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Green extraction using deep eutectic solvents of flavonoids from orange peels

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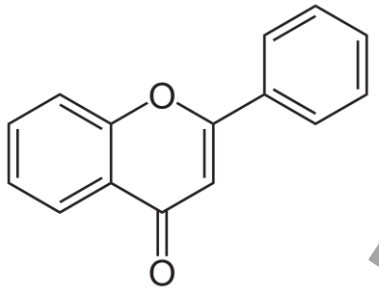
INTRODUCTION

3 million tonnes per year of orange waste

-Fermentable sugars
-Carbohydrate polymers
-Flavonoids
-Polyphenols
-Vitamins
-Essential oils
-Carotenoids



Flavonoids

Traditional extraction

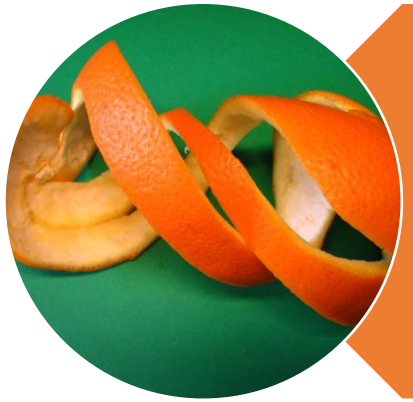
- Organic solvents
- Pollution
- Toxic effects

Green extraction

Natural Deep eutectic solvents (NADES)

The aim of this study was optimized and compare four different NaDEs for the extraction of flavonoids in orange peels (Naveline cultivar) from Valencia-Spain.

MATERIAL AND METHODS



Raw material: Navel cultivar oranges purchased at a local supermarket (Valencia, Spain)

Deep eutectic solvents: Four different NADESs systems with two components were obtained in specific ratios with 10, 30, 50, 75, 85% of NADES in water (w/w).NADES-1: Choline chloride: Fructose (1.9:1), NADES-2: Choline chloride: Glycerol (1:2), NADES-3: Proline: Malic acid (1:1), NADES-4: Betaine:Citric acid (1:1).

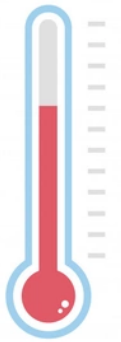
MATERIAL AND METHODS

Total flavonoid content:

Orange peels

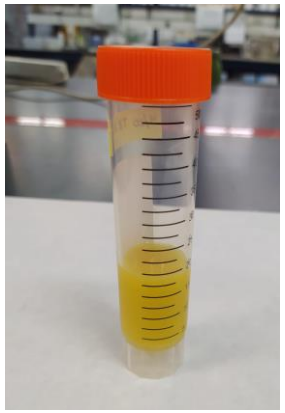
NADES

1:10g/mL for 30 min.



45°C

5° C, 3000 rpm for 10 minutes.



100µl of the sample
48µl of sodium nitrite
48µl of aluminum chloride
320µl of sodium hydroxide

Measured at 510 nm

MATERIAL AND METHODS

Experimental design

- Optimization parameters of the optimum NADES were examined using Response surface methodology (RSM) (Design Expert Software 11.0). A Box-Behnken design was performed with three independent variables of X1, (liquid–solid ratio), X2 (% NADEs in water), X3 (extraction time).

Table 1. 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

RESULTS AND DISCUSSION

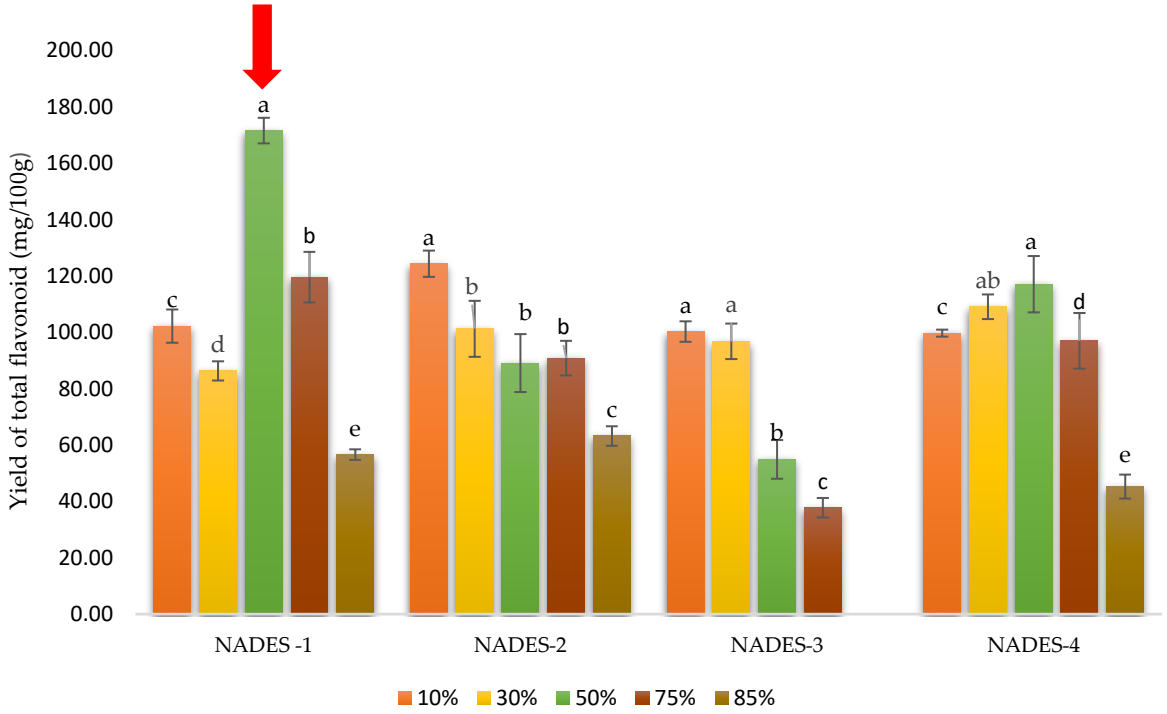


Figure 1. Total flavonoid extraction yields for deep eutectic solvents (NADES-1 to NADES-4) according to the % of NADES. a-b: in the same row, different letters indicate that there are statistically significant differences ($p < 0.05$).

NADES-1 (Choline chloride/fructose) was found to be the most effective NADES for extraction flavonoids from orange peels.

Table 1. Box-Behnken design with the independent variables and responses data

Run	Extraction conditions			Extraction yield TFC
	X_1	X_2	X_3	
1	15	50	10	51.43 ± 3.48
2	25	10	15	224.37 ± 2.34
3	5	50	5	37.27 ± 1.86
4	15	30	15	147.02 ± 1.16
5	5	20	20	23.57 ± 2.96
6	25	30	5	60.54 ± 3.95
7	10	50	30	103.53 ± 1.73
8	15	30	15	75.16 ± 3.59
9	15	30	15	150.18 ± 3.47
10	15	10	30	102.25 ± 5.86
11	25	30	5	79.00 ± 1.76
12	25	10	30	114.51 ± 5.75
13	5	20	20	26.14 ± 3.23
14	10	10	5	140.18 ± 5.33
15	5	40	20	59.81 ± 2.35
16	25	30	30	86.35 ± 3.37
17	25	50	15	81.84 ± 2.5
18	20	10	5	92.88 ± 5.29
19	15	30	15	95.74 ± 4.35
20	5	30	5	80.40 ± 2.48
21	15	75	10	429.81 ± 1.74
22	5	75	5	316.10 ± 10.42
23	10	75	15	516.83 ± 2.85
24	25	75	15	499.21 ± 2.79
25	15	85	10	28.95 ± 1.84
26	5	85	5	30.04 ± 1.27
27	10	85	15	56.66 ± 1.90
28	25	85	15	41.30 ± 2.51

RESULTS AND DISCUSSION

Table 2. ANOVA for response surface polynomial model of all independent variables.

Source	TFC[a]				
	Sum of Squares	df	Mean Square	F-value	p-Value
Model	3.87	9	43011.77	4.00	0.0060**
X_1	5763.58	1	5763.58	0.53	0.4735^{ns}
X_2	54931.89	1	54931.89	5.11	0.0364*
X_3	113.64	1	113.64	0.01	0.9193^{ns}
$X_1 X_2$	42.98	1	42.98	0.01	0.9503^{ns}
$X_1 X_3$	2339.63	1	2339.63	0.21	0.6465^{ns}
$X_2 X_3$	1088.49	1	1088.49	0.10	0.7540^{ns}
X_1^2	4240.27	1	4240.27	0.39	0.5379^{ns}
X_2^2	2666.07	1	2666.07	0.24	0.6246^{ns}
X_3^2	6922.22	1	6922.22	0.64	0.4328^{ns}
Residual	1.93	18	10753.36		
Lack of Fit	1.89	13	14552.39	16.61	0.0030**
Pure Error	4379.41	5	875.88		
Cor Total	5.80	27			

X_1, X_2 and X_3 represent liquid-solid ratio, % NADES in water and extraction time, respectively; df represents degree of freedom. Level of significance: **Significant at $P<0.01$, *Significant at $P<0.05$, ns Not significant al $P>0.05$.

[a]TFC: Total flavonoids content from orange peels.

The optimum extraction conditions obtained from the software analysis were 50% of NADEs, solid: liquid ratio of 1:25 and extraction time of 23 min.

CONCLUSSION

- The results demonstrate that the % of NADES in water has a significant effect in the extraction of total flavonoids in oranges peels. Our results showed that extraction using natural deep eutectic could be efficient and an ecofriendly alternative to extract flavonoids from orange peels. The variables studied had significant effects on measured responses.

ACKNOWLEDGMENTS

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