

Article

Cyclodextrins-Assisted Extraction for the Recovery of Bioactive Compounds from Rosemary Post-Distillation Residues—In Vitro Antioxidant Activity, Comparisons to Conventional Liquid Extracts

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Abstract

This study investigated the valorization of rosemary (*Rosmarinus officinalis* L.) post-distillation residues—by-products derived from essential oil production—using cyclodextrins (CDs) as green co-solvents for the efficient extraction of bioactive compounds. This work aimed to explore key extraction parameters, i.e., extraction time, liquid-to-solid ratio, type of CD (β -CD or HP- β -CD), and CD concentration, and assess the antioxidant potential of the resulting extracts. Total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) assays were performed to evaluate the composition and antioxidant potency of the extracts. Regression analysis identified CD concentration and liquid-to-solid ratio as the most influential factors. Both β -CD and HP- β -CD significantly enhanced polyphenol recovery and antioxidant activity compared to conventional solvents (water, ethanol, methanol), with HP- β -CD showing slightly superior performance. The β -CD-assisted extract exhibited up to four-fold higher DPPH radical scavenging capacity than ethanol-based extracts. Among the extracts, the activity to scavenge superoxide and peroxy (AAPH) radicals notably varied depending on the type of solvent. The findings demonstrated that rosemary post-distillation residues can be valorized to produce extracts rich in bioactive compounds suitable for food, cosmetic, and pharmaceutical applications. CD-assisted extraction offers an efficient, low-cost, and environmentally friendly approach to achieve this. As a continuation of this work, future studies should include LCA, thermodynamic, and techno-economic analyses to confirm the reduced environmental impact and operational costs indicated by the green metrics.

Keywords: *Rosmarinus officinalis* L.; distillate residues; by-products; cyclodextrins; bioactive compounds; green extraction; antioxidants; in vitro assays



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1. Introduction

The global essential oil (EO) market is experiencing significant growth, with a projected valuation of \$16 billion by 2026 [1]. This expansion is driven by a shift in consumer preference toward healthier ingredients with few or no artificial preservatives and products with functional properties that have a low environmental impact [2]. However, industrial

EO production—primarily through hydro-distillation and steam distillation—is characterized by low yields, typically ranging from 1.0% to 2.5% [3]. This inefficiency results in the generation of vast quantities of solid post-distillation residues, posing a substantial disposal challenge and highlighting the need for sustainable valorization strategies [4].

Rosmarinus officinalis L. (rosemary) is one of the most heavily exploited aromatic plants in this sector. While EO and the extracts from rosemary, classified as permitted food additives (E392) in the EU [5], are highly traded, rosemary solid residues remain a rich source of bioactive polyphenols but with limited (if any) exploitation, leading to its classification as a permitted food additive (E392) in the EU [5]. The antioxidant capacity of rosemary is mainly governed by a diverse profile of phenolic compounds, most notably rosmarinic acid, carnosic acid, and its degradation products, carnosol and rosmanol [6,7]. These compounds exhibit prominent antioxidative potency; for instance, the hydrophilic rosmarinic acid and the lipophilic carnosic acid primarily stabilize free radicals through hydrogen atom transfer (HAT) mechanisms [8]. Conversely, carnosol acts as a potent inhibitor of lipid peroxidation, functioning similarly to tocopherols [9].

Over the past few years, agricultural wastes of plant origin have received considerable attention as potential sources of bioactive compounds [10–13]. The developed countries currently promote strategies for sustainable product development approaches by adopting cyclic economy principles to minimize the environmental impact. Therefore, the recovery of high-value compounds from distillation biowaste not only addresses environmental concerns but also provides a cost-effective source of natural antioxidants for the food, cosmetic, and pharmaceutical industries.

Cyclodextrins (CDs) are inexpensive cyclic oligosaccharides with 6, 7 or 8 glucose residues joined by α -(1-4) glycosidic linkages. β -CD has been GRAS-listed since 1998 and is also approved as a food additive in the EU with E 459. Derivatives like 2-hydroxypropyl- β -cyclodextrin (HP- β -CD) further enhance water solubility, binding ability and molecular selectivity [14]. Structurally, CDs form a truncated cone with a hydrophilic exterior and a lipophilic central cavity, enabling them to form inclusion complexes with hydrophobic molecules [15]. The formation of these host–guest complexes, primarily stabilized by hydrophobic interactions and van der Waals forces, allows for the encapsulation of diverse plant polyphenols, including essential oils and flavonoids [16,17]. This complexation is largely driven by the displacement of enthalpy-rich water molecules from the cavity [17]. By utilizing this mechanism, CDs significantly improve the solubility and stability of lipophilic bioactive compounds in aqueous environments.

There is a large body of literature demonstrating the improved water solubility, stability and bioavailability of various polyphenolic compounds due to the inclusion of complex formations with CDs for food and pharmaceutical applications [18]. Cyclodextrins function as effective vehicles for solubilizing and protecting lipophilic polyphenols through inclusion complex formation. Consequently, aqueous CD solutions represent a non-toxic, ‘green’ extraction medium for polyphenols from agricultural wastes [15]. Once trapped, these bioactive compounds can be successfully transferred into food matrices to be released as active antioxidants [18]. The formation of the inclusion complex of rosmarinic acid (RA) with β -cyclodextrin (β -CD), occurring at a 1:1 molar stoichiometry, has been previously verified with proton nuclear magnetic resonance (^1H NMR) spectroscopy [19] and fluorescent methods [14]. Moreover, Andreadelis et al. [20] reported that rosmarinic acid can complex with HP- β -CD (1:1 stoichiometry) through hydrophobic interactions, with the phenolic molecule entering within the hydrophobic cavity of the CD via its aromatic ring. In the same context, effective complexation of carnosic acid with β -CD resulted in improved water solubility, antioxidant activity, and antimicrobial activity of the bioactive compound [21]. In addition, a previous study predicted the inclusion complexation ability of CDs (α -, β - and

γ -) with carnosic acid through docking and quantum chemistry calculations, suggesting that carnosic acid fits better into the cavity of β -CD and γ -CD than α -CD [22].

Several *in vitro* assays have been frequently used to evaluate the antioxidant potential of plant extracts, encompassing both single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms. The DPPH radical scavenging assay assesses the ability of antioxidants to donate hydrogen atoms to the stable DPPH radical [23]. Similarly, the ferric reducing antioxidant power (FRAP) assay evaluates the reduction in ferric tripyridyltriazine (Fe^{3+} -TPTZ) to its ferrous form (Fe^{2+} -TPTZ) and is indicative of the electron-donating capacity of the sample [24]. These methods are rapid and cost-effective but are primarily limited to hydrophilic antioxidants and do not necessarily reflect free radical scavenging in biological systems. Moreover, these techniques have shown different results among various plants tested and across laboratories [25]. The assays that measure total phenolic content (TPC) and total flavonoid content (TFC) also estimate the abundance of bioactive polyphenols, but they are non-specific and do not directly measure antioxidant efficacy. Furthermore, their results can be skewed by other reducing agents in the extract, such as sugars or carboxylic acids [26]. Additional biological relevance can be obtained through functional assays targeting free radical scavenging and lipid peroxidation inhibition catalyzed by enzymes. The superoxide anion radical ($\text{O}_2^{\cdot-}$) scavenging assay is sensitive and widely accepted, as it enables a measurement of scavenging of biologically relevant radicals [27]. The soybean lipoxygenase (LOX) inhibition assay also models oxidative enzyme activity linked to inflammation, with antioxidant activity inferred from reduced hydroperoxide formation as monitored at 234 nm by probing linoleic acid oxidation [28]. Moreover, inhibition of linoleic acid peroxidation induced by AAPH reflects the extract's ability to mitigate lipid oxidation processes via hydrogen atom transfer (HAT) mechanism [29]. These assays offer more physiologically relevant insights but are also subject to variability due to enzyme source or radical generation kinetics.

The present work is the first to design a liquid–solid extraction process for recovering phenolic compounds from rosemary post-distillation residues using aqueous cyclodextrin (CD) solutions and low-cost conventional equipment. It is hypothesized that CD-assisted extraction, a cost-effective and “green” procedure, can successfully valorize these by-products into potent, bioactive-rich extracts. The study aims to evaluate four key extraction parameters: time, liquid-to-solid ratio, type of cyclodextrin and cyclodextrin concentration. The resulting extracts were characterized by their total phenolic (TPC) and flavonoid (TFC) content. Their antioxidant potency was further assessed using DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. After the screening process, multiple regression analysis was conducted to explore the relationships among the examined process variables and to identify those variables that significantly affect the extraction outcome. To the best of the authors' knowledge, this is the first study comparing aqueous CD-aided extraction of rosemary residues against conventional ethanol and methanol methods. Furthermore, it provides a comprehensive characterization of these extracts through multiple *in vitro* assays, including DPPH, soybean lipoxygenase-LOX, scavenging activity of superoxide anion $\text{O}_2^{\cdot-}$, and inhibition of linoleic acid peroxidation-AAPH.

2. Materials and Methods

2.1. Chemicals and Reagents

Folin–Ciocalteu phenol reagent was obtained from Merck (Darmstadt, Germany). Sodium carbonate anhydrous (molecular mass ~ 106.0 g/mol) was supplied by Panreac Quimica SA (Barcelona, Spain). β -cyclodextrin (β -CD, molecular mass of ~ 1135 g/mol), HP- β -cyclodextrin (HP- β -CD, molecular mass of ~ 1542 g/mol), gallic acid (molecular mass ~ 188.1 g/mol, Alfa Aesar, Haverhill, MA, USA), Trolox (molecular mass ~ 250.3 g/mol,

Sigma-Aldrich, Burlington, MA, USA), and 2,2-diphenyl-picrylhydrazyl (DPPH) stable radical (molecular mass ~394.3 g/mol, TCI Chemicals, Japan) were obtained from Sigma-Aldrich, Chemie GmbH (Taufkirchen, Germany). AlCl_3 was supplied by Fisher Scientific (Fair Lawn, NJ, USA). All organic solvents were of high purity (>99%), analytical grade, and purchased from Che-Lab NM (Zedelgem, Belgium). Distilled water was used for all extraction experiments.

2.2. Plant Material

Aerial parts of rosemary (*Rosmarinus officinalis* L.), mainly leaves, were obtained from Physis Ingredients; the plants were organically cultivated in the region of Serres, in northern Greece, and dried at ambient temperature until reaching a constant weight. Then, the dried material was stored at ambient temperature in heat-sealed bags until further use. For preparation of post-distillation rosemary residues, several hydro-distillations of rosemary leaves were conducted on a lab scale according to the 10th edition of the European Pharmacopoeia [30]. Dry-rubbed rosemary (25 g) was placed in a 500 mL round-bottom flask containing 300 mL of distilled water and then submitted to hydro-distillation for 2 h using a Clevenger apparatus. After recovery of the essential oil, the remaining solid rosemary residues were collected. The residual leaves were dried in a forced-air drier for 24 h at 40 °C until the rosemary biomass reached a constant weight (~10% moisture content). The dried post-distillation plant material was subsequently ground to pass through a 2.0 mm sieve and stored in sealed bags at ambient temperature until further analysis.

2.3. Extraction Procedure

2.3.1. Cyclodextrin-Assisted Liquid/Solid Extraction

A conventional liquid–solid extraction method was adapted for the present experimental investigation, adopting four controllable factors at two levels each: the ratio of weight of solvent to plant material (L/S ratio), extraction time, type of cyclodextrin (β -CD and HP- β -CD) used and concentration of the respective cyclodextrin in the aqueous medium. All the parameters and the ranges shown in Table 1 were based on literature information and some preliminary experimental work; the liquid/solid ratio used for the extractions was set at 8 and 16 as per previous studies on rosemary-distillate by-product [31].

Table 1. Conditions for extraction of rosemary post-distillation residues using either β -CD or HP- β -CD aqueous media under stirring at 50 °C and 500 rpm.

Extract	Extraction Time (min) A	L/S Ratio B	CD Type	CD Concentration (mg/mL) C
β 30/16/18.5	30	16	β -CD	18.5
β 180/16/18.5	180	16	β -CD	18.5
β 30/16/9.25	30	16	β -CD	9.25
β 180/16/9.25	180	16	β -CD	9.25
β 30/8/18.5	30	8	β -CD	18.5
β 180/8/18.5	180	8	β -CD	18.5
β 30/8/9.25	30	8	β -CD	9.25
β 180/8/9.25	180	8	β -CD	9.25
HP β 30/16/37	30	16	HP- β -CD	37
HP β 180/16/37	180	16	HP- β -CD	37
HP β 30/16/18.5	30	16	HP- β -CD	18.5
HP β 180/16/18.5	180	16	HP- β -CD	18.5
HP β 30/8/18.5	30	8	HP- β -CD	18.5
HP β 180/8/18.5	180	8	HP- β -CD	18.5
HP β 30/8/37	30	8	HP- β -CD	37
HP β 180/8/37	180	8	HP- β -CD	37

Preliminary experiments, held in the laboratory, also demonstrated that a liquid/solid ratio lower than 8 cannot be facilitated as solvent diffusion and stirring were substantially obstructed. It is well evidenced that extraction time can affect both the yield and the selectivity of the extraction; while recent research works mostly focus on shorter extraction times, in the present study two extraction times of 30 and 180 min were adopted since it had been previously reported that extraction times longer than 180 min using cyclodextrins did not result in any further changes in the total phenols content irrespective of the extraction temperature [32–34]. The maximum solubility of the two types of cyclodextrins in water was provided by the manufacturer's safety data sheets, and it was chosen to test the highest level for this factor. All series of aqueous extractions were held at 50 °C, under stirring at 500 rpm, as it has been previously shown that mild temperatures are optimal for the extraction of polyphenols from medicinal aromatic plants [32,35], and high temperatures can cause degradation of bioactive compounds [34]. Following extraction, the extracts were obtained by filtration through filter paper (Whatman No. 1), which removed the main solid biomass, and subsequently centrifuged at 6000 rpm for 10 min using a bench centrifuge (Hettich Universal 32, DJB Labcare Ltd., Buckinghamshire, UK). The clarified supernatants were subsequently placed in a stoppered glass bottle and stored at −18 °C until required for further analysis.

2.3.2. Determination of Antiradical Activity (AAR) Using DPPH Radical (DPPH Assay)

For the assessment of antiradical activity, the DPPH radical inhibition efficiency was determined using a UV-visible spectrophotometer according to a previously described protocol [36]. Briefly, an aliquot of 0.025 mL of aqueous extract (suitably diluted) was added to 0.975 mL of DPPH solution (100 µM in MeOH), and the absorbance was measured at $t = 0$ and $t = 30$ min. Trolox™ equivalents (mM TRE) were determined from linear regression after plotting %ΔA₅₁₅ of known solutions of Trolox™ against concentration, where

$$\% \Delta A_{515} = \frac{(A_{515}^{t=0} - A_{515}^{t=30})}{A_{515}^{t=0}} \times 100 \quad (1)$$

The results were expressed as mM TRE per g of dry plant material weight. Measurements were performed in triplicate.

2.3.3. Determination of Ferric Reducing Antioxidant Power (FRAP Assay)

The reducing ability of aqueous extracts was determined by the ferric reducing/antioxidant potential (FRAP) assay. This procedure involves the reduction of ferric tripyridyltriazine (Fe³⁺-TPTZ) complex to a blue-colored Fe²⁺-TPTZ by sample antioxidants. For the determination, the protocol described by Arnous et al. [36] was followed. Aqueous extracts (0.05 mL), previously diluted with distilled water, and 0.05 mL of ferric chloride (3 mM in 5 mM citric acid) were vortexed in an Eppendorf tube and then incubated for 30 min in a water bath at 37 °C. Subsequently, 0.90 mL of 1 mM TPTZ solution (in 0.05 M HCL) was added to the mixture and vortexed again. After 10 min of rest, the absorbance was read at 620 nm using a UV-visible spectrophotometer (1800 Shimadzu; Shimadzu, Kyoto, Japan). Ascorbic acid was used as a positive control to construct a reference curve, and the results were expressed as mmol ascorbic acid per g of dry weight (AAE/g DW). Measurements were performed in triplicate.

2.3.4. Determination of Total Phenolic Content (TPC)

The total phenolic content of the samples was determined according to a published protocol [36] using the Folin–Ciocalteu methodology and gallic acid as the standard; the results were expressed as milligrams of gallic acid equivalents per g sample on a dry-

weight basis (mg GAE/g DW) through a calibration curve. Measurements were performed in triplicate.

2.4. Determination of Total Flavonoid Content (TFC)

A previously published protocol was used [37]. An aliquot of 0.5 mL of sample (suitably diluted) was mixed with 500 μ L of AlCl_3 reagent (2% [w/v] AlCl_3 in 5% [v/v] acetic acid in methanol) and 700 μ L of 5% [v/v] acetic acid in methanol and allowed to stand for 30 min at room temperature. The absorbance was obtained at 415 nm (A415) using deionized water as a blank solution, and the TFC was calculated from a calibration curve constructed with quercetin as the calibration standard [37]. TFC was expressed as micrograms of quercetin equivalent (QE) per gram of dry weight. Measurements were performed in triplicate.

2.4.1. Comparison Study (HP- β -CD and β -CD Aqueous Versus Conventional Solvents)

To evaluate the performance of the improved β -CD and HP- β -CD-assisted extractions, comparative extracts were prepared using conventional solvents (ethanol, methanol, water, and 70% ethanol) under two distinct sets of extraction conditions. Specifically, conventional extractions were performed using parameters established for β -CD (L/S = 8, extraction time = 124 min) and HP- β -CD (L/S = 16, extraction time = 78.5 min). All series of extractions were conducted at 50 °C and 500 rpm. Following filtration, aliquots were stored in stoppered glass bottles at -18 °C prior to analysis, as described in Section 2.4.2. The remaining fractions were nitrogen-dried and hermetically sealed for characterization according to Sections 2.4.3–2.4.5.

2.4.2. Determination of Antiradical Activity (AAR) Using DPPH Radical (DPPH Assay) and Total Phenolic Content (TPC)

The extracts were evaluated for their DPPH antiradical activity and total phenolic content using the methods described in Sections 2.3.2 and 2.3.4, respectively. Extracts were prepared and analyzed in liquid form for the characterization of their antiradical activity, while dried extracts, obtained using nitrogen gas, were used for the in vitro assays described in Sections 2.4.3–2.4.5.

2.4.3. Soybean Lipoxygenase Inhibition (LOX%)

The tested extracts were dissolved in DMSO and incubated at room temperature with sodium linoleate (0.1 mL) and 0.2 mL of enzyme solution (1 part of enzyme 1×10^{-4} w/v in saline, and 9 parts of saline) in Tris buffer solution (pH 9.0), which was added to a final volume of 1 mL. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor. In this study, the protocol described in a previous study of natural product extracts was followed, and lipoxygenase inhibition was evaluated using linoleic acid as a substrate [38]. In brief, for this method, two reaction mixtures were prepared in UV cells for each sample; the first reaction mixture contained LOX solution (dissolved in Tris buffer), Tris buffer, pH 9.0 and the sample under examination (10 μ L), while the second reaction mixture also contained 100 μ L of linoleic acid (LLA) solution (dissolved in 0.9% NaCl). Both reaction mixtures were measured within two minutes of mixing, at room temperature, at 234 nm. LOX inhibition was measured in the presence of the same level of DMSO, which served as a control against which the calculations were made. Measurements were performed in triplicate, and the results were presented as percentages obtained via the following Equation [39]:

$$\% \text{ LOX Inhibition} = \frac{(\text{Standard} - \text{Blank}) - (\text{Reference Sample} - \text{Measurement Sample})}{(\text{Standard} - \text{Blank})} \times 100 \quad (2)$$

2.4.4. Scavenging Activity of Superoxide Anion Using the Xanthine–Xanthine Oxidase System (OH%)

This protocol focuses on assessing the inhibitory effect of various compounds on xanthine oxidase (XO), an enzyme that oxidizes hypoxanthine and xanthine to uric acid. The assay has relevance to oxidative tissue damage, thus providing an evaluation of the antioxidant potential of the tested samples. The method employs a phosphate buffer at pH 7.5 to dissolve both xanthine (0.42 mM) and XO (0.25 U/mL), ensuring freshly prepared solutions, and the preparation is done in light-protected conditions due to the photosensitivity of the reactants.

The assay included a standard control (DMSO, XO, xanthine, and buffer) and a blank control (DMSO, xanthine, and buffer), using allopurinol to reduce uric acid levels. The reaction mixture was prepared in a 96-well plate by combining 50 µL of extract (in 5% DMSO), 50 µL of sodium phosphate buffer (pH 7.5) and 10 µL of enzyme solution (0.25 U/mL XO in phosphate buffer). After adding the buffer and the test compounds at specific concentrations, XO was introduced selectively to the designated wells. The plate was incubated at 37 °C for 30 min, followed by the addition of 150 µL xanthine (0.42 mM), and absorbance was measured at 290 nm after 10 min. The XO inhibition was calculated using a standardized formula that compares mean absorbance values across the different well types [38].

$$\% \text{ Inhibition} = \frac{(\text{Mean STD} - \text{Mean BLANK}) - (\text{Mean SMPLwXO} - \text{Mean SMPLwoXO})}{(\text{Mean STD} - \text{Mean BLANK})} \times 100 \quad (3)$$

where *STD* = Standard sample, *BLANK* = Blank sample, *SMPLwXO* = Tested sample with XO, and *SMPLwoXO* = Tested sample without XO.

2.4.5. Inhibition of Linoleic Acid Peroxidation Induced by the Dihydrochloric Acid of 2,2-Azobis-2-Amidinopropane (AAPH%)

For this experimental procedure, a protocol previously described was used [40]. In brief, each tested extract had a reference and a measurement sample. For the reference sample in a UV cuvette, 10 µL of the test sample (100 µM diluted in DMSO), 10 µL linoleic acid (LA) (16 mM), and buffer solution (pH 7.4) were added to a final volume of 1 mL. For the measurement sample, in a UV cuvette, 10 µL of the test sample, 50 µL of AAPH (40 mM), 10 µL of LA, and buffer solution were added to a final volume of 1 mL. Standard and blank samples were prepared in the same way using DMSO (solvent) instead of the tested extract. The absorbance was measured after 2 min at 37 °C at 234 nm. Measurements were performed in triplicate; Trolox was tested as reference, and the results were obtained via the following equation:

$$\% \text{AAPH Inhibition} = \frac{(\text{Standard} - \text{Blank}) - (\text{Reference Sample} - \text{Measurement Sample})}{(\text{Standard} - \text{Blank})} \times 100 \quad (4)$$

2.5. Statistical Analysis

The results are presented as means ± standard error. Analyses were performed using one-way ANOVA, followed by Tukey's post hoc test for comparisons between the extracts and their respective spectrophotometric responses. Unless otherwise stated, *p*-values < 0.05 were considered statistically significant. Statistical analyses were performed using Minitab 19.

3. Results

3.1. β -Cyclodextrin-Assisted Liquid/Solid Extraction

Extraction conditions play a crucial role in determining the yield and composition of extracts [41]. Rosemary (*Rosmarinus officinalis*) contains various phenolic metabolites [6,7] that are prone to oxidation and degradation, thus making the selection of optimal extraction parameters essential. In the first part of this work, the dissolving efficiency of aqueous solutions of β -CD was tested at two concentrations, two liquid/solvent ratios and two extraction times. The TPC and TFC recovered after 30 and 180 min of extraction, together with the obtained DPPH radical scavenging capacity and the ferric reducing antioxidant power of the extracts obtained by the aqueous- β -CD mixtures are presented in Figure 1a–d, respectively, while the extraction parameters for each extract, along with their assigned code names, are presented in Table 1. The concentration of phenolic compounds in the aqueous extract of distilled rosemary ranged from 23.48 to 44.98 (mg GAE/g DW). The highest amount of phenolic compounds was obtained in extracts containing the highest concentration of β -CD irrespective of the liquid-to-solid ratio and the extraction time. Several studies [31,42–44] in the literature employ different extraction methodologies and conditions to recover bioactive compounds from the post-distillation of rosemary residues. The higher amounts of phenolic compounds were obtained with 70% acetone and 70% ethanol (142.4 ± 5.2 and 96.1 ± 2.3 mg GAE/g, respectively) using the conventional solid–liquid extraction process, followed by accelerated solvent extraction ($82\text{--}94$ mg GAE/g) and ultrasound-assisted extraction (25.84 and 59.74 mg GAE/g), and finally by solid–liquid extraction using 80% ethanol or water as a solvent (24.14 ± 0.54 mg GAE/g and 28.3 ± 3.0 mg GAE/g, respectively) [31,42–44]. The latter values are in line with the results of phenolic compounds from the post-distillation rosemary residues determined in the present study.

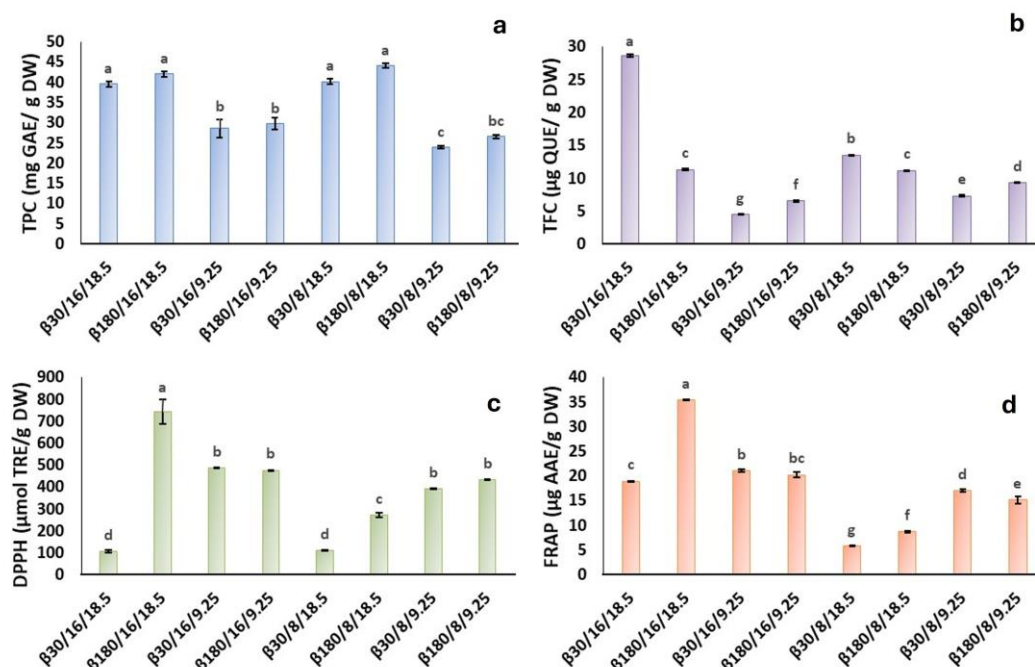


Figure 1. Content of total (a) phenolic compounds (TPC), (b) flavonoids (TFC), (c) antiradical activity by DPPH assay (DPPH), and (d) ferric reducing antioxidant power (FRAP) in rosemary-distillate extracts using β -CD as co-solvent. The abbreviated names of the extracts are presented in Table 1. The experiments were performed in triplicate. The values are shown as means $\pm$ standard error. Differences among means of the extracts are indicated by small letters (a–g) above the columns for each specified frame; values with different letters are statistically different (Tukey's test, $p < 0.05$).

Figure 1b elucidates the total flavonoid content (TFC) results, which, consistent with the TPC trend, though less pronounced, indicate that an increase in β -CD concentration resulted in extracts containing a greater proportion of flavonoids. In this work, the aqueous extract containing 18.5 mg/mL of β -CD obtained after 30 min of extraction and applying the highest liquid-to-solid ratio ($L/S = 16$) showed the most efficient extraction ($\beta_{30/16/18.5}$) for the recovery of flavonoids. These findings indicate that increased amounts of β -CD in the solvent provide a significantly higher total polyphenol content, which is in line with the findings of Kalantari et al. (2020) [45], who applied similar extraction conditions for the recovery of bioactive compounds of pomegranate peel (1.8% concentration of β -CD at 55 °C and ultrasound). The combination of phenolic compounds with β -cyclodextrin (β -CD) most likely enhances their solubility in water by forming an inclusion complex. This occurs through the interaction of hydrophobic polyphenols with the lipophilic cavity of β -CD, as previously noted by Del Valle (2004) [46]. The non-polar cavity of β -CD is well-suited for accommodating aromatic and heterocyclic compounds with molecular weights ranging from 200 to 800 g/mol. Specifically, phenolic acids and flavonoids have molecular weights of approximately 194–354 g/mol and 302–440 g/mol, respectively, making them suitable candidates for this complexation. In addition, it has been reported that the extraction of flavonoid compounds from post-distillation rosemary residues decreased with increasing extraction time (180 min) and β -CD concentration, which might be due to solvent saturation with the extracted compounds and their degradation/polymerization over a longer extraction time [45].

The antioxidant capacity of the extracts, as assessed by the DPPH radical scavenging activity (Figure 1c) and ferric reducing antioxidant power (FRAP) assays (Figure 1d), reflects the impact of the adopted experimental conditions. The DPPH radical scavenging activity ranged from 107.55 to 743.25 $\mu\text{mol TRE/g}$ plant material and from 5.77 μg to 35.5 $\mu\text{g AAE/g}$ plant material for FRAP across the β -CD extracts. Overall, it appears that the liquid-to-solid ratio had a significant impact on both antioxidant capacity tests, as it seems that a high liquid-to-solid ratio resulted in extracts with higher antioxidant capacity and vice versa. The concentration of β -CD had little to no effect on the antioxidant potency of the β -CD extracts of the distilled rosemary residues, as has also been noted in a previous study on β -cyclodextrin-assisted extracts of oregano essential oil [10]. Among the β -CD aqueous extracts, the $\beta_{180/16/18.5}$ exhibited a significantly higher antioxidant capacity in both assays.

Considering the results obtained from the determination of total phenolic and flavonoid content, one might expect the extract $\beta_{30/16/18.5}$ to possess the highest antioxidant capacity. Instead, this aqueous extract was found to have the lowest DPPH radical scavenging potency and a moderate ferrous reducing power under the defined experimental conditions. Consistent with the established literature, the data demonstrate that the highest content of total phenolics does not necessarily confer the highest antioxidant capacity [44]; variations in the phenolic contribution to the total antioxidant activity, as determined using different assays, can also be attributed to the specific assays employed, the tissue structure and composition of the plant raw material tested, the presence of specific high-potency polyphenols and the experimental conditions adopted for the preparation of plant extracts [47–49]. Further, the DPPH and FRAP assays, which are commonly used to study the antioxidant capacity of complex samples, are based on different principles of action; the DPPH assay measures radical scavenging activity, whereas the FRAP assay measures the reducing power of Fe^{3+} to Fe^{2+} under acidic conditions [50]. Therefore, the differences in the experimental results noted for the $\beta_{30/16/18.5}$ extract may suggest that the extraction conditions led to a recovery of phenolic constituents with comparatively lower antioxidant potency.

3.2. HP- β -Cyclodextrin-Assisted Liquid/Solid Extraction

In the case of the HP- β -CD-assisted extraction process, the total polyphenolic compounds and total flavonoids extracted ranged from 18.21 to 54.81 mg GAE/g DW and from 3 to 13 μ g QUE/g DW, respectively (Figure 2a,b). The highest polyphenolic extraction efficiency (54.81 mg GAE/g DW and 13 μ g QUE/g DW) was achieved by employing a high liquid-to-solid ratio (L/S = 16) with 37 mg/mL HP- β -CD under constant stirring for 180 min (HP β 180/16/37). Instead, the aqueous extract prepared with 37 mg/mL HP- β -CD for 180 min at a low liquid-to-solid ratio (HP β 180/8/37) had the lowest content of TPC and TFC (18.21 mg GAE/g DW and 3 μ g QUE/g DW). This extraction outcome has been observed in previous studies [51] and attributed to the fact that a decrease in the liquid-to-solid ratio leads to a solvent saturated with bioactive compounds, preventing extensive extraction of phenolic compounds and thereby leading to lower extraction efficiency.

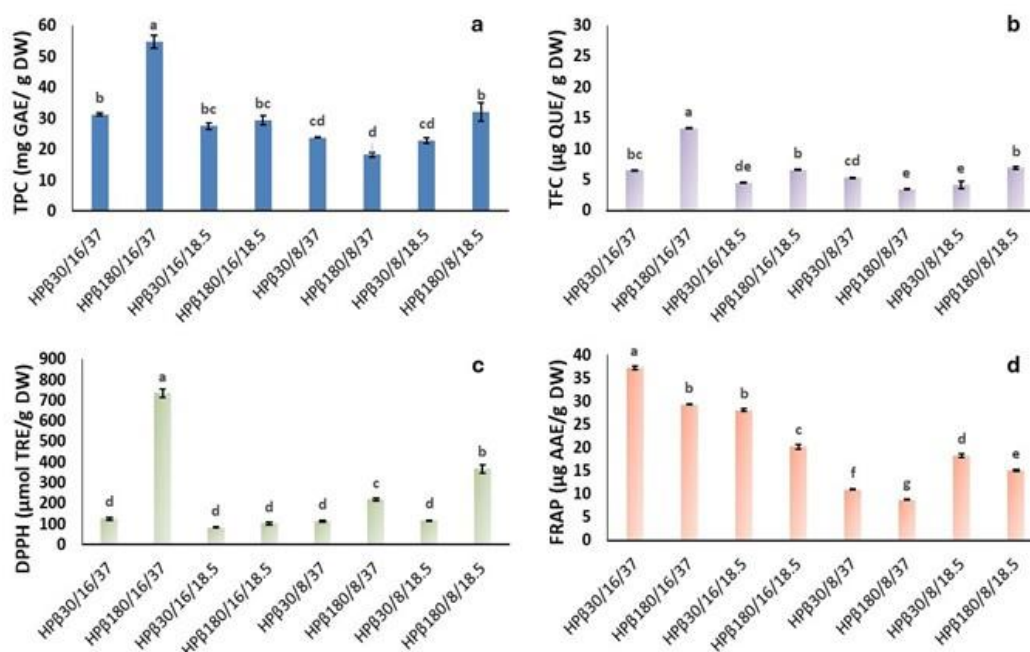


Figure 2. (a) Total phenolic content (TPC), (b) total flavonoid content (TFC), (c) total antiradical activity by DPPH assay (DPPH), and (d) ferric reducing antioxidant power (FRAP) in rosemary-distillate extracts using HP- β -CD as co-solvent. The abbreviated extract names are presented in Table 1. The experiments were performed in triplicate. The values are shown as means $\pm$ standard error. Differences among means of the extracts are indicated by small letters (a–g) above the columns for each specified frame; values with different letters are statistically different (Tukey's test, $p < 0.05$).

As for the antioxidant capacity of the HP- β -CD-assisted extracts, the extract prepared with 37 mg/mL HP- β -CD at a high liquid-to-solid ratio (L/S = 16) for 180 min exhibited the greatest DPPH radical scavenging activity (734.3 μ mol TRE/g DW) and one of the higher ferrous reducing power values (29.3 μ g AAE/g DW). Interestingly, the remaining HP- β -CD-aided extracts showed quite low DPPH radical scavenging activity, whilst the ferrous reducing power of the extracts was quite diversified (Figure 2c,d).

Overall, a slightly higher TPC was observed in the case of extraction with the HP- β -CD derivative under the same conditions as the β -CD-aided extracts. This may originate from the formation of more stable complexes between the phenolics and the HP- β -CD than with β -CD [52]. Overall, the findings presented herein are in line with numerous studies suggesting that both HP- β -CD and β -CD are excellent agents for complexation with phenolic compounds, since the polyphenol moieties are effectively stabilized upon

entrapment in the CD hydrophobic cavity and thereby exhibit improved solubility in an aqueous medium, particularly in the case of less polar compounds [32,53].

3.3. Multiple Linear Regression Analysis

The employed extraction conditions appear to affect the yield of TPC, TFC, and the antioxidant activity of the rosemary post-distillation extracts. To explore the relationships between the dependent (TPC, TFC, antioxidant activities) and independent variables (extraction time, L/S ratio, cyclodextrin concentration), regression analysis was performed, and the corresponding models are presented in Tables 2 and 3 for the β -CD- and HP- β -CD-assisted extractions, respectively; the statistically significant factors ($p < 0.05$) are marked in bold font.

Table 2. Equations (models) constructed by regression analysis for each response of β -CD-assisted extractions.

Response	Equation (Model)	R ²	p
TPC (mg GAE/g DW)	$-2.78 + \mathbf{0.0183} \times \mathbf{A} + 1.278 \times \mathbf{B} + \mathbf{23.00} \times \mathbf{C} - 0.00121 \times \mathbf{A} \times \mathbf{B} + 0.0094 \times \mathbf{A} \times \mathbf{C} - \mathbf{0.714} \times \mathbf{B} \times \mathbf{C}$	0.9594	0.000
TFC (μg QUE/g DW)	$-0.00003 + \mathbf{0.000167} \times \mathbf{A} - \mathbf{0.000995} \times \mathbf{B} + \mathbf{0.00204} \times \mathbf{C} - \mathbf{0.000006} \times \mathbf{A} \times \mathbf{B} - \mathbf{0.000085} \times \mathbf{A} \times \mathbf{C} + \mathbf{0.001407} \times \mathbf{B} \times \mathbf{C}$	0.9279	0.000
DPPH (μmol TRE/g DW)	$1.210 - \mathbf{0.00455} \times \mathbf{A} - \mathbf{0.0305} \times \mathbf{B} - \mathbf{0.708} \times \mathbf{C} + \mathbf{0.000175} \times \mathbf{A} \times \mathbf{B} + \mathbf{0.002761} \times \mathbf{A} \times \mathbf{C} + \mathbf{0.02235} \times \mathbf{B} \times \mathbf{C}$	0.8711	0.000
FRAP (μg AAE/g DW)	$0.05205 - \mathbf{0.000156} \times \mathbf{A} - \mathbf{0.001983} \times \mathbf{B} - 0.03447 \times \mathbf{C} + \mathbf{0.000006} \times \mathbf{A} \times \mathbf{B} + \mathbf{0.000080} \times \mathbf{A} \times \mathbf{C} + 0.002071 \times \mathbf{B} \times \mathbf{C}$	0.9610	0.000

A = extraction time, B = L/S ratio and C = β -CD concentration.

Table 3. Equations (models) constructed by regression analysis for each response of HP- β -CD-assisted extractions.

Response	Equation (Model)	R ²	p
TPC (mg GAE/g DW)	$63.8 - \mathbf{0.0925} \times \mathbf{A} - \mathbf{3.45} \times \mathbf{B} - 16.18 \times \mathbf{C} + \mathbf{0.00899} \times \mathbf{A} \times \mathbf{B} + 0.0121 \times \mathbf{A} \times \mathbf{C} + \mathbf{1.424} \times \mathbf{B} \times \mathbf{C}$	0.7762	0.000
TFC (μg QUE/g DW)	$0.01470 - \mathbf{0.000024} \times \mathbf{A} - \mathbf{0.001036} \times \mathbf{B} - \mathbf{0.00360} \times \mathbf{C} + \mathbf{0.000003} \times \mathbf{A} \times \mathbf{B} + 0.000000 \times \mathbf{A} \times \mathbf{C} + \mathbf{0.000371} \times \mathbf{B} \times \mathbf{C}$	0.8235	0.000
DPPH (μmol TRE/g DW)	$1.083 - \mathbf{0.00193} \times \mathbf{A} - 0.0821 \times \mathbf{B} - \mathbf{0.3483} \times \mathbf{C} + 0.000113 \times \mathbf{A} \times \mathbf{B} + \mathbf{0.000800} \times \mathbf{A} \times \mathbf{C} + \mathbf{0.02790} \times \mathbf{B} \times \mathbf{C}$	0.8006	0.000
FRAP (μg AAE/g DW)	$0.03077 + \mathbf{0.000011} \times \mathbf{A} - \mathbf{0.000599} \times \mathbf{B} - \mathbf{0.012507} \times \mathbf{C} - \mathbf{0.000004} \times \mathbf{A} \times \mathbf{B} + 0.000002 \times \mathbf{A} \times \mathbf{C} + \mathbf{0.001075} \times \mathbf{B} \times \mathbf{C}$	0.9978	0.000

A = extraction time, B = L/S ratio and C = HP- β -CD concentration.

All models were best described with a first-order equation with interaction terms. For β -CD-assisted extractions (Table 2), the β -CD concentration positively influenced TPC and TFC, and its role was substantial for both responses. This finding suggested that polyphenol extraction was facilitated by the presence of β -CD, in agreement with earlier investigations on cyclodextrin-aided polyphenol extraction from solid onion waste [33] and red grape pomace [54]. In addition, the β -CD concentration displayed interaction effects, in combination with the remaining process variables (extraction time and L/S ratio), positively affecting the antiradical activity of the extracts.

In the case of HP- β -CD-assisted extractions (Table 3), the individual process variables (extraction time, L/S ratio, and HP- β -CD concentration) negatively affected the yield of TPC and TFC, as well as the antioxidant activity. However, the interactions between

these variables were found to have a positive influence on the responses. In this respect, the ANOVA showed that variable B, which corresponds to the liquid-to-solid weight ratio, had a significant impact on the yield of TPC, TFC, and the antioxidant activity of the extracts as per the FRAP assay, while the presence of HP- β -CD positively interacted with the liquid-to-solid ratio, affecting the extraction efficiency indicators for all tested dependent variables.

To assess model fitting and the statistical significance of the obtained models, ANOVA was performed (Supplementary Materials), and F-test and p -values were calculated as shown in Table S1. The significance of the selected models, as well as their suitability for the interpretation of the experimental data, was confirmed by the high F-values, while the p -values were lower than 0.001. The determination coefficients (R^2) for the β -CD-assisted extractions were higher compared to those of HP- β -CD-assisted extractions; the R^2 for TPC of the HP- β -CD-assisted extractions was the lowest (0.7726) but still satisfactory, confirming that the observed values were explained by the models. Variance Inflation Factor (VIF) values were calculated as well. All VIF values were found to be exactly 1, indicating a complete absence of multicollinearity among the predictor variables. This result confirms that the estimated regression coefficients are stable and not inflated due to redundancy among variables, thus further supporting the reliability of the models [55,56]. The independence among predictors further enhances the interpretability of each variable's contribution to the extraction outcomes.

The regression models were developed with the primary aim of describing trends and factor interactions within the experimental design space rather than providing extrapolative predictive models. To confirm and validate the obtained regression models within the studied experimental range, additional experiments were conducted employing the D-optimality criterion (Minitab 19); considering that the effect of β -CD and HP- β -CD on all responses (TPC, TFC, DPPH and FRAP) did not match (or did not coincide), it was regarded as a fair compromise to come up with an extract enriched in phenolic compounds with enhanced DPPH antiradical activity [32,57]. The conditions applied for the preparation of the two improved extracts, the predicted and the observed response values as a function of standard error are presented in Table 4. The observed values of the responses (dependent variables) in the prepared extracts were well matched with the predicted ones, confirming the suitability of the calculated models to adequately describe and predict the extraction outcomes of the currently examined process parameters and their range.

Table 4. Predicted and observed response values for the extracts prepared using β -CD and HP- β -CD.

Independent Variables				Predicted Responses (Prediction Intervals at 95% Confidence Interval)		Observed Response Values	
Extraction Time (min)	L/S Ratio	CD Type	CD Concentration (mg/mL)	TPC (mg GAE/g DW)	DPPH (μ mol TRE/g)	TPC (mg GAE/g DW)	DPPH (μ mol TRE/g)
124	8	β -CD	9.25	25.61 $\pm$ 2.466	410 $\pm$ 80.5	26.99 $\pm$ 0.149	403 $\pm$ 17
78.5	16	HP- β -CD	18.5	46.63 $\pm$ 13.2	423 $\pm$ 98.6	45.94 $\pm$ 0.247	480.1 $\pm$ 2

3.4. Comparison Study of HP- β -CD and β -CD Aqueous Versus Conventional Solvents

For comparative analysis, the efficiency of β -CD and HP- β -CD extracts was evaluated against that of various conventional solvents (ethanol, methanol, water and 70% ethanol). All conventional solvent extracts were prepared under identical, fixed extraction parameters (time and liquid-to-solid ratio) established for the improved cyclodextrin-aided extracts. The results for TPC and DPPH antiradical activity are presented in Figure 3a,b for β -CD and Figure 3c,d for HP- β -CD, respectively. The findings indicate that the addition of β -CD resulted in phenolic extraction efficiency that matched or even surpassed the

efficiency of 70% (*w/w*) ethanol, a common aqueous organic solvent employed for the extraction of phenolic compounds from medicinal plants. Ethanol–water mixtures have been used by several researchers [35,58–60], and they are considered green solvents as smaller amounts of organic solvents are utilized; at 70% ethanol concentration there is a balance of polarity for the solvent, suitable for dissolving a broad range of phenolic compounds (e.g., flavonoids), while leaving in the matrix highly non-polar compounds. Moreover, water, ethanol and methanol solutions were found to be less efficient extraction solvents for the phenolic compounds present in the hydro-distillation residues of rosemary. Impressively, the β -CD-assisted extract was found to be the most active with regard to DPPH antiradical activity, exhibiting a four-fold higher capacity compared to the remaining extracts. Likewise, the HP- β -CD-assisted extract displayed a higher extraction yield of antioxidant components than the 70% (*w/w*) ethanolic extract, while the remaining extracts were found to have significantly lower total phenolic content (Figure 3c). These findings were subsequently reflected in the obtained DPPH radical scavenging capacity (Figure 3d), as the HP- β -CD-assisted extract exhibited a two-fold higher antioxidant activity among the examined extracts. Our findings are consistent with those of the study conducted by Barbieri et al. [61], who reported that the deep eutectic (DES) solvent-based ultrasound-assisted extraction of polyphenols from rosemary exhibited higher polyphenolic yield and antioxidant capacity (DPPH and FRAP assays) than the pure alcohol extract. These findings were attributed to the enhanced stability of phenolic compounds within the DES matrix compared to the ethanol-based extract.

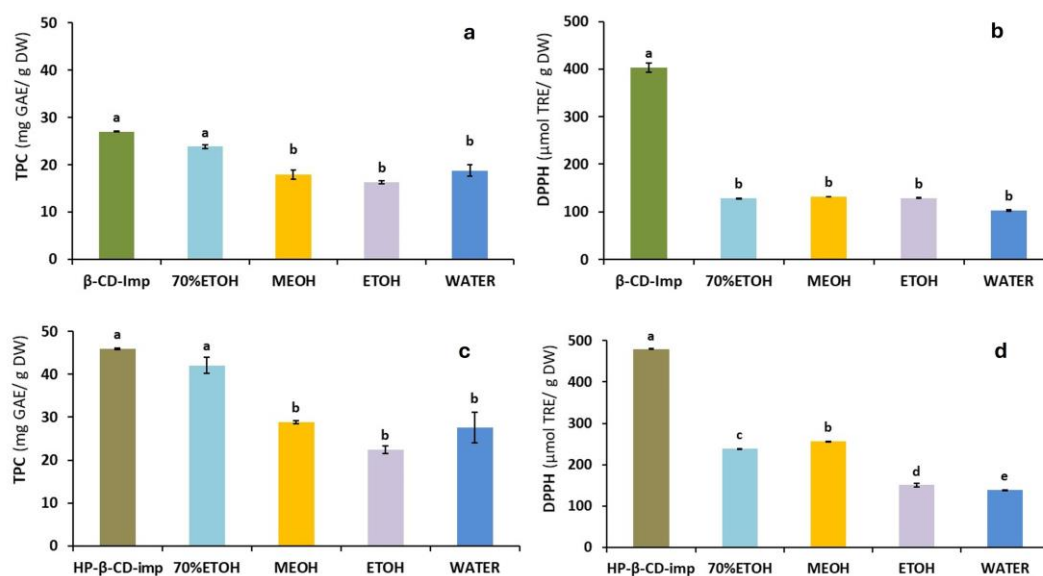


Figure 3. Comparison of the effectiveness of the β -CD- (a,b) and HP- β -CD-assisted extraction (c,d) with 70% aqueous ethanol (70%ETOH), ethanol (ETOH), methanol (MEOH) and water with regard to total polyphenol content (a,c) and DPPH antiradical activity (b,d). For the β -CD-assisted extraction, a β -CD concentration of 9.25 mg/mL was used at L/S = 8 for 124 min. For the HP- β -CD-assisted extraction, an HP- β -CD concentration of 18.5 mg/mL was used at L/S = 16 for 78.5 min. The abbreviated names of the extracts are presented in Table 1. The experiments were performed in triplicate. The values are shown as means $\pm$ standard error. Differences among means of the extracts are indicated by small letters (a–e) above the columns for each specified frame; values with different letters are statistically different (Tukey's test, $p < 0.05$).

Considering all the above experimental data, it could be suggested that β -CD and HP- β -CD assisted in the extraction of phenolics from rosemary hydro-distillation residues. These CD-assisted extractions provided polyphenolic yields and DPPH radical scavenging capacity equivalent to those obtained with aqueous ethanol (70%) and significantly higher

than those achieved with pure aqueous extraction. These results are in agreement with the study by Marijan et al. [53] in which HP- β -CD enhanced the phenolic acid content of *Helichrysum italicum* extract; the work of Kalantari et al. [45], who reported that the presence of β -CD improved the extraction efficiency of bioactive compounds from pomegranate peel; and the investigation of 'green' extracts from coffee pulp [62], which also showed an enhancement of DPPH radical scavenging by the addition of β -CD. In a previous kinetic investigation on the recovery of phenolic compounds by hydro-distillation of the residues of rosemary, Psarrou et al. reported that rosemary phenolic compounds appear to exhibit enhanced solubility in solvents having intermediate polarity, such as aqueous mixtures of alcohols and acetone, in contrast to more polar water or less polar absolute organic solvents [35]. Furthermore, it has been previously reported, in the case of pure polyphenols, that the encapsulation of polyphenols like rosmarinic and chlorogenic acids in CDs has been shown to enhance their antioxidant potency compared to their free states [19,63]. This enhancement is thought to occur because the hydrophobic cavity of the CD stabilizes polyphenol radicals more effectively, thereby increasing their capacity to scavenge free radicals. In a previous study conducted by our laboratory, LC-MS quantification revealed that the β -CD-assisted extract contains 35.3% rosmarinic acid and 24.33% phenolic diterpenes, specifically 14.4% carnosic acid and 9.9% carnosol [64], which is hydrophilic; the non-polar phenolic diterpenes, carnosic acid (14.4%) and carnosol (9.9%) are primary contributors to the extract's antioxidant activity [65]. The formation of inclusion complexes, demonstrated by XRD, FT-IR, and FESEM for carnosic acid [21] and proton nuclear magnetic resonance (^1H NMR) spectroscopy and fluorescence for rosmarinic acid [14,19], likely increases the apparent solubility and stability of these compounds in the assay medium. Therefore, we hypothesize that the superior DPPH activity of CD-assisted extracts is a synergistic result of enhanced phenolic solubility and the localized stabilization of antioxidant radicals within the CD cavity.

As a part of the comparative analysis, the ability of the extracts prepared (under the same, controlled extraction conditions) using conventional solvents and the improved β -CD and HP- β -CD extracts to inhibit soybean lipoxygenase, lipid peroxidation induced by AAPH, and xanthine oxidase was evaluated, and the results of these assays are presented in Table 5. The lipoxygenase inhibitory activity was investigated at a concentration of 2.5 mg/mL of each tested extract. The soybean lipoxygenase (LOX) assay is suggested as a preliminary indication of anti-inflammatory activity [66]. LOX is well known for its crucial role in the inflammatory cascade, whose inhibition is correlated to the ability of the inhibitors to reduce Fe^{3+} at the active site to the catalytically inactive Fe^{2+} . A "non-heme" iron is contained in LOXs per molecule in the enzyme active site as high-spin Fe^{2+} in the native state and the high-spin Fe^{3+} in the activated state. While phenolic derivatives are known to suppress LOX inhibitors [67] and are excellent ligands for Fe^{3+} , the tested extracts exhibited weak lipoxygenase inhibitory activity ranging from 0 to 12.9%, with the lowest activity displayed by the β -CD-assisted extract. Extracts prepared from plants belonging to the *Lamiaceae* family have been reported as weak LOX inhibitors [68].

As a water-soluble azo compound and a reliable free radical producer, AAPH is utilized in an assay for lipid peroxidation. This test directly measures an antioxidant's efficacy in preventing the lipid oxidation chain reaction by donating a hydrogen atom to the peroxy radicals attacking the linoleic acid [38]. All examined extracts were tested in three different concentrations for the assay of lipid peroxidation induced by AAPH. All extracts exerted 100% inhibition rates when tested at concentrations of 5.0 and 10 mg/mL whereas a variation in the inhibitory activity of lipid peroxidation (induced by AAPH) was noted at the tested concentration of 2.5 mg/mL, ranging from 10.0 to 46.3%; among the extracts prepared using the β -CD, the ethanolic extract (ETOH) exhibited the highest

inhibitory activity at a rate of 43.2%, followed by the methanolic extract with a rate of 40.0%. The β -CD-aided extract managed to inhibit the lipid peroxidation of linoleic acid by 11.3%, an activity that is slightly higher than that of the aqueous extract without the presence of β -CD. In contrast, the aqueous extract in the presence of HP- β -CD showed slightly higher inhibitory activity at a rate of 19.4%. Regarding the extracts prepared using the HP- β -CD, the highest inhibition rate was shown by the methanolic extract (MEOH, 46.3% rate), followed by a notably lower value observed from the 70% ethanolic extract (70%ETOH, 28.0% rate).

Table 5. Comparison of β -CD- and HP- β -CD-assisted extractions against extracts using conventional solvents (70%ETOH—70% ethanol, ETOH—ethanol, MEOH—methanol and water) under the same extraction conditions with regard to % soybean lipoxygenase inhibitory activity (LOX%), % inhibition of linoleic acid peroxidation (AAPH) and superoxide anion scavenging activity using the XO system % (OH%). Data were expressed as the mean $\pm$ standard deviation (SD) of $n = 3$ tests.

Concentration (mg/mL)	LOX (%)		AAPH (%)		OH (%)	
	2.5	10	5	2.5	10	
β -CD against conventional solvents at L/S = 8 and Time 124 min *						
β -CD-Imp	0	>100	>100	11.3 $\pm$ 0.4	41.2 $\pm$ 0.3	
70%ETOH	2.8 $\pm$ 0.3	>100	>100	22.8 $\pm$ 0.4	16.5 $\pm$ 0.2	
MEOH	5.4 $\pm$ 0.5	>100	>100	40.0 $\pm$ 0.1	62.4 $\pm$ 0.5	
ETOH	10.9 $\pm$ 0.4	>100	>100	43.2 $\pm$ 0.5	25.9 $\pm$ 0.2	
WATER	10.5 $\pm$ 0.1	>100	>100	10.0 $\pm$ 0.4	71.8 $\pm$ 0.3	
HP- β -CD against conventional solvents at L/S = 16 for 78.5 min **						
HP- β -CD-Imp	2.9 $\pm$ 0.5	>100	>100	19.4 $\pm$ 0.5	57.6 $\pm$ 0.5	
70%ETOH	7.3 $\pm$ 0.3	>100	>100	28.0 $\pm$ 0.3	61.2 $\pm$ 0.5	
MEOH	12.9 $\pm$ 0.4	>100	>100	46.3 $\pm$ 0.5	50.6 $\pm$ 0.5	
ETOH	7.6 $\pm$ 0.5	>100	>100	10.7 $\pm$ 0.3	18.8 $\pm$ 0.4	
WATER	1.4 $\pm$ 0.3	>100	>100	21.4 $\pm$ 0.5	29.4 $\pm$ 0.3	

* In case of comparisons to β -CD improved extract, all extracts were prepared using L/S = 8 for 124 min. ** In case of comparisons to HP- β -CD improved extract, all extracts were prepared using L/S = 16 for 78.5 min.

The evaluation of superoxide anion radical scavenging activity can be determined using assays involving non-enzymatic or enzymatic production of superoxide anions. In this case, the enzymatic assay was applied using xanthine oxidase (XO). Generation of superoxide contributes to the formation of highly damaging reactive oxygen species (ROS), specifically singlet oxygen and hydroxyl radicals. These species promote lipid peroxidation and the resulting cellular injury [69]. All extracts were tested at the concentration of 10 mg/mL and demonstrated considerable superoxide anion scavenging activity (Table 5). Among the extracts prepared using the β -CD conditions, the order of scavenging efficacy was as follows: WATER (71.8%) > MEOH (62.4%) > β -CD (41.2%) > ETOH (25.9%) > 70% ETOH (16.5%), whereas the order of superoxide anion scavenging activity was 70% ETOH (61.2%) > HP- β -CD (57.6%) > MEOH (50.6%) > WATER (29.4%) > ETOH (18.8%) for the extracts prepared using the HP- β -CD conditions. Comparing the results of aqueous extracts, it could be suggested that primarily the extraction conditions and secondarily the type of solvent affect the superoxide scavenging capacity of the extracts. In addition, the β -CD (41.2%) and HP- β -CD (57.6%) assisted extracts showed a noticeable superoxide anion scavenging activity, confirming that the polyphenolic compounds complexed with cyclodextrin could effectively neutralize superoxide radicals to an equal to or greater degree than extracts prepared using conventional solvents. It has been previously reported that

the scavenging activity of xanthine oxidase produced superoxide anion by crude extracts correlates with total polyphenol content [70].

Overall, the results for TPC and DPPH antiradical activity showed that the β -CD and HP- β -CD-assisted extracts resulted in a phenolic extraction efficiency that matched or even surpassed the efficiency of 70% (*w/w*) ethanol, exhibiting great DPPH antiradical activity. On the other hand, β -CD and HP- β -CD-assisted extracts exhibited lower ability to inhibit soybean lipid peroxidation induced by AAPH and moderate activity in scavenging the superoxide anion generated by the xanthine/xanthine oxidase system in comparison to the extracts prepared with conventional solvents. It is hypothesized that the observed low inhibitory activity of lipid peroxidation induced by AAPH results from the non-polar nature of the reaction medium (fatty acid), which favors the hydrogen atom transfer (HAT) mechanism. This stands in contrast to the high SET activity exhibited by CD-assisted extracts in the earlier DPPH assay [71,72].

4. Conclusions

This study demonstrated that rosemary post-distillation residues can be effectively valorized for the recovery of bioactive compounds with notable antioxidant potency, with β -cyclodextrin-assisted aqueous extractions representing an efficient and sustainable alternative to conventional solvent-based methods. Key extraction parameters (time, liquid-to-solid ratio, type of cyclodextrin and cyclodextrin concentration) were evaluated based on their responses to the variables TPC, TFC, DPPH radical scavenging and FRAP; subsequently, a multiple linear regression analysis was conducted. Through the developed regression models, it was observed that β -CD concentration positively influenced TPC and TFC, while the presence of HP- β -CD positively interacted with liquid-to-solid ratio, affecting the extraction efficiency indicators for all dependent variables. Consequently, the efficiency of the β -CD and HP- β -CD extracts was evaluated against that of various conventional solvents (ethanol, methanol, water and 70% ethanol). TPC and the assays of DPPH radical scavenging activity, soybean lipoxygenase inhibition, lipid peroxidation induced by AAPH and superoxide anion radical scavenging activity were employed to assess a broad spectrum of antioxidant activity and identify the primary mechanism of action of the phenolic compounds extracted with various solvents. While the β -CD-aided extract exhibited higher phenolic content and DPPH radical scavenging activity, compared with conventional extracts, it showed no inhibitory activity against the soybean lipoxygenase and a minimal inhibition rate of lipid peroxidation induced by AAPH when tested at a concentration of 2.5 mg/mL. Moreover, the β -CD-aided extract exerted enhanced ability to scavenge the superoxide anion generated by the xanthine/xanthine oxidase system as opposed to the ethanolic extracts (ethanol and 70% ethanol). In the case of the HP- β -CD-aided extract, similar trends were observed across the employed assays, but a more pronounced superoxide anion scavenging activity was noted. These findings indicate that the inclusion of cyclodextrins into aqueous extraction media offers promising potential for producing rosemary-distillate extracts with enhanced functional and antioxidant properties, primarily mediated by a single electron transfer mechanism.

Overall, phenolic extracts derived from the valorization of essential oil industry by-products via green extraction represent promising alternatives for the food and pharmaceutical sectors. The proposed process aligns with circular economy goals by transforming distillation residues into high-value bioactive extracts and facilitating a straightforward transition to large-scale production through the use of standard industrial equipment. Furthermore, based on the green parameters of this study, a formal Life Cycle Assessment and techno-economic analysis would likely highlight a significant reduction in environ-

mental impact and operational costs, potentially offering a more sustainable alternative to traditional solvent-based extraction methods.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/foods15040627/s1>, Table S1: Analysis of variance (ANOVA) and coefficients of determination (R²) for the fitted model equations of contents of TPC and DPPH for β -CD and HP- β -CD extractions.

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

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Abbreviations

The following abbreviations are used in this manuscript:

CD	Cyclodextrin
β -CD	β -cyclodextrin
HP- β -CD	HP- β -cyclodextrin
TPC	Total phenolic content
TFC	Total flavonoid content
LLA	Linoleic acid
AAPH	dihydrochloric acid of 2,2-azobis-2-amidinepropane
L/S	Liquid-to-solid ratio
FRAP	Ferric reducing antioxidant power
EO	Essential oil
AMPs	Aromatic and medicinal plants
GRAS	Generally Recognized as Safe
EU	European Union
¹ H NMR	Proton nuclear magnetic resonance
α -CD	α -cyclodextrin
γ -CD	γ -cyclodextrin
SET	Single electron transfer
HAT	Hydrogen atom transfer
TPTZ	Tripyridyltriazine
LOX	Lipoxygenase
AAR	Antiradical activity
ANOVA	Analysis of variance
GAE	Gallic acid equivalent
DW	Dry weight
TRE	Trolox equivalent
AAE	Ascorbic acid equivalent
QUE	Quercetin equivalent

70%ETOH	70% ethanol
MEOH	Methanol
ETOH	Ethanol
DES	Deep eutectic solvent
LC-MS	Liquid chromatography–mass spectrometry
XRD	X-ray diffraction
FT-IR	Fourier-transform infrared spectroscopy
FESEM	Field-emission scanning electron microscopy

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