, M.; Ferrara, F.; Falco, S.; Santabarbara, G.; De Rosa, M.; Schiraldi, C. Valorization of Olive Mill Wastewater by Membrane Processes to Recover Natural Antioxidant Compounds for Cosmeceutical and Nutraceutical Applications or Functional Foods. *Antioxidants* **2018**, *7*, 72. [CrossRef] [PubMed]
6. Tucker, C.M. *Coffee Culture: Local Experiences, Global Connections*, 2nd ed.; Routledge: Abingdon, UK, 2017; ISBN 9781317392255.
7. Gebreeyessus, G.D. Towards the sustainable and circular bioeconomy: Insights on spent coffee grounds valorization. *Sci. Total Environ.* **2022**, *833*, 155113. [CrossRef]
8. Mussatto, S.I.; Carneiro, L.M.; Silva, J.P.A.; Roberto, I.C.; Teixeira, J.A. A study on chemical constituents and sugars extraction from spent coffee grounds. *Carbohydr. Polym.* **2011**, *83*, 368–374. [CrossRef]

9. Zarrinbakhsh, N.; Wang, T.; Rodriguez-Uribe, A.; Misra, M.; Mohanty, A.K. Characterization of wastes and coproducts from the coffee industry for composite material production. *BioResources* **2016**, *11*, 7637–7653. [CrossRef]
10. Getachew, A.T.; Chun, B.S. Influence of pretreatment and modifiers on subcritical water liquefaction of spent coffee grounds: A green waste valorization approach. *J. Clean. Prod.* **2017**, *142*, 3719–3727. [CrossRef]
11. Temple, J.L.; Bernard, C.; Lipshultz, S.E.; Czachor, J.D.; Westphal, J.A.; Mestre, M.A. The Safety of Ingested Caffeine: A Comprehensive Review. *Front. Psychiatry* **2017**, *8*, 80. [CrossRef]
12. Mostafa, H.S. Assessment of the caffeine-containing beverages available in the local markets, and development of a real energy drink based on the date fruit. *Food Sci. Technol.* **2022**, *42*, e51820. [CrossRef]
13. González-Calderón, D.; González-Romero, C.; González-González, C.A.; Fuentes-Benítes, A. Synthesis of caffeine from theobromine: Bringing back an old experiment in a new setting. *Educ. Química* **2015**, *26*, 9–12. [CrossRef]
14. Bravo, J.; Juaniz, I.; Monente, C.; Caemmerer, B.; Kroh, L.W.; de Peña, M.-P.; Cid, C. Evaluation of Spent Coffee Obtained from the Most Common Coffeemakers as a Source of Hydrophilic Bioactive Compounds. *J. Agric. Food Chem.* **2012**, *60*, 12565–12573. [CrossRef] [PubMed]
15. Mitraka, G.-C.; Kontogiannopoulos, K.; Batsioula, M.; Banias, G.; Assimopoulou, A. Spent Coffee Grounds' Valorization towards the Recovery of Caffeine and Chlorogenic Acid: A Response Surface Methodology Approach. *Sustainability* **2021**, *13*, 8818. [CrossRef]
16. Seo, H.S.; Park, B.H. Phenolic compound extraction from spent coffee grounds for antioxidant recovery. *Korean J. Chem. Eng.* **2019**, *36*, 186–190. [CrossRef]
17. Panusa, A.; Zuorro, A.; Lavecchia, R.; Marrosu, G.; Petrucci, R. Recovery of Natural Antioxidants from Spent Coffee Grounds. *J. Agric. Food Chem.* **2013**, *61*, 4162–4168. [CrossRef]
18. Santana-Gálvez, J.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. Chlorogenic Acid: Recent Advances on Its Dual Role as a Food Additive and a Nutraceutical against Metabolic Syndrome. *Molecules* **2017**, *22*, 358. [CrossRef]
19. Haider, K.; Haider, R.; Neha, K.; Yar, M.S. Free radical scavengers: An overview on heterocyclic advances and medicinal prospects. *Eur. J. Med. Chem.* **2020**, *204*, 112607. [CrossRef]
20. Kang, N.J.; Lee, K.W.; Shin, B.J.; Jung, S.K.; Hwang, M.K.; Bode, A.M.; Heo, Y.-S.; Lee, H.J.; Dong, Z. Caffeic acid, a phenolic phytochemical in coffee, directly inhibits Fyn kinase activity and UVB-induced COX-2 expression. *Carcinogenesis* **2008**, *30*, 321–330. [CrossRef]
21. Coelho, J.P.; Robalo, M.P.; Boyadzhieva, S.; Stateva, R.P. Microwave-Assisted Extraction of Phenolic Compounds from Spent Coffee Grounds. Process Optimization Applying Design of Experiments. *Molecules* **2021**, *26*, 7320. [CrossRef] [PubMed]
22. Coelho, J.P.; Filipe, R.M.; Robalo, M.P.; Boyadzhieva, S.; Cholakov, G.S.; Stateva, R.P. Supercritical CO2 extraction of spent coffee grounds. Influence of co-solvents and characterization of the extracts. *J. Supercrit. Fluids* **2020**, *161*, 104825. [CrossRef]
23. Lakka, A.; Grigorakis, S.; Kaltsa, O.; Karageorgou, I.; Batra, G.; Bozinou, E.; Lalas, S.; Makris, D.P. The Effect of Ultrasonication Pretreatment on the Production of Polyphenol-Enriched Extracts from Moringa oleifera L. (Drumstick Tree) Using a Novel Bio-Based Deep Eutectic Solvent. *Appl. Sci.* **2019**, *10*, 220. [CrossRef]
24. Athanasiadis, V.; Palaiogiannis, D.; Poulianiti, K.; Bozinou, E.; Lalas, S.I.; Makris, D.P. Extraction of Polyphenolic Antioxidants from Red Grape Pomace and Olive Leaves: Process Optimization Using a Tailor-Made Tertiary Deep Eutectic Solvent. *Sustainability* **2022**, *14*, 6864. [CrossRef]
25. Gigliobianco, M.R.; Campisi, B.; Peregrina, D.V.; Censi, R.; Khamitova, G.; Angeloni, S.; Caprioli, G.; Zannotti, M.; Ferraro, S.; Giovannetti, R.; et al. Optimization of the Extraction from Spent Coffee Grounds Using the Desirability Approach. *Antioxidants* **2020**, *9*, 370. [CrossRef]
26. Solomakou, N.; Loukri, A.; Tsafrakidou, P.; Michaelidou, A.-M.; Mourtzinos, I.; Goula, A.M. Recovery of phenolic compounds from spent coffee grounds through optimized extraction processes. *Sustain. Chem. Pharm.* **2022**, *25*, 100592. [CrossRef]
27. Tavagnacco, L.; Schnupf, U.; Mason, P.E.; Saboungi, M.-L.; Cesàro, A.; Brady, J.W. Molecular Dynamics Simulation Studies of Caffeine Aggregation in Aqueous Solution. *J. Phys. Chem. B* **2011**, *115*, 10957–10966. [CrossRef]
28. Shang, Y.-F.; Xu, J.-L.; Lee, W.-J.; Um, B.-H. Antioxidative polyphenolics obtained from spent coffee grounds by pressurized liquid extraction. *S. Afr. J. Bot.* **2017**, *109*, 75–80. [CrossRef]
29. Andrade, K.S.; Gonçalvez, R.T.; Maraschin, M.; Ribeiro-Do-Valle, R.M.; Martínez, J.; Ferreira, S.R. Supercritical fluid extraction from spent coffee grounds and coffee husks: Antioxidant activity and effect of operational variables on extract composition. *Talanta* **2012**, *88*, 544–552. [CrossRef]