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Natural Deep Eutectic Solvents as Main Solvent for the Extraction of Total Polyphenols of Orange Peel †

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Abstract: Normally, for the extraction of bioactive compounds organic solvents are used, because of that this research is focused on the extraction with Natural deep eutectic solvents (NADES) which are natural solvents with a low melting point. In this work, we seek to optimize the extraction process of total polyphenols from orange peel using 4 types of NADES with different water concentrations, different solid/liquid ratios, and different extraction times. Results shown the best percentage of NADES were 10, 30 and 50%, solvent/liquid ratio differs depending on the compound, and the optimal extraction time is generally estimated to be 30 min.

Keywords: natural deep eutectic solvents; orange peel; total polyphenol content

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1. Introduction

Every year food industry produces a great amount of waste, mainly in citric industry, where 18% of the citric fields are made for industrial processes, large amounts of waste is generated as a result, this waste has a high biological value, so it can be used for functional food. Plant-based foods contain large amounts of bioactive, phytochemical or phytonutrient compounds, and they are classified in four groups: nitrogenous substances, sulphurated substances, terpenes and phenolic compounds [1].

The concept of green chemistry and green economy have increased in the recent years, conventionally the use of organic solvents, such as methanol or dichloromethane, and non-green extraction methods for the extraction of bioactive compounds from plants and vegetables have generated a big amount of non-ecofriendly solvents residues and high energy use [2].

Recently, various alternatives to organic solvents are being used for the extraction of bioactive compounds, such as supercritical fluids (SCF), ionic liquids (IL) or deep eutectic solvents (DES). Natural deep eutectic solvents (NADES) are DES composed of small natural molecules such as sugars, organic acids, or amino acids, among others. Their physiochemistry properties given by their molecular interactions, hydrogen bonds, such as their low fusion point and high viscosity make them an alternative to organic solvents [3].

Orange peel is a by-product of the agri-food industry which have a high biological value, since it is the part of the fruit that contains significant amounts bioactive compounds that act by delaying oxidation, in addition to avoiding the presence of insects, and protecting the fruit from possible mechanical damage [4].

The aim of this study is to optimize the extraction procedure for the total polyphenol content (TPC) with NADES.

2. Materials and Methods

2.1. Raw Material

Orange peels were obtain from Navel variety, purchased at a local market. The peels were removed from the pulp and crushed with a blender and used immediately

2.2. Chemical and Reagents

Choline chloride (≥98%), D-(−)-fructose (≥99%), Folin-Ciocalteau reagent, were purchased from Sigma-Aldrich (Steinheim, Germany). Glycerol from Glentham Life Science (Corsham, UK). DL-malic acid (≥98%) from Thermo Fisher (Kendel, Germany). Citric acid (≥99,9%), anhydrous sodium carbonate (Na_2CO_3) was purchased from VWR Chemicals (Leuven, Belgium). L-Proline from Guinama (Spain). Betaine from Fluorochem (Hadfield, UK).

2.3. Preparation of Natural Deep Eutectic Solvents

NADES were prepared following the method described by Dai et al. (2015) [5], with modifications. They were prepared by mixing the reagent in different molar ratio (according to their molar mass) and heated and stirred at 60–80 °C in a water bath until a liquid was formed.

Four combinations of NADES were made, where Choline Chloride (ChChl), Betaine (Bet) and the amino acid L-Proline (LP) acted as Hydrogen Bond Acceptors (HBA) and the sugar Fructose (Fruc), two organic acids as Malic Acid (MA) and Citric Acid (CA), and the polyalcohol Glycerol (Gly) acted as Hydrogen Bond Donor (HBD) (Table 1). They were mixed with different amount of water (NADES in water 10%, 20%, 30%, 40%, 50%, 75% and 85%) to reduce the viscosity.

Table 1. Materials and molar ratios of the NADES.

Acronym	HBA	HBD	Molar Ratio
ChChl:Fruc	Choline Chloride	Fructose	1.9:1
ChChl:Gly	Choline Chloride	Glycerol	1:2
Bet:CA	Betaine	Citric Acid	1:1
LP:MA	L-Proline	Malic Acid	1:1

HBA: Hydrogen Bond Acceptor. HBD: Hydrogen Bond Donor.

2.4. Extraction Procedure

Bioactive compounds were determinated in NADES extract from orange peel, taking care of the concentration (ratio solid/liquid), kind of NADES and percentage of water, and the extraction time. It was carried out by stirring and heated to 45 ± 5 °C. After the extraction, the samples were centrifuged (Eppendorf, Germany) at 5 °C, 3000 rpm and 10 min. The supernatant was stored and used for the determination of TPC. The results were optimized using the Response Surface Methodology (RSM), to determine the best condition (Table 2).

Table 2. Coded levels of independent variables.

Independent Variable		Level		
		−1	0	+1
Liquid/solid ratio	X_1	5	15	25
NADES (%, *v*/*v*)	X_2	10	50	85
Extraction time	X_3	5	15	30

Independent variables of study.

2.5. Determination of Total Polyphenol Content by UV-Vis Spectroscopy

Determination of TPC was carried out using the method described by Singleton y Rossi (1965) [6]. 100 µL of the sample was mixed with 3 mL of sodium carbonate (2%, *w*/*v*) and 100 µL of Folin-Ciocalteu reagent (1:1, *v*/*v*). Gallic acid calibration curve was done under the same conditions as samples. Once the reaction was over (1 h), the absorbance was measured at 765 nm (Perkin Elmer ®, Boston, MA, USA). TPC was done in milligrams of gallic acid equivalent (GAE) per 100 g of dry weight of orange peel.

2.6. Statistics

All measures were repeated three times. The optimization results were generated with Design-Expert 8.0 for Windows® (Stat-Ease, Minneapolis, MN, USA). Mean differences were analyzed by one-way analysis of variance (ANOVA) to compare two or more values to check if the differences were statistically significative**.** After this test, Tukey's test was made using the statistic programe SPSS® 26.0 for Windows® (SPSS, Chicago, IL, USA).

3. Results and Discussion

For the optimization of the extraction of TPC a Box-Behnken design was done as it shows Table 3, the optimization model selected one of NADES and the extraction yields for these NADES. ANOVA showed *p* values < 0.0001, indicating that the model selected was highly significant.

Table 3. Box-Behnken design with the independent variables and responses data.

Runs	Extraction			Total Polyphenol Content (mg GAE/100 g DW)			
	X_1	X_2	X_3	LP:MA	ChChl:Fruc	ChChl:Gly	Bet:CA
1	15	50	10	2804.7 ± 146.6	1045.4 ± 73.6	296.3 ± 22.6	1767.4 ± 221.5
2	25	10	15	2341.5 ± 149.3	1688.0 ± 107.0	512.3 ± 3.3	1711.7 ± 117.5
3	5	50	5	2029.4 ± 57.5	4128.3 ± 109.0	535.2 ± 32.5	1445.9 ± 47.8
4	15	30	15	3582.8 ± 506.3	1503.2 ± 32.2	611.4 ± 13.8	1889.4 ± 181.9
5	5	20	20	ND	3337.5 ± 339.4	1706.1 ± 125.7	ND
6	25	30	5	2569.1 ± 59.4	913.8 ± 76.6	177.4 ± 15.7	1254.7 ± 136.6
7	10	50	30	3712.2 ± 1222.6	7577.3 ± 385.1	596.1 ± 16.3	3004.4 ± 238.1
8	15	30	15	3079.6 ± 221.3	1482.8 ± 80.0	609.7 ± 23.7	1852.6 ± 87.2
9	15	30	15	4334.1 ± 441.5	1360.8 ± 37.6	649.9 ± 53.1	1698.9 ± 52.9
10	15	10	30	2130–6 ± 43.3	3220.8 ± 245.0	1088.2 ± 20.2	1630.3 ± 47.9
11	25	30	5	2645.8 ± 50.3	778.9 ± 24.1	190.2 ± 6.6	1194.1 ± 183.9
12	25	10	30	2598.8 ± 133.6	3241.5 ± 40.3	631.4 ± 15.1	1713.4 ± 243.7
13	5	20	20	ND	3637.5 ± 118.2	1717.0 ± 56.4	ND
14	10	10	5	2187.8 ± 92.8	2744.4 ± 768.2	927.2 ± 9.5	1317.2 ± 76.7
15	5	40	20	ND	1480.6 ± 72.4	266.1 ± 13.0	ND
16	25	30	30	4121.5 ± 231.1	515.7 ± 10.3	285.2 ± 16.9	1657.4 ± 93.0
17	25	50	15	1161.1 ± 11.2	354.9 ± 7.8	214.4 ± 10.0	694.1 ± 70.4
18	20	10	5	2134.5 ± 186.4	729.9 ± 11.1	438.8 ± 14.3	1318.4 ± 9.7
19	15	30	15	4706.2 ± 592.9	1773.3 ± 32.1	594.4 ± 15.3	1742.2 ± 142.3
20	5	30	5	486.7 ± 14.7	2563.9 ± 10.8	944.7 ± 36.6	1455.4 ± 63.0
21	15	75	10	3208.8 ± 573.9	55.7 ± 1.2	27.9 ± 1.2	1458.3 ± 194.0
22	5	75	5	1754.8 ± 79.0	28.2 ± 1.8	16.5 ± 1.4	1090.2 ± 53.2
23	10	75	15	3104.5 ± 68.7	114.4 ± 14.4	87.2 ± 109.1	1707.0 ± 99.4
24	25	75	15	2924.7 ± 214.1	158.9 ± 7.9	35.1 ± 3.0	1199.4 ± 73.9
25	15	85	10	3643.2 ± 279.9	103.8 ± 12.8	37.8 ± 10.0	ND
26	5	85	5	1581.4 ± 87.1	41.3 ± 4.9	216.1 ± 14.3	ND
27	10	85	30	3232.1 ± 308.2	68.8 ± 13.3	212.2 ± 18.5	ND
28	25	85	15	3895.8 ± 213.0	188.6 ± 34.8	115.8 ± 3.3	ND

X_1, solid-liquid ratio; X_2, %NADES; X_3, extraction time; ND: Not detected; LP:MA, L-Proline:Malic Acid; ChChl:Fruc, Choline Chloride:Glycerol ; ChChl:Gly, Choline Chloride:Glycerol; Bet:CA, Betaine:Citric Acid.

3.1. NADES Mixed with Water

In order to decrease the viscosity, each NADES was mixed with different amoung of water. Dai et al. (2015) [5] observed that > 50% of water, hydrogen bonds may break giving lower extraction, in addition NADES mixed with more than 50% of water are considered aquos solutions and not eutectic mixtures [6]. In this study, for two of the NADES studied, the best extraction efficiency was with an aquos solution (ChChl:Gly 10%, LP:MA 30%) (Figure 1), observing the same in the results given by Mouratoglou et al. (2016) [7], studying the extraction of polyphenols with 90% aqueos NADES. ChChl:Fruc and Bet:CA show the best extraction at 50% with water.

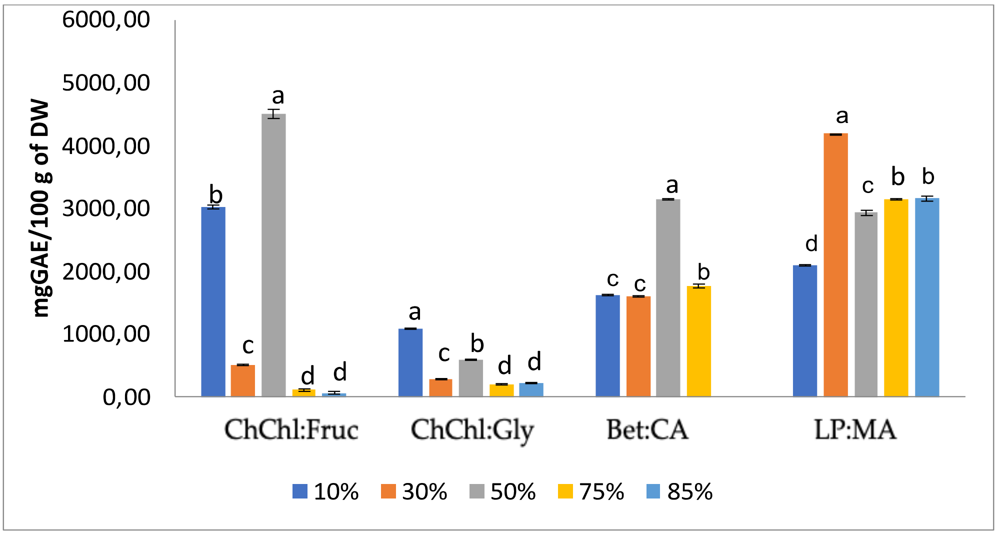

Figure 1. Total phenolic content in NADES extracts with different amount of water. ChChl:Fruc Choline Chloride fructose. ChChl:Gly Choline Chloride Glycerol. Bet:CA Betaine Citric Acid. LP:MA L-Proline Malic Acid. a–e: different letters indicate that there are statistically significant differences ($p < 0.05$).

3.2. Optimization

Optimum conditions for the extración of TPC with each one of the NADES studied is shown in Table 4.

Table 4. Optimum conditions of extraction.

Acronym	Ratio	Extraction Time	Max	Desirability
ChChl:Fruc	5.000	30.000	6530.839	0.830
ChChl:Gly	5.234	23.339	1833.512	1.000
Bet:CA	6.000	28.458	3218.766	1.000
LP:MA	16.689	29.041	5389.107	1.000

4. Conclusions

The extraction of total polyphenols content with NADES was viable. The best percentage of NADES in water was differents for two of the NADES, ChChl:Gly and LP:MA obtained the higher extraction with a high content in water, considering these solvents aqueos solutions, moreover ChChl:Fruc and Bet:CA, both showed the best extraction at 50% (eutectic mixture).

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

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

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