EXTRACTION OF ANTIOXIDANT NON-EXTRACTABLE POLYPHENOLS FROM MANGOSTEEN PEEL USING GREEN SOLVENTS

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Introduction

Mangosteen (*Garcinia mangostana* L.) peel byproduct contains phenolic compounds with beneficial health effects.

Mainly, phenolic compounds are extracted by conventional extraction techniques. However, these techniques do not have the capacity to release phenolic compounds strongly attached to the matrix's cell wall, which are called non-extractable polyphenols (NEPs).

New sustainable solvents known as natural deep eutectic solvents (NaDES) have emerged increasing the extraction yields and protecting the degradation of bioactive compounds.

NaDES are environmentally friendly, easily synthesized, biodegradable, non-volatile, highly stable, and have a low cost.

Objectives

- To develop a sustainable analytical methodology for the recovery of antioxidant NEPs from *Garcinia mangostana* L. peels based on the combination of NaDES with ultrasound-assisted extraction (UAE) technology.
- To optimize the extraction conditions (extraction time, percentage of water on the NaDES, and ultrasound amplitude) to obtain extracts with the high content of antioxidant NEPs, using the Box-Behnken experimental design.

Materials and Methods

Mangosteen

PEEL

Extraction of extractable polyphenols (EPPs)

15 g of mangosteen peel was shaken with 20 mL of methanol/water (50:50, v/v, 1 h, 25°C). After centrifugation, the residue was re-extracted with 20 mL of acetone/water (70:30, v/v, 1 h, 25°C).

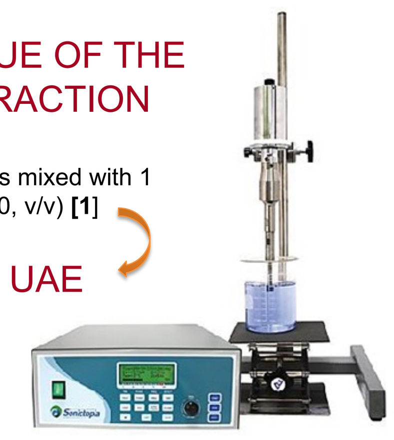
RESIDUE OF THE EXTRACTION

Extraction of NEPs

RESIDUE OF THE EXTRACTION

50 mg of extraction residue was mixed with 1 mL of NaDES:water (70:30, v/v) [1]

1 min at 30% amplitude UAE



Selection of the best NaDES and molar ratio between NaDES components to obtain NEPs

Choline chloride was used as hydrogen bond acceptor (HBA) and tested with several hydrogen bond donors (HBD) at different ratios

(HBA)	(HBD)	Abbreviation	Molar ratio
Choline chloride	Glycerol	ChCl-gly	1:2
Choline chloride	Ethylene glycol	ChCl-EG	1:2
Choline chloride	Urea	ChCl-urea	1:2
Choline chloride	Sorbitol	ChCl-sorb	1:1
Choline chloride	Lactic acid	ChCl-LA	1:2
Choline chloride	Citric acid	ChCl-CA	2:1
Choline chloride	Formic acid	ChCl-FA	1:2

Determination of the best NaDES to obtain NEPs

- **Total proanthocyanidins content**
  - DMAC and butanol/HCl assays
- **Antioxidant capacity**
  - Hydroxyl radical and ABTS

Box-Behnken design

Optimization of the extraction conditions with the selected NaDES

Factors

- Percentage of water (10, 20, 30%, v/v)
- Ultrasound amplitude (30, 45, 60%)
- Time (1, 8, 15 min)

Response variables

- **Total proanthocyanidins content**
  - DMAC and butanol/HCl assays
- **Antioxidant capacity**
  - Hydroxyl radical, ABTS, and FRAP assays

15 experimental runs

Experimental determination of total proanthocyanidins content and antioxidant capacity with the optimal extraction conditions

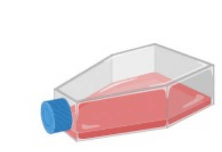
Size exclusion chromatography (SEC)

Column: PolySep-GFC-P2000 (300 × 7.8 mm).
Column temperature: 25°C.
Flow: 0.3 mL/min
Mobile phase: water
Elution gradient: isocratic **[2]**

Molecular weight determination of conventional extract and optimum extract obtained by UAE with NaDES

Citotoxicity

Effect of NEPs extract obtained by UAE with the selected NaDES on viability in HeLa cell line.



Results

Determination of the best NaDES to obtain NEPs

Table 1. Total proanthocyanidin content and total antioxidant capacity, obtained from the extraction residue of mangosteen peel by UAE with the different NaDES. a,b,c,d,e,f,g *Letters show the significant differences among the NEPs extraction with different NaDES ($p \leq 0.05$).*

NaDES	DMAC (mg epicatechin/100 g sample)	Butanol/HCl (mg epicatechin/100 g sample)	Hydroxyl radical (% of hydroxyl radical inhibition)	% scavenging of ABTS radicals
ChCl:FA	18 ± 1^{b}	$1767 \pm 197^{b,c}$	71 ± 17^{a}	41 ± 2^{d}
ChCl:LA	25.4 ± 0.6^{a}	2447 ± 237^{a}	23 ± 5^{d}	34 ± 2^{e}
ChCl:sorb	5 ± 1^{f}	759 ± 52^{d}	58 ± 10^{b}	49 ± 5^{c}
ChCl:EG	9.2 ± 0.9^{e}	623 ± 110^{e}	39 ± 8^{c}	65 ± 4^{a}
ChCl:CA	15 ± 2^{d}	1493 ± 72^{c}	--	25 ± 2^{g}
ChCl:urea	--	415 ± 57^{e}	9 ± 4^{e}	61 ± 2^{b}
ChCl:gly	16.5 ± 0.5^{c}	1954 ± 184^{b}	10 ± 4^{e}	30 ± 6^{f}

Selection of molar ratio between HBA and HBD for the best NaDES

Table 2. Total proanthocyanidin content and total antioxidant capacity, obtained from the extraction residue of mangosteen peel by UAE with the different NaDES. a,b,c,d,e *Letters show the significant differences among the NEPs extraction with different NaDES ($p \leq 0.05$).*

NaDES		DMAC (mg epicatechin/100 g sample)	Butanol/HCl (mg epicatechin/100 g sample)	Hydroxyl radical (% of hydroxyl radical inhibition)	% scavenging of ABTS radicals
ChCl:LA					
	1:1	13.7 ± 0.5^{d}	1230 ± 182^{b}	33 ± 5	13.9 ± 0.8^{d}
	1:2	25.4 ± 0.6^{a}	2447 ± 237^{a}	$24 \pm 5^{a,b}$	34 ± 2^{c}
	1:3	18.7 ± 0.6^{b}	2277 ± 386^{a}	24 ± 4^{b}	15 ± 1^{d}
ChCl:EG					
	1:2	9.2 ± 0.9^{e}	623 ± 110^{c}	39 ± 8^{a}	65 ± 4^{a}
	1:3	16.9 ± 0.5^{c}	2187 ± 436^{a}	31 ± 3^{a}	39 ± 1^{b}

Box-Behnken design with the NaDES ChCl:LA with a molar ratio between components of 1:2

Optimal extraction conditions → Water percentage: 18.8% UAE amplitude: 60.0% Extraction time: 15.0%

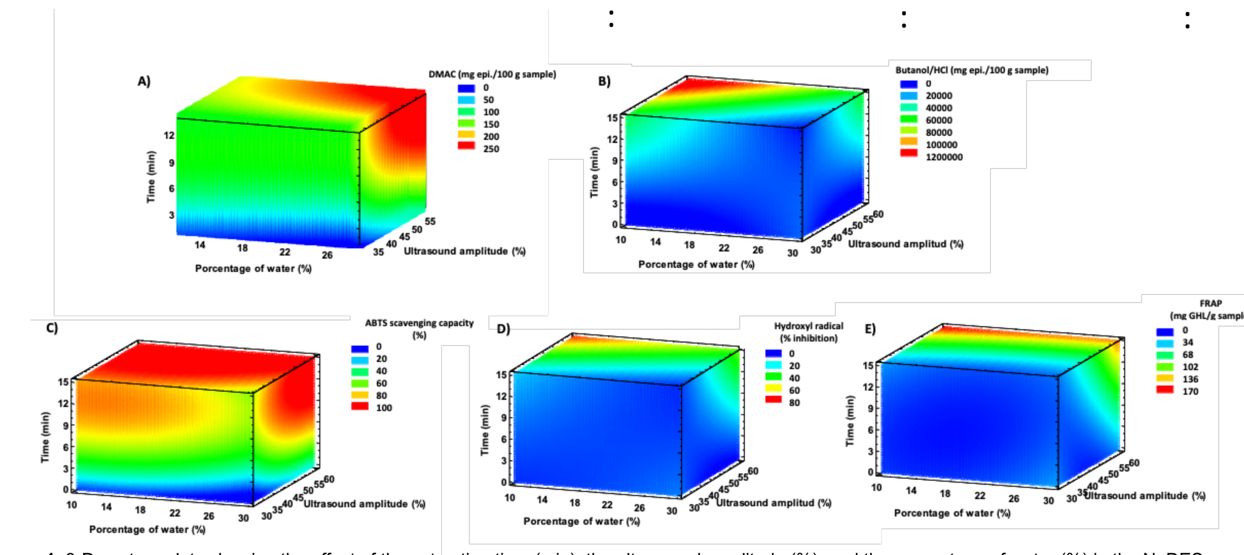
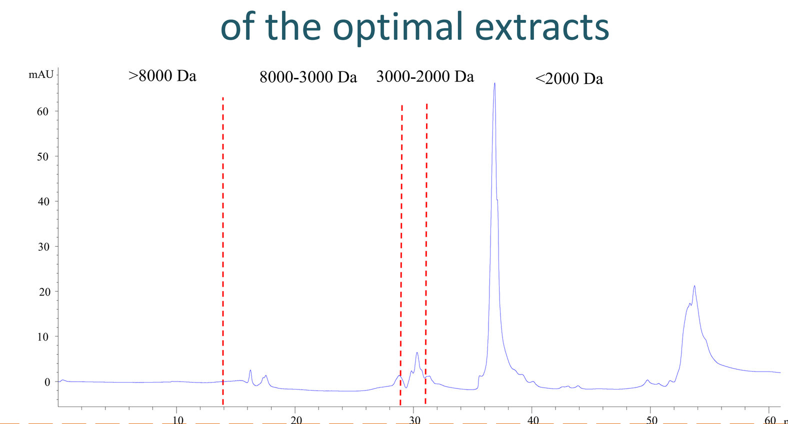


Figure 1. 3-D contour plots showing the effect of the extraction time (min), the ultrasound amplitude (%), and the percentage of water (%) in the NaDES on the total PA content (DMAC and butanol/HCl assays) and total antioxidant capacity (ABTS scanvenging capacity, capacity to inhibit the formation of hydroxyl radical, and FRAP assays) in the extracts.

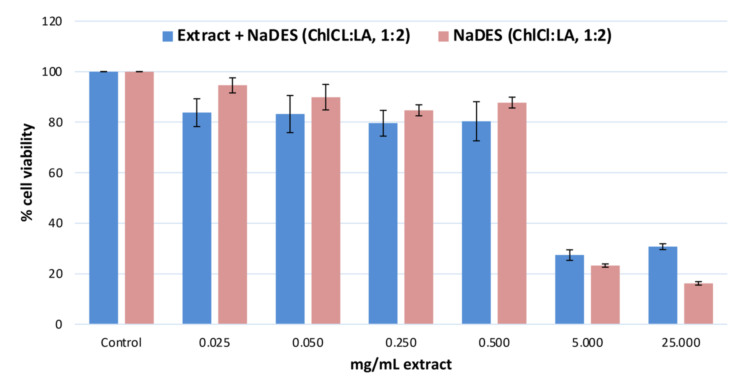
Table 3. Theoretical and experimental values of total proanthocyanidin content and total antioxidant capacity obtained under the optimal UAE conditions, as well as the results displayed of the extraction of EPPs by a conventional extraction method. a,b *Letters show significant differences between UAE with NaDES and conventional extraction.*

Response variables	Theoretical values	Experimental values	Conventional extraction
DMAC (mg epicatechin/100 g sample)	219.95	238 ± 21^{a}	2.4 ± 0.2^{b}
Butanol/HCl (mg epicatechin/100 g sample)	102200	37638 ± 6389^{a}	1017 ± 57^{b}
Scavenging capacity of ABTS radical (%)	105.57	113 ± 10^{a}	14 ± 1^{b}
Hydroxyl radical (% of hydroxyl radical inhibition)	61.14	66 ± 9^{a}	5.0 ± 0.4^{b}
FRAP (mg GSH/ g sample)	153.28	283 ± 17^{a}	1.58 ± 0.08^{b}

Size exclusion chromatography of the optimal extracts



Citotoxicity



Conclusions

- Choline chloride: lactic acid with a molar ratio of 1:2 was the NaDES selected as the best extraction solvent to release antioxidant NEPs from mangosteen peel.
- Box-Behnken experimental design showed that ultrasound amplitude and extraction time have a positive effect on the extraction of antioxidant NEPs from mangosteen peel while the percentage of water presented a negative effect.
- SEC allowed to observe that UAE with NaDES provided NEPs with high molecular weight (from 2000 to 8000 Da).
- UAE with NaDES is an efficient, nontoxic and sustainable alternative to recover NEPs from mangosteen peles.

References

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[2] Domínguez-Rodríguez, G., Marina, M.L., Plaza, M. (2021). Enzyme-assisted extraction of bioactive non-extractable polyphenols from sweet cherry (*Prunus avium* L.) pomace. *Food Chemistry.* 339, 128086.

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