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β-Cyclodextrin-Aided Aqueous Extraction of Antioxidant Polyphenols from Peppermint (*Mentha* × *piperita* L.)

Vassilis Athanasiadis, Dimitrios Palaiogiannis, Eleni Bozinou, Stavros I. Lalas and Dimitris P. Makris *

Department of Food Science & Nutrition, School of Agricultural Sciences, University of Thessaly, N. Temponera Street, 43100 Karditsa, Greece
* Correspondence: dimitrismakris@uth.gr; Tel.: +30-24410-64792

Abstract: This study explored the use of β-cyclodextrin (β-CD) as an additive to improve the aqueous extraction of antioxidant polyphenols from peppermint (*Mentha* × *piperita*). For this purpose, an initial single-factor screening was performed to test the effect of β-CD concentration on the yield of polyphenol extraction. In the following step, the extraction process was optimized through response surface methodology, considering β-CD and temperature as the process variables. The experimental design included the yield in total polyphenols and total flavonoids, the ferric-reducing power and the antiradical activity as the responses. The optimization showed that each response was maximized at different levels of β-CD concentration, but in all cases, 80 °C was the optimum extraction temperature. The composition of the extracts produced was profiled by high-performance liquid chromatography (HPLC). A comparison of the β-CD extract with the aqueous and hydroethanolic extracts revealed that the addition of β-CD at a specified concentration might boost aqueous polyphenol extraction. On the other hand, the hydroethanolic extract exhibited the richest polyphenolic profile. It was also shown that the β-CD extracts might possess improved antiradical activity. It was concluded that β-CD-aided polyphenol extraction from *M. piperita* may provide extracts with enriched polyphenolic composition and improved antioxidant characteristics, and this technique may be considered an alternative to solvent extraction.

Keywords: antioxidants; β-cyclodextrin; extraction; *Mentha piperita*; polyphenols

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1. Introduction

Medicinal and aromatic plants (MAPs) have been used globally for centuries by many populations as folk remedies and food condiments. The scientific data accumulated to date concur with the nutritional and pharmacological properties attributed to MAPs and have substantiated several claims pertaining to their biofunctionality [1]. The ever-increasing interest in MAPs and MAP-based products driven by consumer preferences and demands for natural commodities with multifunctional characteristics has stimulated an important trend for the development of novel botanical-derived ingredients for cosmetics, foods, and pharmaceuticals [2,3].

Mentha × *piperita* L., collectively known as peppermint, is a plant species belonging to the Lamiaceae family and occurs in temperate areas of Europe, Asia, North America, Africa, and Australia. This species is exceptionally rich in polyphenolic phytochemicals, which may account for up to 19–23% of its dry weight [4], and they are represented mainly by hydroxycinnamates, flavanone, and flavone glycosides [5]. There is a significant body of information evidencing the high biological potential of *M. piperita*, including cytotoxicity activities, anticarcinogenic and antioxidant activities, antimicrobial activities, and anti-inflammatory properties; several of these attributes have been ascribed to polyphenolic substances [6,7]. However, to date, the development of sustainable extraction processes to produce polyphenol-containing extracts from *M. piperita* is very limited.

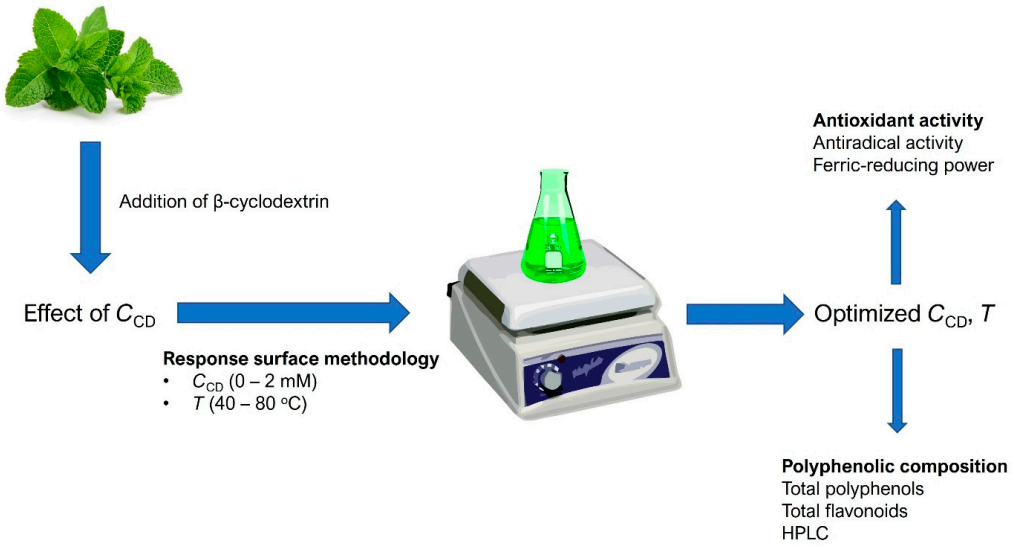
The great interest in the polyphenolic composition of MAPs has currently led to the development and implementation of sustainable, cutting-edge extraction methodologies,

and in this direction, an assortment of low-cost, green techniques has been proposed as more precise and efficient compared to conventional ones [8,9]. On the basis of Green Chemistry principles, there is a dire necessity for the establishment of eco-friendly extraction processes with bio-based alternative solvents to replace petroleum-based, conventional volatile ones. Towards this direction, the utilization of a benign and environmentally friendly extraction medium is imminent to the sustainable character of an extraction process. This medium should be highly effective for polyphenol extraction, non-toxic, readily available, and inexpensive and should be produced from recyclable materials [10,11].

Cyclodextrins (CDs) are a group of cyclic oligosaccharide supramolecules composed of D-glucopyranose subunits linked with α(1→4) glycosidic bonds. The most commonly used CDs are α-, β- and γ-CDs, comprised of six, seven, and eight glucose units, respectively. These compounds are produced through enzymic degradation of starch, and they have a shape of a truncated cone with the hydroxyl groups being oriented towards the outer surface of the cavity. The major characteristic of this 3D configuration of the CD molecules is the hydrophilic outer surface and the hydrophobic internal cavity. These structural features endow CDs with water solubility but and the ability to encapsulate molecules of appropriate polarity and suitable size to form inclusion complexes [12,13].

Although the use of CDs may have a spectrum of applications in chemical, pharmaceutical, and other disciplines, there is a steady annual increase in uses related to food [14,15]. CDs in food products may serve to stabilize flavors, solubilize poorly water-soluble substances, protect labile additives, etc. However, over the last years, the utilization of CDs for polyphenol extraction has attracted significant interest, making CD-based polyphenol extraction a state-of-the-art trend, which might offer innovative opportunities in the development of green processes. Despite the fact that conventional solvents routinely used for polyphenol extraction (e.g., acetone, ethyl acetate, ethanol, etc.) may exhibit excellent efficiency, their use may be restricted due to, e.g., cost, State laws, environmental concerns, etc. Thus, aqueous CD systems may be viewed as alternative green extraction media, with a high perspective in this regard [16].

This study investigated the efficiency of β-cyclodextrin (β-CD) aqueous solutions on polyphenol extraction from *M. piperita*. The examination was based on an experiment designed to include two critical process variables, the β-CD concentration and temperature. The extracts' produced characteristics were assessed by estimating their polyphenolic load, antioxidant properties, and polyphenolic composition. To the best of the authors' knowledge, this is the first report on the extraction of *M. piperita* polyphenols employing aqueous β-CD. The sequence of the procedures followed is depicted in Scheme 1.



Scheme 1. The sequence of the experimental procedures followed in this study. Assignments: C_{CD}, β-cyclodextrin concentration (%); T, temperature.

2. Materials and Methods

2.1. Chemicals–Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Karlsruhe, Germany). Sodium acetate anhydrous, sodium carbonate anhydrous, and aluminum chloride anhydrous were from Penta (Prague, Czech Republic). L-Ascorbic acid was purchased from Carlo Erba (Milano, Italy). 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was from Fluka (Steinheim, Germany). Gallic acid monohydrate, absolute ethanol, and Folin-Ciocalteu regent were from Panreac (Barcelona, Spain). Iron (III) chloride hexahydrate ($FeCl_3$) was from Merck (Darmstadt, Germany). Rutin hydrate was from MP Biomedicals (Illkirch, France). Luteolin 7-*O*-rutinoside, eriocitrin, rosmarinic acid, narirutin, caffeic acid, and hesperidin were from Sigma-Aldrich (Steinheim, Germany). β-Cyclodextrin (98%) was from Acros Organics (Geel, Belgium). Acetonitrile and formic acid used for chromatographic determinations were HPLC grade and obtained from Panreac (Barcelona, Spain).

2.2. Plant Material and Handling

Certified peppermint (*Mentha* × *piperita*) was purchased from a local store (Karditsa, Central Greece) in dried form, in plastic, air-tight packaging. The material was pulverized with a 1400 W domestic blender (Camry, Poland), at room temperature, at intermittent periods of 15 s (2–3 repetitions), and then sieved using a Fritsch Analysette 3 device (Idar-Oberstein, Germany). Powder with around 245 μm particle diameter was collected and stored in the freezer (−40 °C).

2.3. Extraction Procedures

Extractions were accomplished with 10 mL of solvent and 1 g of dry mass (DM) to provide a liquid-to-solid ratio of 10 mL g^{-1}. The dried material and the solvent were placed into a 25-mL Duran™ glass bottle immersed in an oil bath. Stirring and heating of the mixture were carried out with a temperature-controlled hotplate (Witeg, Wertheim, Germany) set at 500 rpm. For the single-factor experiments aimed at screening various β-CD concentrations, extractions were accomplished at 40 °C. The design dictated the extraction temperature for the extractions performed for the response surface methodology (Table 1).

Table 1. Codified and actual values of the process variables employed to construct the experimental design.

Process Variables	Codes	Coded Variable Level		
		−1	**0**	**1**
C_{CD} (mM)	X_1	0	1	2
T (°C)	X_2	40	60	80

Aqueous β-cyclodextrin (β-CD) solutions were prepared in deionized water. Because β-CD has a solubility of 18.5 mg mL^{-1} (approximately 15 mM) at 25 °C, β-CD concentrations tested were 1, 2, 4, 8, and 15 mM [17]. Extractions were then accomplished as described above. Control extracts with 60% (v/v) ethanol and deionized water were prepared at 70 °C, with extraction times of 60 and 180 min, respectively. These settings are average values of extraction conditions reported in a previous thorough study [18]. All extracts were centrifuged at 10,000× g for 10 min prior to chemical analyses.

2.4. Experimental Design and Response Surface Methodology (RSM)

Based on preliminary experiments and previously published data [19], two independent variables—the β-CD concentration (C_{CD}) and temperature (T)—were selected to study their effect on four responses—the total polyphenol yield (Y_{TP}), the total flavonoid yield (Y_{TFn}), the ferric-reducing power (P_R), and the antiradical activity (A_{AR}). The RSM used a three-level, two-variable central composite design (Table 1) consisting of 11 experimental

runs (design points) to investigate the response pattern and to determine the optimum combination of independent (process) variables. Codification of the variables was accomplished using the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i},\ x_i = 1,\ 2 \quad (1)$$

x_i is the dimensionless value of an independent variable; X_i is the real (actual) value of an independent variable; X_0 is the real (actual) value of an independent variable at the center point, and ΔX_i = step change. The data from the experimental design were elaborated with second-order polynomial regression analysis employing the least square regression methodology to determine the mathematical model parameters. The experimental data were fitted to the second-order polynomial model using the equation shown below:

$$Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad (2)$$

where $X_1, X_2, \ldots, X_k$ are the independent (process) variables which affect the responses Y_i; β_0, β_i (i = 1, 2, …, k), β_{ii} (i = 1, 2, …, k), and β_{ij} (i = 1, 2, …, k; j = 1, 2, …, k) are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively; k is the variables number. The responses from the experimental design were subjected to multiple nonlinear regression analyses to determine the second-order polynomial model coefficients. Model evaluation was based on analysis of variance (ANOVA) and lack-of-fit test. Three-dimensional surface response plots were constructed to provide a visual projection of the model equation.

2.5. Spectrophotometric Analyses

2.5.1. Total Polyphenols

All measurements were performed with a Shimadzu UV-1700 (Shimadzu Europa GmbH, Duisburg, Germany) spectrophotometer. For the analysis of total polyphenols (TP), a micro-scale methodology was employed, as previously described [20]. In short, 0.1 mL of Folin-Ciocalteu reagent was added to an equal extract volume and allowed to react for 2 min. Then, a 0.8 mL Na_2CO_3 solution (5% w/v) was added, followed by incubation of the mixture in a water bath for 20 min at 40 °C. Total polyphenol content was calculated from the absorbance at 740 nm, and a calibration curve was constructed using gallic acid standard solutions (10–80 mg L^{-1}). The expression of the results was as mg gallic acid equivalents (GAE) L^{-1}.

2.5.2. Total Flavonoids

For the analysis of total flavonoids, a protocol described elsewhere was implemented [21]. The sample (0.1 mL) was combined with 0.04 mL of a reagent containing 5% (w/v) $AlCl_3$ and 0.5 M CH_3COONa, and 0.86 mL 35% (v/v) ethanol. The mixture was left for 30 min at ambient temperature before measuring the absorbance at 415 nm. Content in total flavonoid was determined as mg rutin equivalents (RtE) per DM, using a rutin calibration curve (30–300 mg L^{-1}).

2.5.3. Antioxidant Activity

Two tests were used to evaluate the antioxidant activity of the extracts produced, the ferric-reducing power (P_R) and the antiradical activity (A_{AR}). Briefly, A_{AR} was measured by mixing a 0.025 mL sample with 0.975 mL DPPH solution and taking the absorbance at 515 nm, at t = 0 and t = 30 min. A_{AR} was expressed as a µmol DPPH g^{-1} DM, as described elsewhere [22]. The ferric-reducing power was measured by incubating a 0.05 mL sample with 0.05 mL of iron chloride, for 30 min, at 37 °C. Then, 0.9 mL TPTZ solution was added, and the absorbance was read at 625 nm. Results were given as µmol ascorbic acid equivalents (AAE) per g DM, employing a calibration curve (50–300 µM) with ascorbic acid as standard [22].

2.6. Quantitative High-Performance Liquid Chromatography (HPLC)

The liquid chromatograph used for the analyses was a Shimadzu CBM-20A (Shimadzu Europa GmbH, Duisburg, Germany), equipped with a CTO-20AC column oven, a SIL-20AC autosampler, and a Shimadzu SPD-M20A detector. The system was controlled by the Shimadzu LC solution software. Details regarding the column used and other analytical parameters have been provided elsewhere [22]. Quantification was performed at 280, 320, and 345 nm for flavanones, hydroxycinnamates, and flavones, respectively. For the quantitative determinations, calibration curves of eriocitrin (R^2 = 0.9990), narirutin (R^2 = 0.9999), hesperidin, caffeic acid (R^2 = 0.9980), rosmarinic acid R^2 = 0.9990), and luteolin rutinoside (R^2 = 0.9980) were constructed (1–50 μg mL^{-1}), from standard solutions prepared in HPLC-grade methanol.

2.7. Statistical Elaboration

The JMP™ Pro 13 (SAS, Cary, NC, USA) software was used to derive the experimental design and the statistical analyses associated with the response surface methodology. Linear regression analyses were conducted with SigmaPlot™ 12.5 (Systat Software Inc., San Jose, CA, USA). The extraction procedures were performed at least twice, and the quantitative analyses were run in triplicate. The values reported are means ± standard deviation.

3. Results and Discussion

3.1. The Effect of β-CD Concentration

Prior to setting up the experimental design, an issue of high significance was the range of C_{CD} that should be tested. Thus, a single-factor experiment was judged necessary in the light of previous recent investigations, which indicated that C_{CD} increases beyond a certain limit did not provide significantly higher total polyphenol yield (Y_{TP}) [19,23]. Figure 1 depicts the results of the testing of the effect of C_{CD} on Y_{TP}, and it can be seen that for extractions with C_{CD} higher than 1 mM, the increase in Y_{TP} was either lower or statistically non-significant ($p < 0.05$). This was emphasized by the finding that a solution with C_{CD} of 15 mM afforded a Y_{TP} higher by only 5.4% compared to that with C_{CD} of 1 mM. On this ground, it was evidenced that a range of C_{CD} from 0 to 2 mM would actually reveal a significant effect on Y_{TP}. Furthermore, considering recent kinetic studies, which showed that extraction time longer than 180 min did not afford significant changes in Y_{TP}, irrespective of the extraction temperature [17,24], this resident period was adopted for all subsequent experiments.

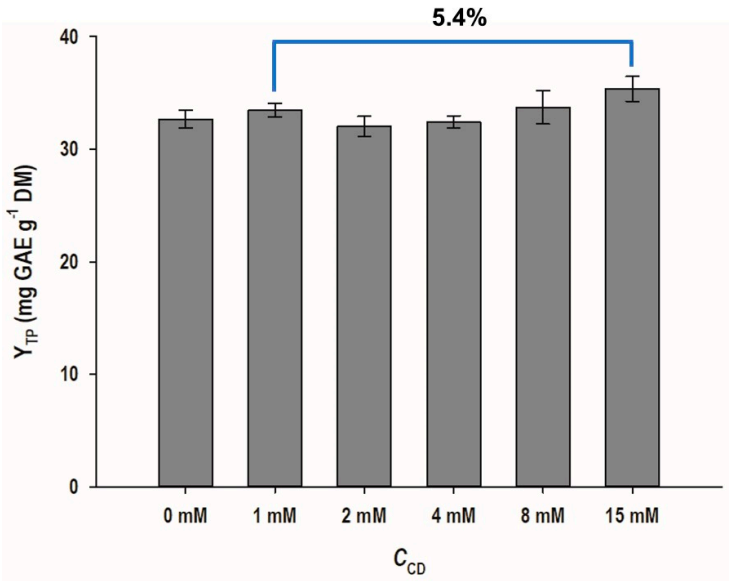


Figure 1. Screening for the effect of β-CD concentration (C_{CD}) on the yield of total polyphenols (Y_{TP}). Extractions were accomplished for 180 min at 40 °C.

3.2. Extraction Optimization by Response Surface Methodology

The assessment of model fitting and the suitability of the response surface was based on the analysis of variance (ANOVA) and lack-of-fit test (Figures S1–S4, supplementary file), taking into consideration the closeness of the predicted and measured response values (Table 2). The relevant statistical processing revealed the significance of the coefficients obtained for each model according to the second-degree polynomial equations (Figure S1, inset table "Parameter estimates"). These equations, composed only of the significant terms, are presented in Table 3. The R^2 determined in each case gave an indication of the total variability around the mean, explained by the model. It can be seen that all R^2 determined were higher than or equal to 0.97, and it could be supported that the models displayed a good fit to the sample data. The *p* value for lack-of-fit (confidence interval of 95%) was also significant for all responses considered, indicating that the fitted models may be very good predictors. For each response, the effect of the variables was depicted as 3-dimensional diagrams, and it can be distinguished that the response pattern for Y_{TP} and P_R was very similar (Figure 2). This was also the case for Y_{TFn} and A_{AR}. These trends might indicate that the conditions that favored obtaining extracts with increased total polyphenol concentration also displayed increased P_R. A similar claim might hold true for Y_{TFn} and A_{AR}.

Table 2. The design of experiment used for the response surface methodology, including the design points and the predicted and measured responses.

Design Point	Process Variable		Responses							
	X_1 (*C*)	X_2 (*T*)	Y_{TP} (mg GAE g^{-1} DM)		Y_{TFn} (mg RtE g^{-1} DM)		A_{AR} (μmol DPPH g^{-1} DM)		P_R (μmol AAE g^{-1} DM)	
			Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted
1	−1	−1	32.66	32.85	17.95	17.23	402.93	368.26	241.17	238.46
2	−1	1	57.66	57.27	27.12	26.63	615.67	613.93	407.22	415.96
3	1	−1	33.02	34.25	18.05	17.97	259.87	272.60	260.55	247.96
4	1	1	54.83	55.49	26.94	27.09	700.70	746.26	355.52	354.46
5	−1	0	40.09	40.29	18.07	19.29	465.04	503.81	266.82	260.58
6	1	0	42.00	40.11	19.96	19.89	571.85	522.14	221.85	234.59
7	0	−1	33.46	32.04	23.00	23.80	522.50	544.14	207.76	222.58
8	0	1	55.14	54.87	32.72	33.06	946.79	903.81	373.28	364.54
9	0	0	38.37	38.69	26.53	25.79	729.40	736.68	245.48	226.95
10	0	0	37.02	38.69	25.05	25.79	733.12	736.68	234.56	226.95
11	0	0	39.04	38.69	27.02	25.79	739.16	736.68	223.24	226.95

Table 3. Equations (models) are constructed by the response surface methodology for each response considered.

Response	Equation (Model)	R^2	*p*
Y_{TP} (mg GAE g^{-1} DM)	$38.69 + 11.42\ X_2 + 4.77\ {X_2}^2$	0.99	<0.0001
Y_{TFn} (mg RtE g^{-1} DM)	$25.79 + 4.63\ X_2 - 6.21\ {X_1}^2 + 2.64\ {X_2}^2$	0.98	0.0005
A_{AR} (μmol DPPH g^{-1} DM)	$736.68 + 179.83\ X_2 - 223.71\ {X_1}^2$	0.97	0.0009
P_R (μmol AAE g^{-1} DM)	$226.95 + 71.00\ X_2 + 66.63\ {X_2}^2$	0.97	0.0006

The *p* value for each of the terms of the equations (models) was determined to investigate the contribution of the linear, cross (interaction), and quadratic effects of the process variables on the responses. In this regard, the ANOVA showed that variable X_1, which corresponds to C_{CD}, had a non-significant impact on Y_{TP} (Table 3). This finding suggested that polyphenol extraction was not facilitated in the presence of β-CD. This was in accordance with data on β-CD-aided polyphenol extraction from *Salvia fruticosa*, where the concentration of β-CD was tested up to 13 mM [24]. On the other hand, it contrasted results from earlier investigations on cyclodextrin-aided polyphenol extraction from solid onion waste, where β-CD was demonstrated to have a significant effect [17]. The same held true for polyphenol extraction from red grape pomace [25]. The addition of β-CD

did not affect the reducing properties of the extracts produced, as implied by the model (equation) given in Table 3. On the contrary, the role of C_{CD} was significant for both Y_{TFn} and A_{AR}. This might indicate that flavonoid extraction was enhanced upon the addition of β-CD, and so did the antiradical activity of the extracts.

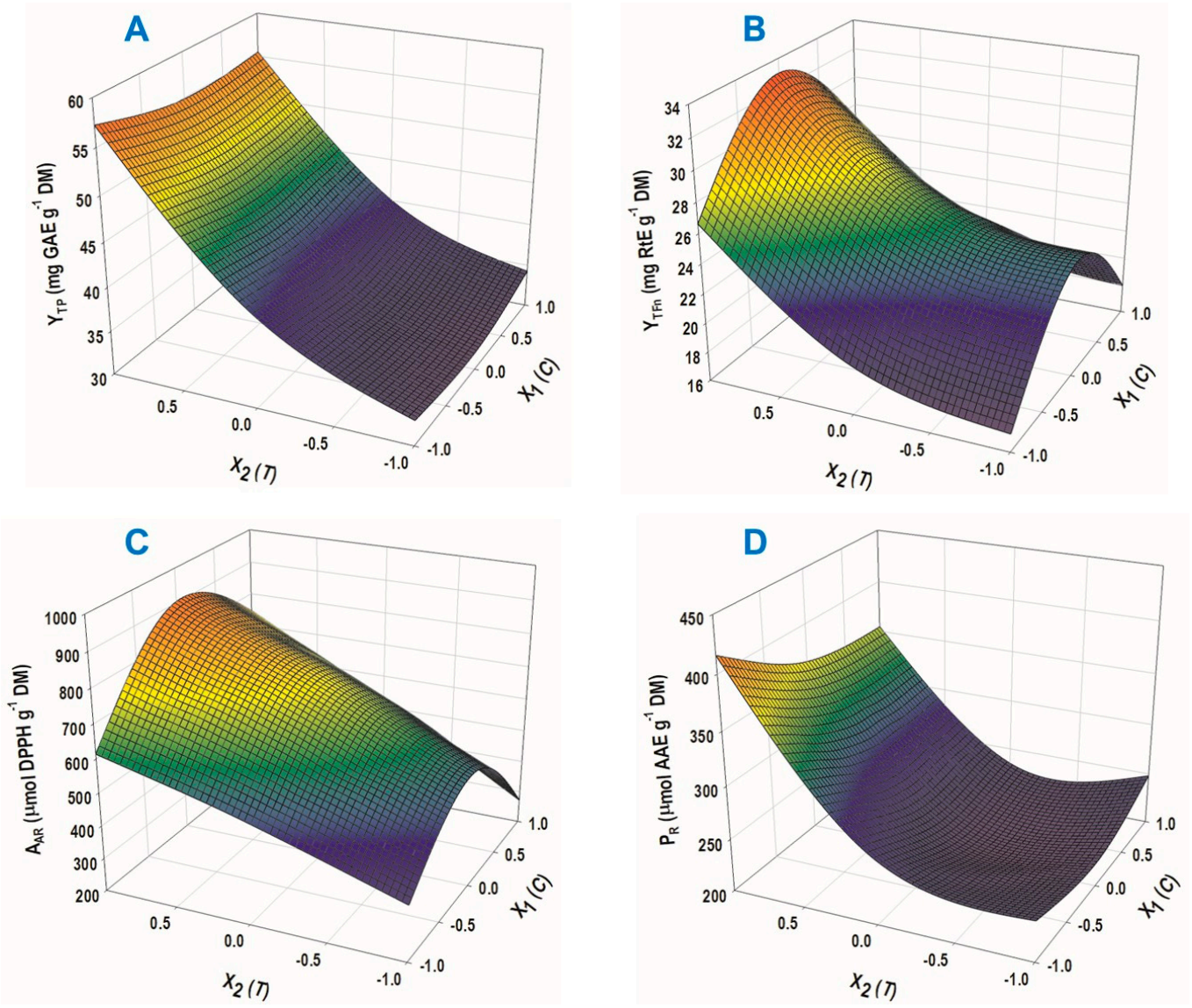


Figure 2. Three-dimensional diagrams showing the effect of independent (process) variables on the responses considered. (**A**), effect on Y_{TP}; (**B**), effect on Y_{TFn}; (**C**), effect on A_{AR}; (**D**), effect on P_R.

With reference to temperature, the optimization for all responses showed that the values were maximized at 80 °C (Table 4). This was in line with a recent thorough investigation, which suggested 76 °C to be the optimum *T* for polyphenols extraction from mint, using various solvents, such as water, aqueous ethanol, aqueous glycerol, and a deep eutectic solvent [18]. Optimizing A_{AR} and P_R required 80 °C was also strong evidence that no loss of antioxidant activity occurred at this temperature. However, an optimum temperature of as low as 50 °C has also been reported for peppermint extractions performed with water/glycerol mixtures [26].

Table 4. Maximum values for each response considered and the optimal conditions, as predicted by the desirability function (Figures S1–S4).

Response	Maximum Predicted Value	Optimal Conditions	
		C **(mM)**	***T*** **(°C)**
Y_{TP} (mg GAE g^{-1} DM)	57.27 ± 3.38	0	80
Y_{TFn} (mg RtE g^{-1} DM)	33.07 ± 1.96	1.02	80
A_{AR} (μmol DPPH g^{-1} DM)	908.70 ± 87.46	1.15	80
P_R (μmol AAE g^{-1} DM)	415.96 ± 36.55	0	80

3.3. Extraction Yield and Antioxidant Properties

Considering that the level of C_{CD} for optimization of all responses (Y_{TP}, Y_{TFn}, A_{AR}, and P_R) did not coincide, a peppermint extract was prepared using a β-CD solution with a concentration of 1.08 mM. This value represented an average of the optimum required to achieve maximum Y_{TFn} and A_{AR} (Table 4), and it was regarded as a fair compromise to come up with an extract enriched in flavonoids with increased antiradical activity. This extract's composition and antioxidant characteristics were then compared to those of extracts prepared with water and 60% aqueous ethanol. It can be viewed in Figure 3A that the extract produced with β-CD had 22 and 31% higher Y_{TP} compared to the aqueous and hydroethanolic extracts, respectively. On the other hand, the β-CD extract displayed 15% lower Y_{TFn} than the hydroethanolic extract but 18% higher than the aqueous extract (Figure 3B). Considering both Y_{TP} and Y_{TFn}, it could be argued that the β-CD-aided extraction of polyphenols from peppermint may provide yields comparable to those obtained with a green organic solvent (aqueous ethanol) and even more enhanced than those produced with aqueous extraction.

With regard to A_{AR}, the β-CD extract was the most active, exhibiting 11 and 27% higher performance compared to the hydroethanolic and aqueous extract, respectively (Figure 3C). However, the pattern seen for P_R was different (Figure 3D) since both the aqueous and hydroethanolic extracts were more powerful than the β-CD extract. This diversified outcome indicated that the use of β-CD might contribute towards obtaining extracts with enhanced A_{AR}, but not P_R. A similar phenomenon has been reported in polyphenol-containing extracts obtained from onion solid wastes with various cyclodextrins, where an enhancement was seen for A_{AR}, as opposed to P_R, which was found to be weakened [17]. Likewise, red grape pomace polyphenol extracts generated with β-CD were shown to possess significantly higher A_{AR} than aqueous or hydroethanolic extracts [25]. Studies with coffee extracts were also in the same line [27], but a kinetic investigation on the expression of A_{AR} by olive leaf extracts suggested the presence of β-CD to be rather inhibitory in this regard [28]. The explanation for these observations may lie in studies on pure polyphenols, such as chlorogenic acid [29], quercetin [30], and rosmarinic acid [31]. These substances were proven to display enhanced antioxidant activity when encapsulated in CDs compared to their non-encapsulated (free) state. This behavior was attributed to the fact that polyphenol radicals may be more effectively stabilized when encapsulated in a CD hydrophobic cavity, which in turn may endow polyphenols with higher radical-scavenging ability.

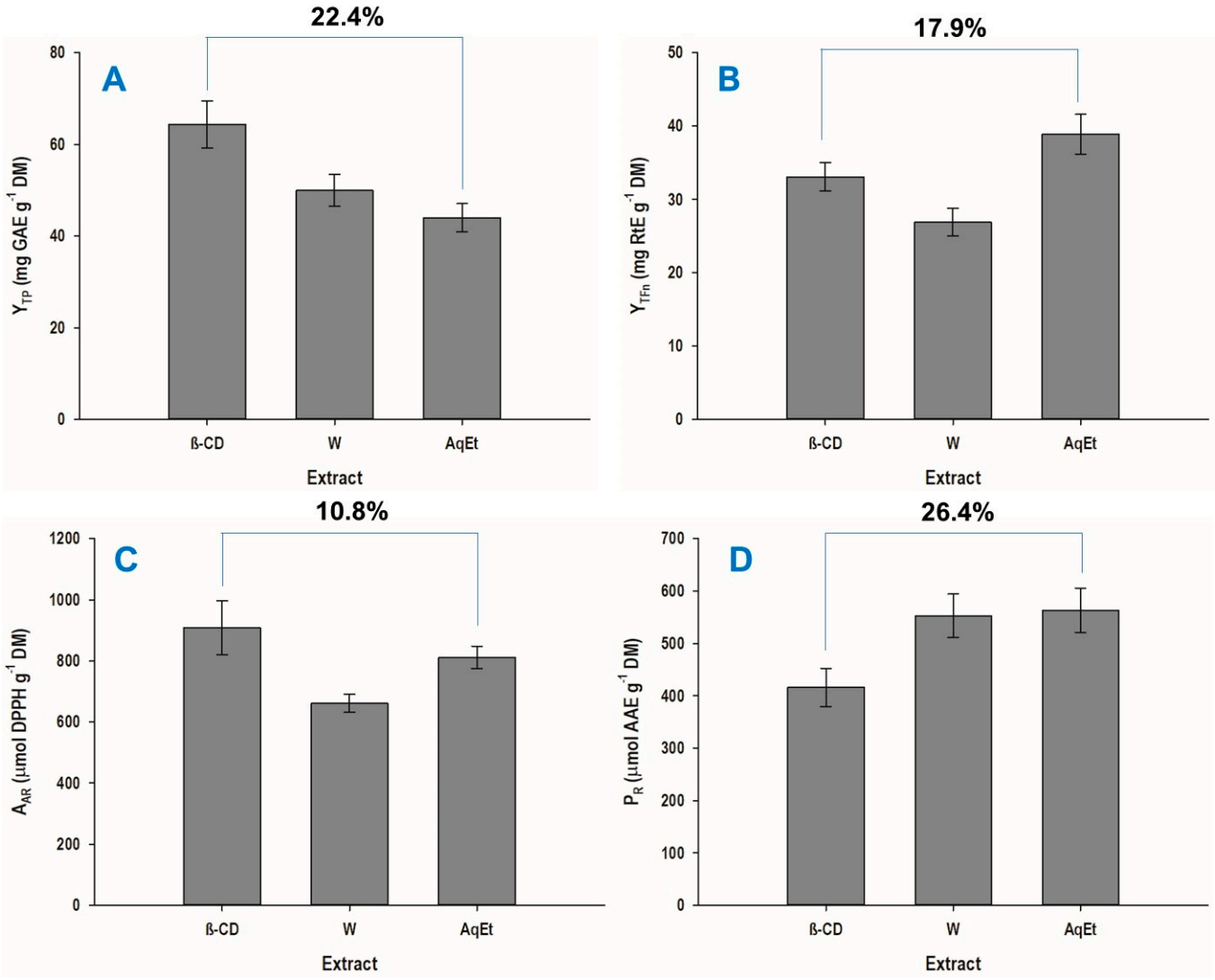


Figure 3. Comparison of the effectiveness of the β-CD-aided extraction with 60% aqueous ethanol (AqEt) and water with regard to total polyphenol yield (**A**), total flavonoid yield (**B**), antiradical activity (**C**) and ferric-reducing power (**D**). For the β-CD-aided extraction, a β-CD concentration of 1.08 mM was used at 80 °C for 180 min.

3.4. Polyphenolic Profile

The extract prepared using a β-CD solution with a concentration of 1.08 mM at 80 °C was analyzed by HPLC to trace its polyphenolic profile and obtain quantitative information regarding the principal constituents. The analyses were also accomplished for the aqueous and the hydroethanolic extracts to evaluate the β-CD-aided extraction thoroughly. The chromatogram monitored at 320 nm revealed the presence of eight major polyphenols (Figure 4), which were tentatively identified by comparing their retention times and UV-vis characteristics with those of original standards. Luteolin 7-*O*-glucuronide was identified by liquid chromatography-mass spectrometry, as previously described [24].

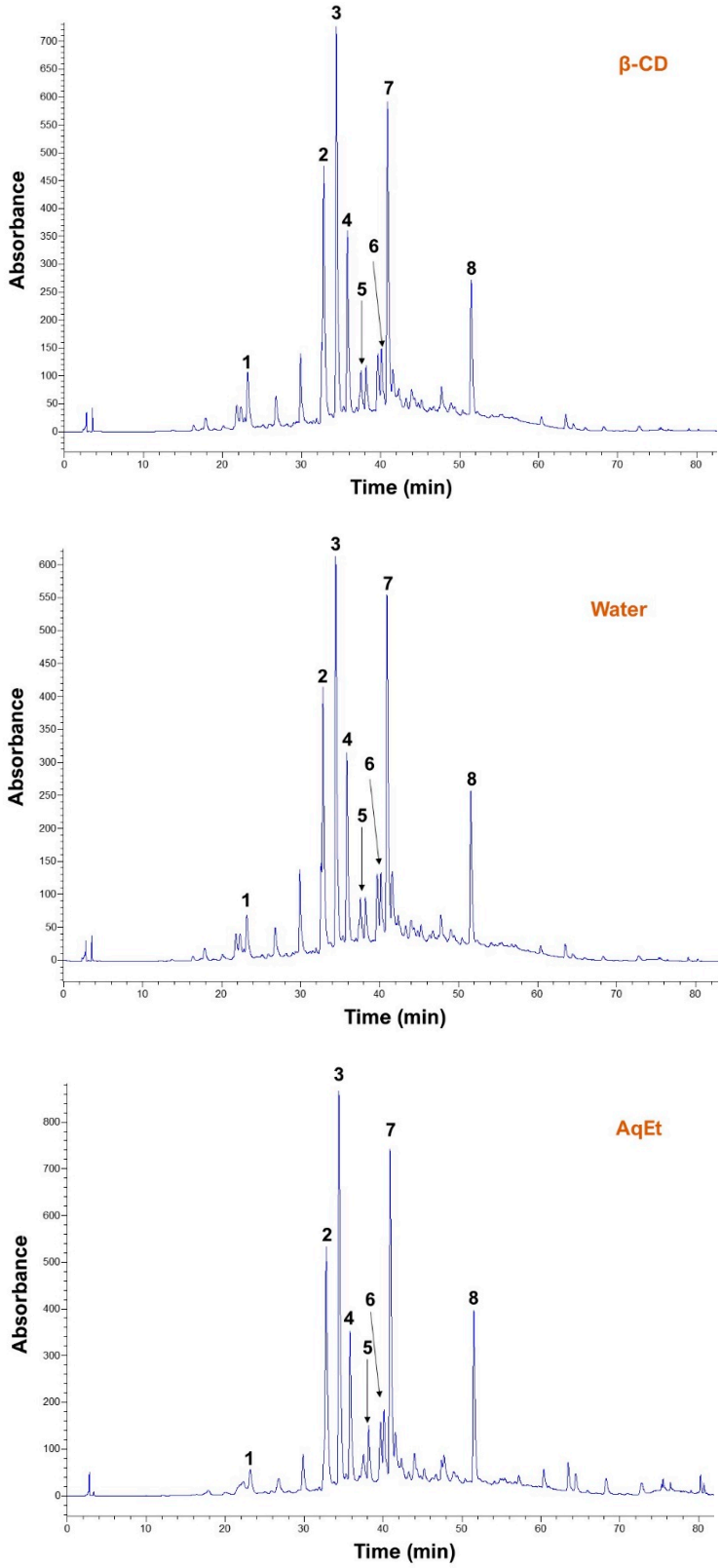


Figure 4. Chromatograms of *M. piperita* extracts were recorded at 320 nm. AqEt extract corresponds to 60% aqueous ethanol. For the β-CD-aided extraction, a β-CD concentration of 1.08 mM was used at 80 °C for 180 min. Peak assignment: 1, caffeic acid; 2, eriocitrin; 3, luteolin 7-*O*-rutinoside; 4, luteolin 7-*O*-glucuronide; 5, narirutin; 6, hesperidin; 7, rosmarinic acid; 8, luteolin derivative.

In Table 5, quantitatively, the principal metabolite in any extract analyzed was eriocitrin, followed by luteolin 7-*O*-rutinoside and rosmarinic acid.

Table 5. Analytical polyphenolic profile of the extracts obtained with β-CD and the control solvents. AqEt denotes 60% aqueous ethanol. Values reported are means ± standard deviation.

#	Compound	Yield (mg g^{-1} dm)		
		Water	AqEt	β-CD
1	Caffeic acid	0.20 ± 0.01	0.18 ± 0.03	0.31 ± 0.00 [a]
2	Eriocitrin	25.65 ± 2.14	36.48 ± 2.97 [a]	29.22 ± 1.97
3	Luteolin 7-*O*-rutinoside	5.80 ± 0.80	9.16 ± 0.87 [a]	7.07 ± 0.37
4	Luteolin 7-*O*-glucuronide	2.89 ± 0.19	3.43 ± 0.01	3.29 ± 0.25
5	Narirutin	0.18 ± 0.00	0.30 ± 0.04 [a]	0.25 ± 0.04
6	Hesperidin	2.23 ± 0.21	3.24 ± 0.44 [a]	2.51 ± 0.05
7	Rosmarinic acid	3.30 ± 0.21	4.60 ± 0.29 [a]	3.44 ± 0.14
8	Luteolin derivative	2.53 ± 0.15	4.11 ± 0.08 [a]	2.61 ± 0.20
	Sum	44.18	63.00 [a]	50.12

[a] Designate statistically different value within rows.

This was in line with previous examinations, which demonstrated these three compounds to be dominant in *M. piperita* extracts [32,33]. Other studies showed that in organic *M. piperita*, caffeic acid occurred at levels higher than 2.40–3.0 mg g^{-1} [34,35]. In this study, the highest yield of caffeic acid was 0.31 mg g^{-1}, achieved with β-CD-aided extraction (Table 5), and it was 42% higher than that obtained with 60% ethanol. Furthermore, rosmarinic acid was reported to occur at 54.52 mg g^{-1}, but in this study, the highest yield of 4.60 mg g^{-1} was determined for the hydroethanolic extract.

In fact, for all other polyphenols considered, the hydroethanolic extraction was shown to afford the highest yields, with the exception of luteolin 7-*O*-glucuronide, for which no statistical difference was found between the yield attained with β-CD and aqueous ethanol. Overall, the extraction with aqueous ethanol yielded 63 mg g^{-1}, which was by almost 20% higher than that obtained with β-CD and 30% than that obtained with water.

4. Conclusions

This examination illustrated the effect of β-CD addition on the performance of aqueous extraction of polyphenolic antioxidants from *M. piperita*. The implementation of response surface methodology suggested that incorporation of β-CD at levels of approximately 1.02–1.15 mM and a temperature of 80 °C may effectively increase yield in total flavonoids, which are some of the major *M. piperita* constituents, but also provide extracts with improved antiradical activity. The chromatographic analyses revealed that extraction with β-CD-produced extracts is enriched in antioxidant polyphenols, but extraction with a hydroethanolic solution was even more effective. In every extract examined, the flavonoid glycoside eriocitrin was the predominant constituent, followed by luteolin 7-*O*-rutinoside and rosmarinic acid. The outcome of the study suggested the β-CD-aided aqueous extraction of *M. piperita* polyphenols to be an alternative to conventional organic solvents. It is proposed that such processes may be improved, e.g., by considering other cyclodextrins (i.e., hydroxypropyl β-CD). A similar investigation is currently in progress.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/oxygen2040029/s1, Figure S1: Desirability function (graph A), and plot of predicted vs. actual values of the response (YTP) (plot B), for the optimization of the extraction of *M. piperita* polyphenols performed with β-CD. Inset tables provide statistics associated with the assessment of the model derived. Values with color and asterisk are statistically significant; Figure S2: Desirability function (graph A), and plot of predicted vs. actual values of the response (YTFn) (plot B), for the optimization of the extraction of *M. piperita* polyphenols performed with β-CD. Inset tables provide statistics associated with the assessment of the model derived. Values with color and asterisk are statistically significant; Figure S3: Desirability function (graph A), and plot

of predicted vs. actual values of the response (AAR) (plot B), for the optimization of the extraction of *M. piperita* polyphenols performed with β-CD. Inset tables provide statistics associated with the assessment of the model derived. Values with color and asterisk are statistically significant; Figure S4: Desirability function (graph A), and plot of predicted vs. actual values of the response (PR) (plot B), for the optimization of the extraction of *M. piperita* polyphenols performed with β-CD. Inset tables provide statistics associated with the assessment of the model derived. Values with color and asterisk are statistically significant.

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