AppliedChem

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Carotenoids Extraction from Orange Peels Using a Thymol-Based Hydrophobic Eutectic Solvent

Konstantinos Terlidis [1], Vassilis Athanasiadis [2], Theodoros Chatzimitakos [2], Eleni Bozinou [2] and Stavros I. Lalas [2,*]

[1] Department of Chemical Engineering, University of Western Macedonia, 50100 Kozani, Greece; kostasterlidis@yahoo.gr
[2] Department of Food Science and Nutrition, University of Thessaly, 43100 Karditsa, Greece; vaathanasiadis@uth.gr (V.A.); tchatzimitakos@uth.gr (T.C.); empozinou@uth.gr (E.B.)
* Correspondence: slalas@uth.gr; Tel.: +30-24410-64783

Abstract: The food industry produces substantial amounts of waste, which can cause a lot of environmental issues. However, such waste is also a valuable source of bioactive substances that can potentially be used either by the food industry or other types of industries, in the production of medicines, nutraceuticals, cosmetics, etc. The present study proposes a novel approach to extract such bioactive compounds from orange peel waste using hydrophobic eutectic solvents synthesized with thymol and fatty acids (hexanoic and octanoic acid). A response surface methodology was employed to optimize the extraction conditions and achieve maximum recovery of carotenoids. The optimal hydrophobic eutectic solvent consisted of thymol and hexanoic acid at a molar ratio of 2:1, and the optimum extraction was achieved using a solvent-to-solid ratio of 12:1 and a temperature of 20 °C for 78 min; this resulted in a recovery of 259.45 μg of total carotenoids per g of dry matter, which is a significantly higher recovery compared to common organic solvents. Based on the above, it is demonstrated that hydrophobic eutectic solvents is a promising solvent that can be used to extract bioactive compounds from orange peel waste.

Keywords: hydrophobic deep eutectic solvents; orange peel waste; carotenoids; antioxidants; color; response surface methodology; extraction

Citation: Terlidis, K.; Athanasiadis, V.; Chatzimitakos, T.; Bozinou, E.; Lalas, S.I. Carotenoids Extraction from Orange Peels Using a Thymol-Based Hydrophobic Eutectic Solvent. *AppliedChem* **2023**, *3*, 437–451. https://doi.org/10.3390/appliedchem3040028

Academic Editor: José P. Coelho

Received: 15 August 2023
Revised: 11 September 2023
Accepted: 19 September 2023
Published: 25 September 2023

1. Introduction

The food industry generates a substantial amount of solid and liquid waste as a result of the food production process. With regard to the orange-related industries, the by-products account for about 50% of the fresh fruit weight [1]. Out of these waste, 60–65% consists of peels [2], while the rest consists of seeds, pulp, membranes, etc. The disposal of orange peels is a potential environmental pollution issue [3]. However, if valorized properly, they can serve as a good source of valuable constituents such as pectin, carotenoids, and flavonoids. These substances can be used as food additives (e.g., colorants, flavorings, and antioxidants), thereby promoting acceptance of food products or improving human health [4], as well as can be utilized in pharmaceuticals, cosmetics, and other industries. To this end, various methods are being applied in order to extract beneficial compounds, such as carotenoids, from orange peels [5]. However, most of the extraction processes use organic solvents such as acetone, hexane, ethanol, etc. According to published research, acetone can provide an extraction yield of 7.88 ± 0.59 μg/g dry weight [6], hexane can provide an yield of 113.5 ± 0.4 mg/100 g fresh weight, and ethanol can provide an yield of 110.3 ± 0.3 mg/100 g fresh weight [7]. These solvents pose risks to human health due to their flammability and toxicity, and are harmful to the environment [8]. To address the issue of organic solvent usage, as well as to promote more "green" practices, in 2010, Anastas and Eghbali [9] proposed 12 principles for greener procedures in chemistry and chemical engineering, highlighting the importance of employing so-called green solvents.

One relatively new and promising category of green solvents is eutectic solvents (ESs), also known as deep eutectic solvents (DESs). A eutectic mixture is a mixture of two or more compounds, with relatively high melting points, that exhibits a significantly lower melting point than the individual components. The decrease in the melting point of DESs mainly stems from the formation of a hydrogen bond network between the hydrogen bond donor (HBD) and the hydrogen bond acceptor (HBA). As such, even though the HBDs and HBAs used are typically solid at room temperature, the resulting mixture becomes liquid and can be utilized as a solvent. Although this property of eutectic mixtures is not new, it was not until 2002 that Abbott et al. [10] showed that eutectic mixtures of choline chloride and urea can be used as green solvents with unique properties. DESs can be easily synthesized via simple methods [11]. DESs exhibit several advantages over conventional organic solvents, including low vapor pressure, easy and cost-effective preparation, and non-toxicity [12]. More importantly, DESs are considered to be tunable solvents as their physicochemical properties can be tailored by properly selecting the HBA and HBD, by adjusting their molar ratio, or even by the introduction of a third compound in the eutectic mixture, rendering it more suitable for the extraction of specific compounds [13]. Finally, since the synthesis of DESs is straightforward, with the synthesis yield being 100% and no requirement of purification steps, DESs are excellent candidates for large-scale industrial applications [14]. Although DESs have been used for the extraction of various compounds, attempts were made to synthesize new DESs based on natural components, already present in food matrices, so as to negate the need to separate the target compounds from the DES. As such, the natural deep eutectic solvents (NADESs) were developed. NADESs exhibit excellent extraction properties, and in some cases, they exhibit even more favorable characteristics, such as the deceleration of anthocyanin degradation [15].

However, DES and NADES have some disadvantages that limit their extraction potential. One major disadvantage is their high viscosity, which limits their extraction efficiency and can lead to challenges in industrial-scale applications due to heat and mass transfer limitations during extraction or dissolution [16]. Although the viscosity of NADES can be significantly reduced by adding water, the increase in water content in the NADES weakens the hydrogen bonding interactions, and might even completely disrupt them if the water content exceeds 50% [17]. Another limitation of (NA)DES is their hydrophilicity resulting in inefficient applicability for non-polar substances. In 2015, Van Osch et al. [18] created a DES based on a quaternary ammonium salt and decanoic acid, and used it for the extraction of volatile fatty acids from aqueous solutions. Since this solvent was hydrophobic, a new category of DES, called hydrophobic DES (HDES) was created, paving the way for a series of applications such as the extraction of pesticides from aqueous environments [19], enzyme activation [20], dye-sensitized solar cells [21], etc.

The aim of this study was to conduct the synthesis of HDES based on thymol and fatty acids (hexanoic and octanoic) for extracting carotenoids from orange peel waste. In recent years, many studies have explored the use of NADES for the extraction of bioactive compounds, such as phenolic compounds [22–25]; however, fewer studies have focused on the extraction of carotenoids [26,27]. Since carotenoids are lipophilic substances, a hydrophobic DES based on thymol could prove to be a good extraction solvent, and to the best of our knowledge, a thymol-based HDES has never been used for the extraction of carotenoids from orange peels. To this end, various syntheses were evaluated, so as to select the optimum HDES. Next, the extraction parameters were optimized, in order to further enhance the extraction process, using response surface methodology (RSM) [28,29]. As carotenoids are soluble in acetone, ethyl acetate, and hexane [30], these solvents were used for comparison.

2. Materials and Methods

2.1. Chemicals and Reagents

Thymol, hexane, iron chloride (III), ascorbic acid, and β-carotene were purchased from Sigma-Aldrich (Steinheim, Germany). Hexanoic acid and octanoic acid were pur-

chased from Fluorochem (Hadfield, UK). Ethyl acetate was purchased from Carlo Erba (Val de Reuil, France). Acetone was purchased from Scharlab (Barcelona, Spain). 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (99%) were purchased from Penta (Prague, Czech Republic).

2.2. Orange Sample Preparation

Fresh oranges of the Merlin (Washington navel) cultivar (*Citrus sinensis* L.), collected at the maturity stage, were purchased from selected orchards in the Argos area (Peloponnese, Greece) in December 2022. The physical and chemical parameters of the oranges were evaluated to ensure that they were at the maturity stage. The color of the fruit limb/twig was green, the fruit rind (epicarp) color was orange, and the weight of the fruits ranged from 150 to 200 g. Furthermore, the total soluble solids (TSSs) (°Brix) were between 10 and 12, the titratable acidity (TA) (expressed as % citric acid) was >0.8, and the TSS/TA ratio was >12.

The oranges were washed with tap water and dried with a paper towel. The peels were removed manually, cut into smaller pieces (~2 × 2 cm), and placed in a Biobase BK-FD10P freeze-dryer (Jinan, China) for 24 h in order to remove water. The freeze-dried peels were then pulverized and placed in sieves, so as to separate them according to size. The powdered orange peels with an average particle diameter of 470 μm were used for the preparation of the extracts (the amount of the powder from the smaller average particle diameter was substantially smaller compared to the used particles, and therefore was not used as we opted for the extraction of compounds from the major fraction of the powder).

2.3. HDES Synthesis

A total of 9 HDES were synthesized. Thymol was used as the HBA, whereas hexanoic acid and octanoic acid were used as the HBDs. Since the fatty acids have hydroxyl groups (−OH), they can either behave as HBAs or HBDs, so the combination of the two fatty acids was also tested. In the case of an HDES consisting of two fatty acids, the one with the longest chain behaves as the HBD [31]. For each combination of HBA and HBD, molar ratios of 2:1, 1:1, and 1:2 were chosen, as shown in Table 1.

Table 1. Constituents, molar ratios, abbreviations, and densities of the prepared HDES.

HBA	HBD	Molar Ratio	Abbreviation	Density (g/mL)
Thymol	Hexanoic acid	2:1	Thy/Hex 2:1	0.838
		1:1	Thy/Hex 1:1	0.862
		1:2	Thy/Hex 1:2	0.855
Thymol	Octanoic acid	2:1	Thy/Oct 2:1	0.870
		1:1	Thy/Oct 1:1	0.830
		1:2	Thy/Oct 1:2	0.863
Hexanoic acid	Octanoic acid	2:1	Hex/Oct 2:1	0.869
		1:1	Hex/Oct 1:1	0.858
		1:2	Hex/Oct 1:2	0.848

To synthesize the HDES, appropriate amounts of HBA and HBD were mixed in 25 mL glass bottles and heated at 70 °C under stirring at 350 rpm until a clear homogeneous liquid was formed. The HDES were then allowed to cool to room temperature, and inspected for the formation of crystals after 24 h.

The experimental methodology for density measurement involved precise sample preparation, temperature calibration, and density determination. All HDES samples were carefully transferred into pre-weighed density bottles, and the masses were recorded. Reproducibility was ensured through multiple measurements.

2.4. Extraction Process

Initially, all HDES were assessed to examine their efficiency for the extraction. To this end, the HDES and the orange peels were mixed at a solvent-to-solid ratio of 10:1, and the mixture was stirred at 500 rpm for 60 min at room temperature. For means of comparison, extracts were also prepared using three conventional organic solvents (i.e., hexane, ethyl acetate, and acetone).

Using the optimum HDES, the overall extraction process was further optimized. For the optimization of the extraction parameters, an RSM was employed. The parameters to be tested were solvent-to-solid ratio, temperature, and time. An experiment using a Box–Behnken design with 15 design points, including 3 central points, served as the foundation for the optimization, and the response was the extraction yield in total carotenoids (Y_{TCn}). The process variables were established in three levels (Table 2). The analysis of variance (ANOVA) and the summary-of-fit tests were used to evaluate the overall model significance (R^2, p) at a minimum level of 95%.

Table 2. Levels of independent variables in actual and coded values for the optimization of the extraction of total carotenoids.

Independent Variables	Coded Units	Coded Levels		
		−1	**0**	**1**
Solvent-to-solid ratio (mL/g)	X_1	10	25	40
t (min)	X_2	30	60	90
T (°C)	X_3	20	35	50

2.5. Total Carotenoid Content (TCC)

A method developed by Biswas et al. [32] was implemented for the determination of the total carotenoid content of the extracts. Briefly, 200 μL of the sample was mixed with 800 μL of ethanol, and the solution was vigorously shaken for 30 s. The absorbance was read at 428 nm using a Shimadzu spectrophotometer (UV-1700, Shimadzu Europa GmbH, Duisburg, Germany). A calibration curve, created with β-carotene (1–10 μg/mL) as a standard compound, was used for the determination of the TCC.

2.6. Antiradical Activity (DPPH Assay)

The antiradical activity of the extracts was determined via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay using a previously described method [33]. An aliquot of 200 μL of the sample was mixed with 800 μL of ethanol, and the solution was vigorously shaken for 30 s. Briefly, 25 μL of the solution was mixed with 975 μL of DPPH solution (100 μM). After mixing, the absorbance of the solution was read at 515 nm ($A_{515(i)}$). The solutions were incubated for 30 min in the absence of light, and the absorbance was read at 515 nm ($A_{515(f)}$). The capacity to scavenge the DPPH radical was expressed as

$$\text{Inhibition } (\%) = \frac{A_{515(i)} - A_{515(f)}}{A_{515(i)}} \times 100 \quad (1)$$

The antiradical activity (A_{AR}) was expressed as μmol ascorbic acid equivalents (AAE), using an ascorbic acid calibration curve (C_{AA}, 50–1.000 μM) and the following Equation (2):

$$A_{AR}(\mu\text{mol AAE/g dm}) = \frac{C_{AA} \times V}{w} \quad (2)$$

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

2.7. Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric-reducing power was determined using a previously described method [34]. An aliquot of 200 µL of the sample was mixed with 800 µL of ethanol, and the solution was vigorously shaken for 30 s. Briefly, 50 µL of the solution was mixed with 50 µL of $FeCl_3$ solution (4 mM in 0.05 M HCl), and the solutions were incubated at 37 °C for 30 min. After incubation, 900 µL of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution (1 mM in 0.05 HCl) was added, and 5 min later, the absorbance was read at 620 nm. A calibration curve, with ascorbic acid (C_{AA}, 50–500 µM) as a standard compound, was used, and the P_R was determined as µmol ascorbic acid equivalents (AAE) per g of dm (dry matter), using the following Equation (3):

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

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

2.8. Color Analysis

The color analysis of the extracts was conducted through measuring the absorbance in three different wavelengths (420, 520, and 620 nm), as well as using a Lovibond CAM-System 500.

2.8.1. Colorimeter Method

A Lovibond CAM-System 500 Imaging Colorimeter was used for the color (CIE L^*, a^*, b^*) analysis of the extracts. The parameter L^* indicates lightness, a^* redness, and b^* yellowness [35]. The values of these parameters were used to calculate Chroma (C^*_{Ab}) and hue-angle (h^o_{Ab}), according to Equations (4) and (5), respectively.

$$C^*_{ab} = \sqrt{(a^*)^2 + (b^*)^2} \quad (4)$$

$$h^o_{Ab} = \tan^{-1}\left(b^*/a^*\right) \quad (5)$$

2.8.2. Absorbance Method

An aliquot of 200 µL of the sample was mixed with 800 µL of ethanol, and the solution was vigorously shaken for 30 s. The absorbance of the solution was read at 420, 520, and 620 nm. The sum of the absorbance at the above wavelengths provides the color intensity (CI), and the ratio of the absorbance at 420 nm over the absorbance at 520 nm provides the hue (Equations (6) and (7)) [36]. Also, the color composition was calculated, i.e., the contribution of the three components (yellow, red and blue, expressed as a percentage), using the following Equations (8)–(10):

$$\text{CI} = A_{420} + A_{520} + A_{620} \quad (6)$$

$$\text{H} = \frac{A_{420}}{A_{520}} \quad (7)$$

$$\text{Yellow (\%)} = \frac{A_{420}}{\text{CI}} \times 100 \quad (8)$$

$$\text{Red (\%)} = \frac{A_{520}}{\text{CI}} \times 100 \quad (9)$$

$$\text{Blue (\%)} = \frac{A_{620}}{\text{CI}} \times 100 \quad (10)$$

2.9. Statistical Analysis

The experimental design, statistical analysis related to the response surface methodology, and distribution analysis were all created using the JMP® Pro 16 (SAS, Cary, NC, USA) software. The results were expressed as mean values, and the standard deviation was also calculated.

3. Results and Discussion

In this study, we investigated the use of eutectic solvents derived from thymol and fatty acids (hexanoic and octanoic acid) at various molar ratios (2:1, 1:1, and 1:2). While the term "DES" typically refers to a specific eutectic composition, we employ it here to encompass a range of compositions within the vicinity of the eutectic point. These ratios were examined, since they are common to form eutectic mixtures for both HBD and HBA, in order to evaluate the potential of the eutectic mixtures as solvents for carotenoid extraction. The relatively high density and viscosity values of DES are limiting factors in their applicability as extraction solvents. Since hydrophobic DES generally have lower densities and viscosities compared to hydrophilic ones [37–39], we proposed the use of a HDES synthesized from thymol and fatty acids to be used as a solvent for the extraction of carotenoids from orange peels.

3.1. Choice of Solvent

A total of nine solvents based on thymol and fatty acids (hexanoic and octanoic) were synthesized at three molar ratios. These HDES, along with three conventional organic solvents were used to extract carotenoids from orange peel waste. The results are shown in Table 3. The absorbance spectra (not depicted) showed a peak at 428 nm, indicating the presence of violaxanthin in the extracts, which is the major carotenoid in orange peels [40].

Table 3. Extraction yield of total carotenoids (Y_{TCn}) obtained via the prepared HDES, and conventional solvents expressed as μg of total carotenoids per g of dry matter.

Solvent	Y_{TCn} (μg CtE/g dm)
Thy/Hex 2:1	184.94 ± 4.69 ^{a}
Thy/Hex 1:1	166.97 ± 4.11 ^{b}
Thy/Hex 1:2	165.39 ± 3.82 ^{b}
Thy/Oct 2:1	180.42 ± 9.02 ^{a,b}
Thy/Oct 1:1	134.98 ± 5.71 ^{c}
Thy/Oct 1:2	164.12 ± 7.7 ^{b}
Hex/Oct 2:1	179.52 ± 5.8 ^{a,b}
Hex/Oct 1:1	171.15 ± 2.76 ^{a,b}
Hex/Oct 1:2	175.17 ± 5.79 ^{a,b}
Hexane	141.58 ± 3.49 ^{c}
Ethyl Acetate	171.2 ± 8.16 ^{a,b}
Acetone	128.15 ± 6.9 ^{c}

Statistically significant differences ($p < 0.05$) are denoted with different superscript letters (e.g., $^{a–c}$).

Although carotenoids are mainly non-polar substances, the epoxy functional group in violaxanthin increases its polarity [41], so more polar solvents would achieve a higher extraction yield. According to Martins et al. [42], the aromatic ring in the structure of thymol results in high values of the solvatochromic parameter π*, which is related to the polarizability/dipolarity of its mixtures; so in our case, the thymol-based HDES should present high extraction yields for the polar carotenoids in the orange peels.

As can be seen in Table 3, the highest extraction yield was achieved with Thy/Hex 2:1, followed by Thy/Oct 2:1. The majority of the other HDES had comparable extraction yields, except for Thy/Oct 1:1, which proved to be the least efficient. When octanoic acid was used as the HBD, the extraction yield was lower than when hexanoic acid was the

HBD. This can be attributed to the fact that when the number of carbon atoms in the fatty acid increases, the polarity of HDES decreases [43].

As far as the organic solvents are concerned, hexane and acetone exhibited the lowest extraction yields 141.58 ± 3.49 and 128.15 ± 6.9 μg CtE/g dm, respectively, while ethyl acetate yielded much better results (171.2 ± 8.16 μg CtE/g dm), similar to most of the HDES (max 184.94 ± 4.69 μg CtE/g dm for Thy/Hex 2:1). This is because ethyl acetate presents higher solubility for polar carotenoids compared to hexane, as predicted via the Conductor-like Screening Model for Real Solvents (COSMO-RS) analysis, using COSMOthermX program (version C30 release 13.01) [44]. HDES synthesized exclusively of fatty acids had very good extraction yields, and is reported to be the most efficient one in a number of recent publications, i.e., a mixture of capric acid and lauric acid at a molar ratio of 1:2 for the extraction of carotenoids from tomato achieving an extraction of 7.90 ± 0.15 mg lycopene/100 g fresh weight [45], and a mixture of caprylic acid and capric acid at a molar ratio of 3:1 for the extraction of 151.41 mg β-carotene/mL from pumpkin [27]. Fan et al. [46] found that an HDES composed of thymol and fenchyl alcohol proved to be the most efficient for the recovery of lutein from microalgae, achieving a yield of 6.26 ± 0.40 mg/g. Based on the above, the synthesized HDES can be used as an extraction solvent, achieving comparable or enhanced extraction yields as compared to organic solvents. Thus, Thy/Hex 2:1 was selected as the optimal HDES.

3.2. Extraction Optimization

The Box–Behnken Design (BBD) was chosen because it is specifically constructed to fit a second-order model, which is the main focus of most RSM investigations. Additionally, a BBD frequently requires fewer experimental runs. Thus, a BBD was applied to test the effect of solvent-to-solid ratio (X_1), extraction time (X_2), and temperature (X_3) on total carotenoid content (TCC). Fifteen experiments were conducted, as seen in Table 4, and the results were analyzed using ANOVA (Figure 1) to evaluate the statistical significance of the model. Model fitting was assessed with the square coefficient of correlation (R^2), which was over 0.96, and suggested a satisfactory agreement between the measured and predicted values. Figure 2 shows the generated response surface plots. The optimum extraction conditions, as calculated using the statistical analysis, were a solvent-to-solid ratio of 12:1, an extraction time of 78 min, and a temperature of 20 °C.

Table 4. Experimental design, measured and predicted responses of the dependent variable expressed as μg of total carotenoids per g of dry matter.

Design Point	Independent Variables			Y_{TCn} (μg CtE/g dm)	
	X_1	X_2	X_3	Measured	Predicted
1	10	30	35	175.6	175.0
2	10	90	35	220.1	222.2
3	40	30	35	182.5	180.4
4	40	90	35	183.3	183.9
5	25	30	20	176.0	184.6
6	25	30	50	178.5	172.7
7	25	90	20	235.7	241.5
8	25	90	50	175.0	166.4
9	10	60	20	250.4	242.4
10	40	60	20	256.8	250.3
11	10	60	50	216.9	223.3
12	40	60	50	174.4	182.4
13	25	60	35	182.5	183.3
14	25	60	35	187.8	183.3
15	25	60	35	179.7	183.3

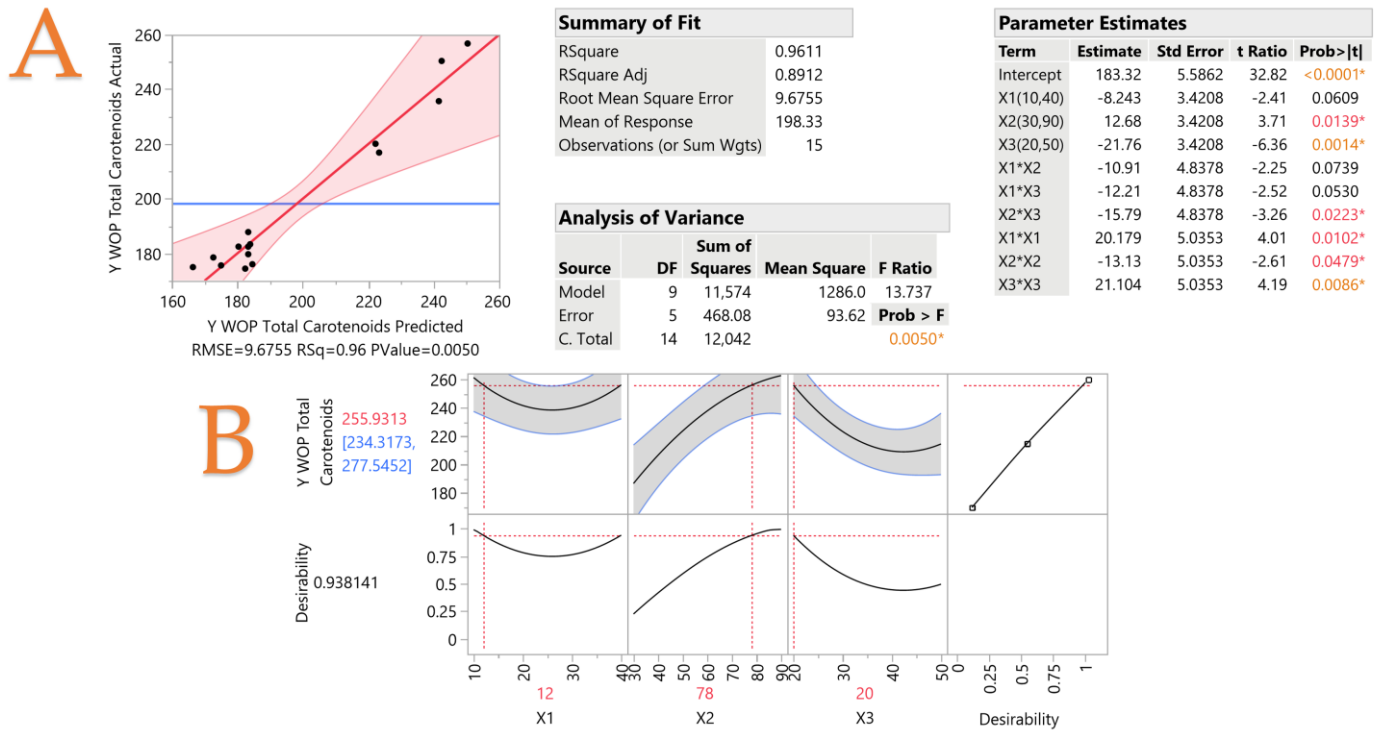


Figure 1. Plot of predicted vs. actual values of the response (Y_{TCn}) (plot **A**), and desirability function (plot **B**), describing the effect of three independent variables considered (solvent-to-solid ratio X_1, extraction time X_2, temperature X_3) on the Y_{TCn} upon simultaneous variation. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.

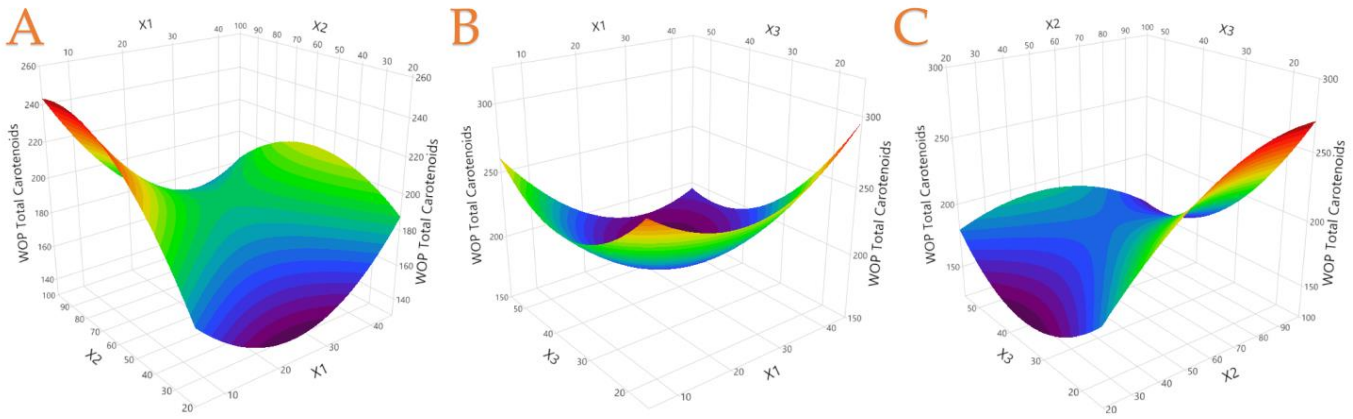


Figure 2. Response surface plots for the total carotenoid yields. (**A**) The interaction between solvent-to-solid ratio (X_1) and extraction time (X_2), (**B**) the interaction between solvent-to-solid ratio (X_1) and temperature (X_3), and (**C**) the interaction between extraction time (X_2) and temperature (X_3).

The final extractions were performed at the optimum conditions using Thy/Hex 2:1, whereas hexane, ethyl acetate, and acetone were also examined for comparison.

3.3. TCC of the Extracts

The determination of the total carotenoids (Table 5) showed that HDES achieved a better extraction yield than all of the conventional organic solvents, with ethyl acetate being the most efficient of the three solvents, followed by hexane and acetone, which was the least efficient. It is significant that the quantity of the TCC extracted via HDES was 59% higher (statistically significant at $p < 0.05$) compared to hexane, which is the most commonly

employed organic solvent for the extraction of carotenoids; it was also 37% and 76% higher compared to ethyl acetate and acetone, respectively. These data are supported by a number of recent publications which report that, as compared to hexane, HDES achieved higher extraction yields of carotenoids from orange peels [26], tomato pomace [47], and kale [48]. Additionally, the TCC of 259.45 ± 3.46 μg/g dm that was obtained under the optimum extraction conditions was very close to the predicted value of 255.93 ± 21.61 μg/g dm, which suggested a good prediction ability of the model. Even though the extraction yield of 259.45 ± 3.46 μg/g dm may not seem substantial, considering the market price of USD 300–3000 per kg of β-carotene and the market volume growth [49], along with the cost of other methods of carotenoid production, e.g., the cost of microbial growth medium for the microbial production of pigments [50], the use of HDES for carotenoid extraction could prove to be more efficient and sustainable.

Table 5. Extraction yield of total carotenoids and antioxidant properties of the extracts obtained with different solvents at optimum extraction conditions.

Solvent	Total Carotenoids Content (Y_{TCn}) (μg CtE/g dm)	Antiradical Activity (A_{AR}) (μmol AAE/g dm)	Reducing Power (P_R) (μmol AAE/g dm)
Thy/Hex 2:1	259.45 ± 3.46 ^{a}	72.32 ± 0.45 ^{a}	29.48 ± 0.15 ^{a}
Hexane	163 ± 4.78 ^{c}	24.24 ± 1 ^{b}	21.27 ± 1.87 ^{b}
Ethyl acetate	188.27 ± 4.54 ^{b}	16.61 ± 0.4 ^{c}	11.66 ± 0.25 ^{c}
Acetone	147.38 ± 6.15 ^{d}	18.16 ± 0.41 ^{c}	10.75 ± 0.12 ^{c}

Within each column, statistically significant differences ($p < 0.05$) are denoted with different superscript letters (e.g., $^{a–d}$).

3.4. Antioxidant Properties of the Extracts

As seen from the results in Table 5, the extract obtained with HDES exhibited better antioxidant properties (statistically significant at $p < 0.05$), quantified using the FRAP assay and the DPPH assay, compared to the three other solvents.

More specifically, the FRAP assay results showed that the extracts obtained with ethyl acetate and acetone exhibited similar reducing power, whereas the hexane extract had almost double reducing power. The extract obtained with Thy/Hex 2:1 had a much higher reducing power compared to ethyl acetate and acetone (152% and 174%, respectively), and was 38% higher compared to hexane.

The comparison of the DPPH assay results further validated that the extract obtained using hexane had better antioxidant activity than the ones obtained with ethyl acetate and acetone, but again, the Thy/Hex 2:1 extract was found to achieve an increased scavenging activity, from 198% up to 335% (statistically significant for $p < 0.05$), compared to the organic solvents.

These results are in accordance with recent publications. Alsaud et al. [51] measured the antioxidant activity of Manuka leaves extract, and found that the extract obtained using HDES showed higher antioxidant activity than the one obtained with hexane. In a study of orange peel, Viñas-Ospino et al. [7] showed that extracts obtained using hydrophobic DES had higher antioxidant activity compared to conventional solvents and hydrophilic DES. According to some researchers, in addition to being better at extracting substances with antioxidant activities, (NA)DES can also enhance the antioxidant activity of the extracts and improve their storage stability [52,53]. In our case, the higher antioxidant activity of the Thy/Hex 2:1 extract could be attributed to the solvent itself, since the antioxidant properties of thymol have been well studied in the past [54–57].

3.5. Color Analysis of the Extracts

The values of the color parameters obtained with the Lovibond colorimeter are shown in Table 6. The extracts obtained with the three organic solvents had very similar color saturation and hue values (expressed by the chroma and hue-angle, respectively). Their hue-

angles were very close to 90 degrees (yellow), whereas the extract obtained with Thy/Hex 2:1 was the farthest (70.8 degrees), which means less yellow and redder (90 degrees hue angle is yellow and zero degrees is red), as can also be seen in Figure 3.

Table 6. Color parameters of the extracts. Measurements were obtained with a colorimeter.

Solvent	*L**	*a**	*b**	*C**	*h*°
Thy/Hex 2:1	64.6 ± 0.8^{b}	11.4 ± 1.4^{a}	32.8 ± 1.6^{b}	34.7 ± 2^{b}	70.9 ± 1.4^{c}
Hexane	69.3 ± 1^{a}	-4 ± 1.2^{c}	67.3 ± 3^{a}	67.4 ± 2.9^{a}	93.5 ± 1.2^{a}
Ethyl acetate	$66.3 \pm 0.8^{a,b}$	3.5 ± 0.8^{b}	65.5 ± 2.8^{a}	65.6 ± 2.8^{a}	86.9 ± 0.7^{b}
Acetone	$67.3 \pm 2^{a,b}$	3.8 ± 1.6^{b}	62.9 ± 2.5^{a}	63 ± 2.4^{a}	86.5 ± 1.6^{b}

Within each column, statistically significant differences ($p < 0.05$) are denoted with different superscript letters (e.g., $^{a–c}$).

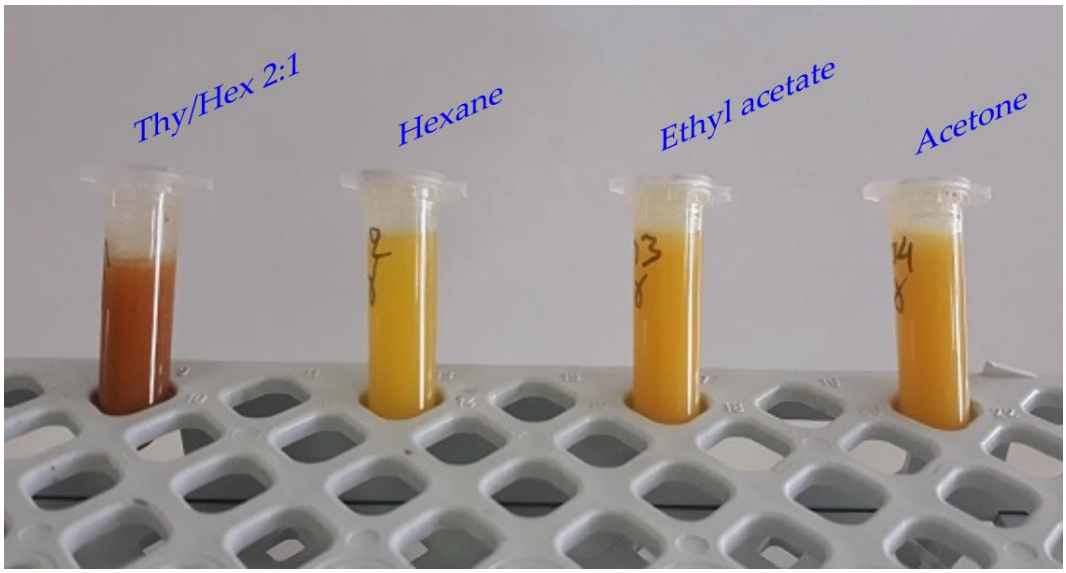


Figure 3. Extracts obtained with Thy/Hex 2:1, hexane, ethyl acetate, and acetone (from left to right) at optimum conditions.

The hue-angle (h°) data support our earlier findings about the carotenoid contents in the extracts, as according to Kishimoto et al. [58], the redness of the extract increases as the carotenoid content increases, which is the case here.

As an alternative method to analyze the color parameters, the absorbance of the extracts (after proper dilution) was measured at 420, 520, and 620 nm. The calculated values for color intensity (CI), hue (h), and the percentage of each color are given in Table 7.

Table 7. Color parameters of the extracts. Measurements obtained with UV-Vis spectrophotometer.

Solvent	CI	Hue	% Yellow	% Red	% Blue
Thy/Hex 2:1	6.5 ± 0.02^{a}	8.16 ± 0.2^{b}	87.91	10.78	1.31
Hexane	4.1 ± 0.07^{c}	11.07 ± 1.84^{a}	91.21	8.24	0.55
Ethyl acetate	4.71 ± 0.02^{b}	11.16 ± 0.48^{a}	91.29	8.18	0.53
Acetone	3.74 ± 0.05^{d}	11.1 ± 0.86^{a}	91.18	8.22	0.60

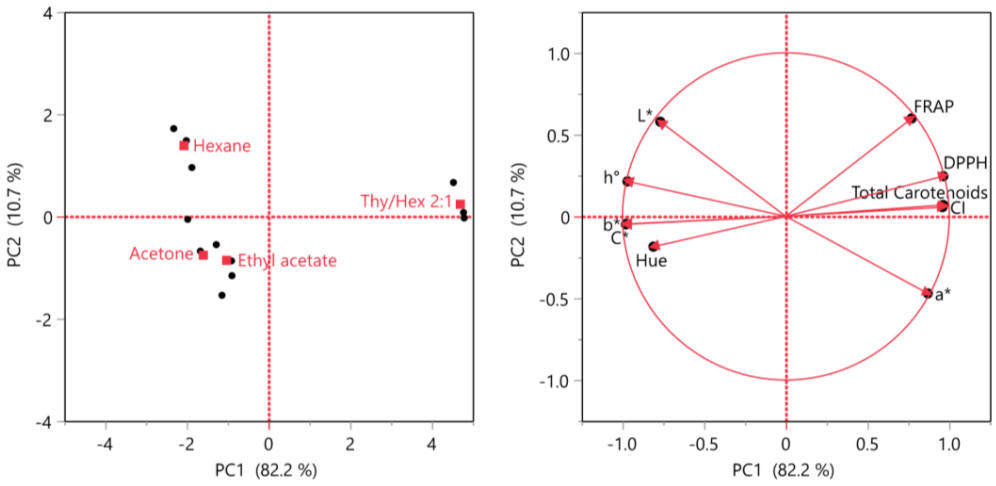
Within each column, statistically significant differences ($p < 0.05$) are denoted with different superscript letters (e.g., $^{a–d}$).

It can be seen that the extracts obtained with the organic solvents had similar color attributes, whereas the one obtained with HDES differed. The Thy/Hex 2:1 extract had a lower percentage of yellow and a higher percentage of red, which again supports our earlier results about the higher concentration of carotenoids. This finding is also corroborated by

the correlation analysis, as described in the next section. The lower percentage of yellow may arise from the decreased co-extraction of other pigments such as anthocyanins or chlorophyll derivatives, that may influence the observed colors.

3.6. Principal Component Analysis (PCA) and Multivariate Correlation Analysis (MCA)

In order to minimize the dimensionality of the multivariate data and obtain an improved understanding of the results, a principal component analysis (PCA) was also performed. Figure 4 shows that the two main components could explain 92.9% of the variation (eigenvalues > 1), which was considered to be a statistically significant parameter ($p < 0.0001$). PC1 demonstrated a positive correlation with total carotenoids, DPPH, FRAP, color intensity (CI), and redness (a^*), and a negative correlation with other color indices (L^*, b^*, C^*, h°, and hue). PC1 also explained 82.2% of the variability. With the exception of several color characteristics (a^*, b^*, C^*, and hue), PC2 can account for 10.7% of the variance, and shows a positive correlation with all of the variables examined. According to the PCA plots in Figure 4, the loading direction of DPPH, FRAP, and the total carotenoids is the same with the Thy/Hex 2:1 sample parameter; however, the loading direction of C^* is different. Because of this, it can be assumed from Figure 5 that the total carotenoids are positively correlated (>0.9) with the antioxidant parameters, but negatively correlated (−0.9) with C^*. The stronger the correlation between the measured variables, the closer it is to 1. Additionally, the best correlation was found between CI and total carotenoids (0.99), which was found to be a statistically significant factor ($p < 0.0001$).



Eigenvalues

Number	Eigenvalue	Percent	Cum Percent	ChiSquare	DF	Prob>ChiSq
1	8.2244	82.244	82.244	341.965	45.813	<0.0001*
2	1.0684	10.684	92.928	223.273	49.717	<0.0001*
3	0.3781	3.781	96.709	171.736	41.367	<0.0001*
4	0.2174	2.174	98.883	136.819	32.893	<0.0001*
5	0.0931	0.931	99.814	100.374	25.207	<0.0001*
6	0.0176	0.176	99.990	58.449	18.550	<0.0001*
7	0.0005	0.005	99.996	13.781	12.734	0.3693
8	0.0004	0.004	99.999	8.930	7.711	0.3213
9	0.0001	0.001	100.000	2.470	3.129	0.5042
10	0.0000	0.000	100.000	0.000	0.421	0.9904

Figure 4. Principal component analysis (PCA) for the measured variables. The axis scores for PC1 and PC2 are displayed. The inset table includes the eigenvalues. Asterisks and colored values denote statistically significant values.

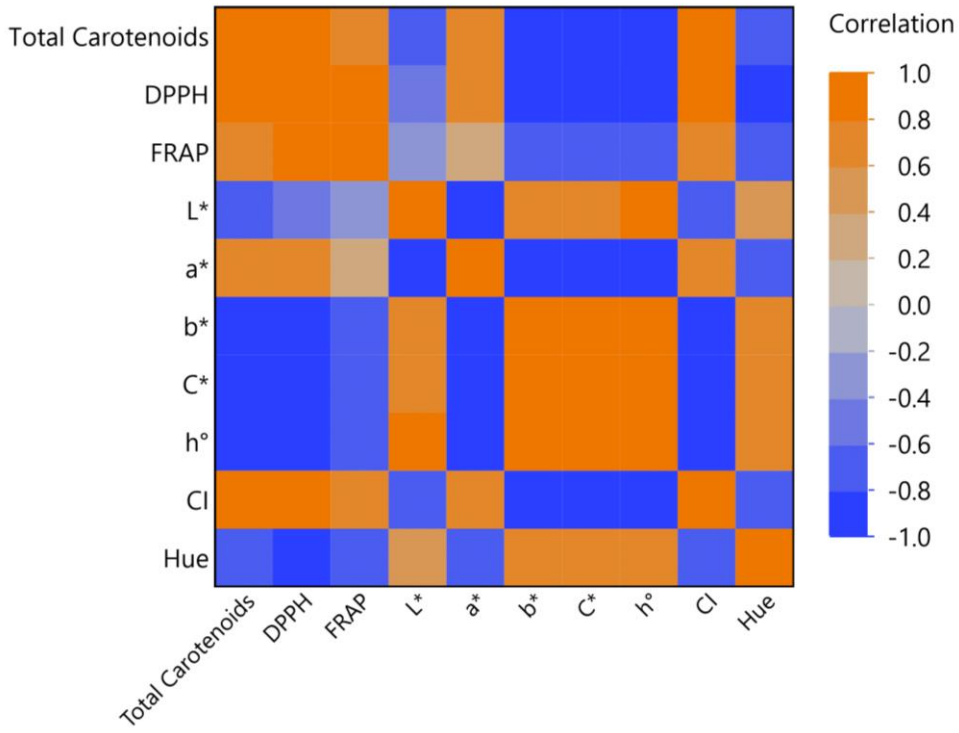


Figure 5. Multivariate correlation analysis of measured variables.

4. Conclusions

In this study, a series of hydrophobic deep eutectic solvents was synthesized to test their efficiency in the recovery of carotenoids from orange peels. The initial extractions indicated that HDES composed of thymol and hexanoic acid at a molar ratio of 2:1 achieved the highest extraction yield, and it was used for further optimization of the extraction parameters. Under optimum conditions, the extract obtained with Thy/Hex 2:1 had a higher carotenoid concentration compared to hexane, acetone, and ethyl acetate; also, the extracted compounds exhibited enhanced antioxidant properties, as evidenced by the DPPH and FRAP assay results. Based on the above results and the fact that the process of HDES preparation is economical, easy, and 100% atom efficient, we can conclude that HDES prepared from thymol and hexanoic acid provides a viable and sustainable alternative to conventional organic solvents for the extraction of carotenoids from orange peels.

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

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

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

References

1. Garzón, C.G.; Hours, R.A. Citrus Waste: An Alternative Substrate for Pectinase Production in Solid-State Culture. *Bioresour. Technol.* **1992**, *39*, 93–95. [CrossRef]
2. Mamma, D.; Christakopoulos, P. Biotransformation of Citrus By-Products into Value Added Products. *Waste Biomass Valorization* **2014**, *5*, 529–549. [CrossRef]
3. Satari, B.; Karimi, K. Citrus Processing Wastes: Environmental Impacts, Recent Advances, and Future Perspectives in Total Valorization. *Resour. Conserv. Recycl.* **2018**, *129*, 153–167. [CrossRef]
4. Wang, Y.-C.; Chuang, Y.-C.; Hsu, H.-W. The Flavonoid, Carotenoid and Pectin Content in Peels of Citrus Cultivated in Taiwan. *Food Chem.* **2008**, *106*, 277–284. [CrossRef]
5. Martins, N.; Ferreira, I.C.F.R. Wastes and By-Products: Upcoming Sources of Carotenoids for Biotechnological Purposes and Health-Related Applications. *Trends Food Sci. Technol.* **2017**, *62*, 33–48. [CrossRef]
6. Murador, D.C.; Braga, A.R.C.; Martins, P.L.G.; Mercadante, A.Z.; De Rosso, V.V. Ionic Liquid Associated with Ultrasonic-Assisted Extraction: A New Approach to Obtain Carotenoids from Orange Peel. *Food Res. Int.* **2019**, *126*, 108653. [CrossRef]
7. Viñas-Ospino, A.; Panić, M.; Bagović, M.; Radošević, K.; Esteve, M.J.; Radojčić Redovniković, I. Green Approach to Extract Bioactive Compounds from Orange Peel Employing Hydrophilic and Hydrophobic Deep Eutectic Solvents. *Sustain. Chem. Pharm.* **2023**, *31*, 100942. [CrossRef]
8. Płotka-Wasylka, J.; Rutkowska, M.; Owczarek, K.; Tobiszewski, M.; Namieśnik, J. Extraction with Environmentally Friendly Solvents. *TrAC Trends Anal. Chem.* **2017**, *91*, 12–25. [CrossRef]
9. Anastas, P.; Eghbali, N. Green Chemistry: Principles and Practice. *Chem. Soc. Rev.* **2010**, *39*, 301–312. [CrossRef]
10. Abbott, A.P.; Capper, G.; Davies, D.L.; Rasheed, R.K.; Tambyrajah, V. Novel Solvent Properties of Choline Chloride/Urea mixtures. *Chem. Commun.* **2003**, *1*, 70–71. [CrossRef]
11. Cannavacciuolo, C.; Pagliari, S.; Frigerio, J.; Giustra, C.M.; Labra, M.; Campone, L. Natural Deep Eutectic Solvents (NADESs) Combined with Sustainable Extraction Techniques: A Review of the Green Chemistry Approach in Food Analysis. *Foods* **2022**, *12*, 56. [CrossRef]
12. Smith, E.L.; Abbott, A.P.; Ryder, K.S. Deep Eutectic Solvents (DESs) and Their Applications. *Chem. Rev.* **2014**, *114*, 11060–11082. [CrossRef]
13. El Achkar, T.; Greige-Gerges, H.; Fourmentin, S. Basics and Properties of Deep Eutectic Solvents: A Review. *Environ. Chem. Lett.* **2021**, *19*, 3397–3408. [CrossRef]
14. Zhang, Q.; De Oliveira Vigier, K.; Royer, S.; Jérôme, F. Deep Eutectic Solvents: Syntheses, Properties and Applications. *Chem. Soc. Rev.* **2012**, *41*, 7108. [CrossRef] [PubMed]
15. Guo, N.; Ping-Kou; Jiang, Y.-W.; Wang, L.-T.; Niu, L.-J.; Liu, Z.-M.; Fu, Y.-J. Natural Deep Eutectic Solvents Couple with Integrative Extraction Technique as an Effective Approach for Mulberry Anthocyanin Extraction. *Food Chem.* **2019**, *296*, 78–85. [CrossRef] [PubMed]
16. Gómez-Urios, C.; Viñas-Ospino, A.; Puchades-Colera, P.; López-Malo, D.; Frígola, A.; Esteve, M.J.; Blesa, J. Sustainable Development and Storage Stability of Orange By-Products Extract Using Natural Deep Eutectic Solvents. *Foods* **2022**, *11*, 2457. [CrossRef]
17. Dai, Y.; Witkamp, G.-J.; Verpoorte, R.; Choi, Y.H. Tailoring Properties of Natural Deep Eutectic Solvents with Water to Facilitate Their Applications. *Food Chem.* **2015**, *187*, 14–19. [CrossRef]
18. Van Osch, D.J.G.P.; Zubeir, L.F.; Van Den Bruinhorst, A.; Rocha, M.A.A.; Kroon, M.C. Hydrophobic Deep Eutectic Solvents as Water-Immiscible Extractants. *Green Chem.* **2015**, *17*, 4518–4521. [CrossRef]
19. Florindo, C.; Branco, L.C.; Marrucho, I.M. Development of Hydrophobic Deep Eutectic Solvents for Extraction of Pesticides from Aqueous Environments. *Fluid Phase Equilibria* **2017**, *448*, 135–142. [CrossRef]
20. Elgharbawy, A.; Syed Putra, S.; Khan, H.; Azmi, N.; Sani, M.; Ab Llah, N.; Hayyan, A.; Jewaratnam, J.; Basirun, W. Menthol and Fatty Acid-Based Hydrophobic Deep Eutectic Solvents as Media for Enzyme Activation. *Processes* **2023**, *11*, 547. [CrossRef]
21. Boldrini, C.L.; Manfredi, N.; Perna, F.M.; Capriati, V.; Abbotto, A. Designing Eco-Sustainable Dye-Sensitized Solar Cells by the Use of a Menthol-Based Hydrophobic Eutectic Solvent as an Effective Electrolyte Medium. *Chem. Eur. J.* **2018**, *24*, 17656–17659. [CrossRef]
22. Ozturk, B.; Parkinson, C.; Gonzalez-Miquel, M. Extraction of Polyphenolic Antioxidants from Orange Peel Waste Using Deep Eutectic Solvents. *Sep. Purif. Technol.* **2018**, *206*, 1–13. [CrossRef]
23. Bajkacz, S.; Adamek, J. Development of a Method Based on Natural Deep Eutectic Solvents for Extraction of Flavonoids from Food Samples. *Food Anal. Methods* **2018**, *11*, 1330–1344. [CrossRef]
24. Gómez-Urios, C.; Viñas-Ospino, A.; Puchades-Colera, P.; Blesa, J.; López-Malo, D.; Frígola, A.; Esteve, M.J. Choline Chloride-Based Natural Deep Eutectic Solvents for the Extraction and Stability of Phenolic Compounds, Ascorbic Acid, and Antioxidant Capacity from Citrus Sinensis Peel. *LWT—Food Sci. Technol.* **2023**, *177*, 114595. [CrossRef]
25. Tejero, A.; Martín, M.E.; López-Malo, D.; Esteve, M.J.; Frigola, A.; Blesa, J. Assessment of the Use of a Selection of Natural Deep Eutectic Solvents in the Extraction of Polar Bioactive Compounds from Orange Peel. *Biol. Life Sci. Forum* **2021**, *6*, 14. [CrossRef]
26. Viñas-Ospino, A.; Panić, M.; Radojčić- Redovniković, I.; Blesa, J.; Esteve, M.J. Using Novel Hydrophobic Deep Eutectic Solvents to Improve a Sustainable Carotenoid Extraction from Orange Peels. *Food Biosci.* **2023**, *53*, 102570. [CrossRef]

27. Stupar, A.; Šeregelj, V.; Ribeiro, B.D.; Pezo, L.; Cvetanović, A.; Mišan, A.; Marrucho, I. Recovery of β-Carotene from Pumpkin Using Switchable Natural Deep Eutectic Solvents. *Ultrason. Sonochem.* **2021**, *76*, 105638. [CrossRef]
28. Khuri, A.I.; Mukhopadhyay, S. Response Surface Methodology. *Wiley Interdiscip. Rev. Comput. Stat.* **2010**, *2*, 128–149. [CrossRef]
29. Dean, A.; Voss, D.; Draguljić, D. *Design and Analysis of Experiments*; Springer Texts in Statistics; Springer International Publishing: Cham, Switzerland, 2017. [CrossRef]
30. Rodriguez-Amaya, D.B. *A Guide to Carotenoid Analysis in Foods*; ILSI Press: Washington, DC, USA, 2001; ISBN 978-1-57881-072-7.
31. Li, H.; Zhao, C.; Tian, H.; Yang, Y.; Li, W. Liquid–Liquid Microextraction Based on Acid–Base-Induced Deep Eutectic Solvents for Determination of β-Carotene and Lycopene in Fruit Juices. *Food Anal. Methods* **2019**, *12*, 2777–2784. [CrossRef]
32. Biswas, A.K.; Sahoo, J.; Chatli, M.K. A Simple UV-Vis Spectrophotometric Method for Determination of β-Carotene Content in Raw Carrot, Sweet Potato and Supplemented Chicken Meat Nuggets. *LWT—Food Sci. Technol.* **2011**, *44*, 1809–1813. [CrossRef]
33. Karvela, E.; Makris, D.P.; Karathanos, V.T. Implementation of Response Surface Methodology to Assess the Antiradical Behaviour in Mixtures of Ascorbic Acid and α-Tocopherol with Grape (Vitis Vinifera) Stem Extracts. *Food Chem.* **2012**, *132*, 351–359. [CrossRef] [PubMed]
34. Paleologou, I.; Vasiliou, A.; Grigorakis, S.; Makris, D.P. Optimisation of a Green Ultrasound-Assisted Extraction Process for Potato Peel (Solanum Tuberosum) Polyphenols Using Bio-Solvents and Response Surface Methodology. *Biomass Convers. Biorefinery* **2016**, *6*, 289–299. [CrossRef]
35. Robertson, A.R. The CIE 1976 Color-Difference Formulae. *Color Res. Appl.* **1977**, *2*, 7–11. [CrossRef]
36. Martínez-Pérez, M.P.; Bautista-Ortín, A.B.; Pérez-Porras, P.; Jurado, R.; Gómez-Plaza, E. A New Approach to the Reduction of Alcohol Content in Red Wines: The Use of High-Power Ultrasounds. *Foods* **2020**, *9*, 726. [CrossRef] [PubMed]
37. Ribeiro, B.D.; Florindo, C.; Iff, L.C.; Coelho, M.A.Z.; Marrucho, I.M. Menthol-Based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents. *ACS Sustain. Chem. Eng.* **2015**, *3*, 2469–2477. [CrossRef]
38. Florindo, C.; Romero, L.; Rintoul, I.; Branco, L.C.; Marrucho, I.M. From Phase Change Materials to Green Solvents: Hydrophobic Low Viscous Fatty Acid–Based Deep Eutectic Solvents. *ACS Sustain. Chem. Eng.* **2018**, *6*, 3888–3895. [CrossRef]
39. Verma, R.; Banerjee, T. Liquid–Liquid Extraction of Lower Alcohols Using Menthol-Based Hydrophobic Deep Eutectic Solvent: Experiments and COSMO-SAC Predictions. *Ind. Eng. Chem. Res.* **2018**, *57*, 3371–3381. [CrossRef]
40. Rodrigo, M.-J.; Marcos, J.F.; Zacarías, L. Biochemical and Molecular Analysis of Carotenoid Biosynthesis in Flavedo of Orange (*Citrus sinensis* L.) during Fruit Development and Maturation. *J. Agric. Food Chem.* **2004**, *52*, 6724–6731. [CrossRef]
41. Saini, R.K.; Keum, Y.-S. Carotenoid Extraction Methods: A Review of Recent Developments. *Food Chem.* **2018**, *240*, 90–103. [CrossRef]
42. Martins, M.A.R.; Crespo, E.A.; Pontes, P.V.A.; Silva, L.P.; Bülow, M.; Maximo, G.J.; Batista, E.A.C.; Held, C.; Pinho, S.P.; Coutinho, J.A.P. Tunable Hydrophobic Eutectic Solvents Based on Terpenes and Monocarboxylic Acids. *ACS Sustain. Chem. Eng.* **2018**, *6*, 8836–8846. [CrossRef]
43. Li, K.; Jin, Y.; Jung, D.; Park, K.; Kim, H.; Lee, J. In Situ Formation of Thymol-Based Hydrophobic Deep Eutectic Solvents: Application to Antibiotics Analysis in Surface Water Based on Liquid-Liquid Microextraction Followed by Liquid Chromatography. *J. Chromatogr. A* **2020**, *1614*, 460730. [CrossRef] [PubMed]
44. Yara-Varón, E.; Fabiano-Tixier, A.S.; Balcells, M.; Canela-Garayoa, R.; Bily, A.; Chemat, F. Is It Possible to Substitute Hexane with Green Solvents for Extraction of Carotenoids? A Theoretical versus Experimental Solubility Study. *RSC Adv.* **2016**, *6*, 27750–27759. [CrossRef]
45. Kyriakoudi, A.; Tsiouras, A.; Mourtzinos, I. Extraction of Lycopene from Tomato Using Hydrophobic Natural Deep Eutectic Solvents Based on Terpenes and Fatty Acids. *Foods* **2022**, *11*, 2645. [CrossRef] [PubMed]
46. Fan, C.; Liu, Y.; Shan, Y.; Cao, X. A Priori Design of New Natural Deep Eutectic Solvent for Lutein Recovery from Microalgae. *Food Chem.* **2022**, *376*, 131930. [CrossRef] [PubMed]
47. Silva, Y.P.A.; Ferreira, T.A.P.C.; Jiao, G.; Brooks, M.S. Sustainable Approach for Lycopene Extraction from Tomato Processing By-Product Using Hydrophobic Eutectic Solvents. *J. Food Sci. Technol.* **2019**, *56*, 1649–1654. [CrossRef]
48. Lee, S.Y.; Liang, Y.N.; Stuckey, D.C.; Hu, X. Single-Step Extraction of Bioactive Compounds from Cruciferous Vegetable (Kale) Waste Using Natural Deep Eutectic Solvents. *Sep. Purif. Technol.* **2023**, *317*, 123677. [CrossRef]
49. Yaqoob, S.; Riaz, M.; Shabbir, A.; Zia-Ul-Haq, M.; Alwakeel, S.S.; Bin-Jumah, M. Commercialization and Marketing Potential of Carotenoids. In *Carotenoids: Structure and Function in the Human Body*; Zia-Ul-Haq, M., Dewanjee, S., Riaz, M., Eds.; Springer International Publishing: Cham, Switzerland, 2021; pp. 799–826. [CrossRef]
50. Grewal, J.; Wołącewicz, M.; Pyter, W.; Joshi, N.; Drewniak, L.; Pranaw, K. Colorful Treasure From Agro-Industrial Wastes: A Sustainable Chassis for Microbial Pigment Production. *Front. Microbiol.* **2022**, *13*, 832918. [CrossRef]
51. Alsaud, N.; Shahbaz, K.; Farid, M. Antioxidant and Antibacterial Evaluation of Manuka Leaves (*Leptospermum scoparium*) Extracted by Hydrophobic Deep Eutectic Solvent. *Chem. Eng. Res. Des.* **2021**, *174*, 96–106. [CrossRef]
52. Durand, E.; Lecomte, J.; Upasani, R.; Chabi, B.; Bayrasy, C.; Baréa, B.; Jublanc, E.; Clarke, M.J.; Moore, D.J.; Crowther, J.; et al. Evaluation of the ROS Inhibiting Activity and Mitochondrial Targeting of Phenolic Compounds in Fibroblast Cells Model System and Enhancement of Efficiency by Natural Deep Eutectic Solvent (NADES) Formulation. *Pharm. Res.* **2017**, *34*, 1134–1146. [CrossRef]
53. Li, Y.; Hu, K.; Huang, C.; Hu, Y.; Ji, H.; Liu, S.; Gao, J. Improvement of Solubility, Stability and Antioxidant Activity of Carotenoids Using Deep Eutectic Solvent-Based Microemulsions. *Colloids Surf. B Biointerfaces* **2022**, *217*, 112591. [CrossRef]

54. Lee, S.-J.; Umano, K.; Shibamoto, T.; Lee, K.-G. Identification of Volatile Components in Basil (*Ocimum basilicum* L.) and Thyme Leaves (*Thymus vulgaris* L.) and Their Antioxidant Properties. *Food Chem.* **2005**, *91*, 131–137. [CrossRef]
55. Youdim, K.A.; Deans, S.G.; Finlayson, H.J. The Antioxidant Properties of Thyme (*Thymus zygis* L.) Essential Oil: An Inhibitor of Lipid Peroxidation and a Free Radical Scavenger. *J. Essent. Oil Res.* **2002**, *14*, 210–215. [CrossRef]
56. Aeschbach, R.; Löliger, J.; Scott, B.C.; Murcia, A.; Butler, J.; Halliwell, B.; Aruoma, O.I. Antioxidant Actions of Thymol, Carvacrol, 6-Gingerol, Zingerone and Hydroxytyrosol. *Food Chem. Toxicol.* **1994**, *32*, 31–36. [CrossRef] [PubMed]
57. Lagouri, V.; Boskou, D. Screening for Antioxidant Activity of Essential Oils Obtained from Spices. In *Developments in Food Science*; Elsevier: Amsterdam, The Netherlands, 1995; Volume 37, pp. 869–879. [CrossRef]
58. Kishimoto, S.; Sumitomo, K.; Yagi, M.; Nakayama, M.; Ohmiya, A. Three Routes to Orange Petal Color via Carotenoid Components in 9 Compositae Species. *J. Jpn. Soc. Hortic. Sci.* **2007**, *76*, 250–257. [CrossRef]