



VNIVERSITAT
DE VALÈNCIA

Foods
2022



Sveučilište u
Zagrebu
University of Zagreb

Hydrophobic and hydrophilic Deep Eutectic Solvents to obtain green extracts with biological activity

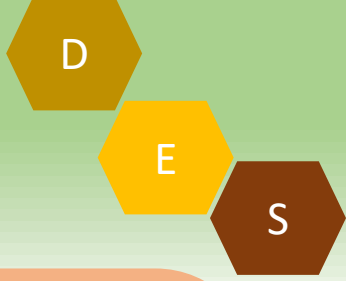
Viñas-Ospino A¹, Panić M², Blesa J¹, López-Malo D³, Frígola A¹, Radojčić-Redovniković I², Esteve MJ¹

¹Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department. Faculty of Pharmacy. University of Valencia. Avda. Vicent Andrés Estellés, s/n, 46100 Burjassot, Valencia.

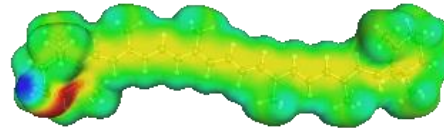
²Laboratory for Cell Culture Technology and Biotransformations, Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb. Pierotti Street 6, Zagreb, Croatia.

³Department of Nursery, European University of Valencia. Paseo de La Alameda, 7, 46010, Valencia (Valencia), Spain.

INTRODUCTION



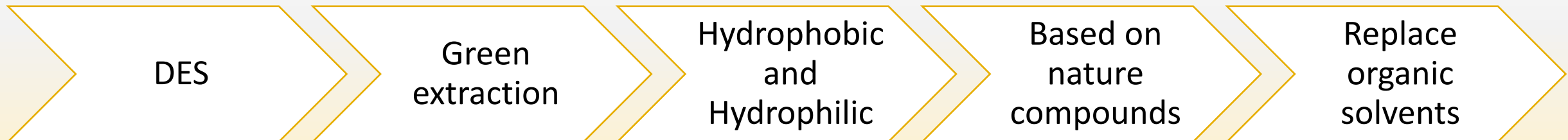
Fermentable sugars
Carbohydrate polymers
Flavonoids
Polyphenols
Vitamins
Essential oils
Carotenoids



THE AIM of the present study was to select the most promising DES for carotenoid extraction from orange peel and obtain green extracts with biological activity following the principles of green chemistry.

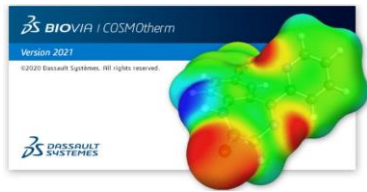
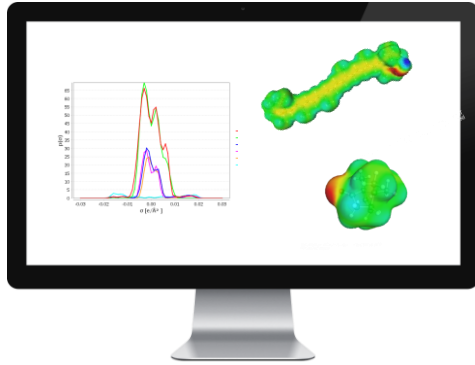


**Deep eutectic solvents
(DES)**

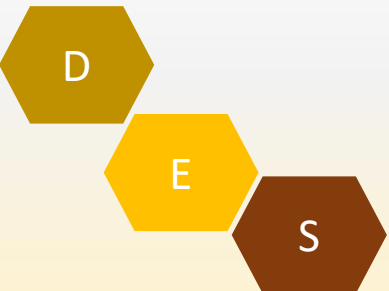


METHODOLOGY

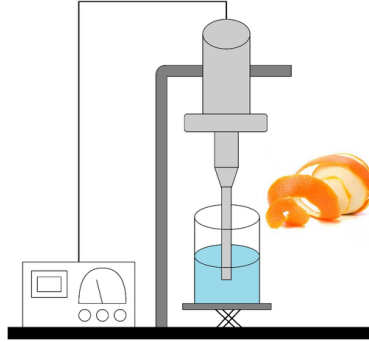
1. COSMOtherm screening



68 Hydrophobic and Hydrophilic DES



2. Experimental validation

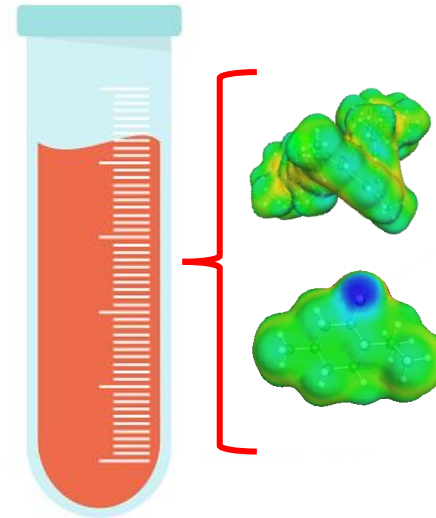


Ultrasound-assisted extraction



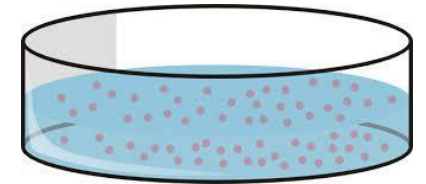
Total carotenoid content spectrophotometrically

3. Green extracts



Menthol: Camphor (Me: Cam)
Menthol: Eucalyptol (Me: Eu)
Lauric Acid: Octanoic acid (C12:C8)
Proline: Malic acid (Pro: MA)
Choline chloride: Urea (ChCl: U)

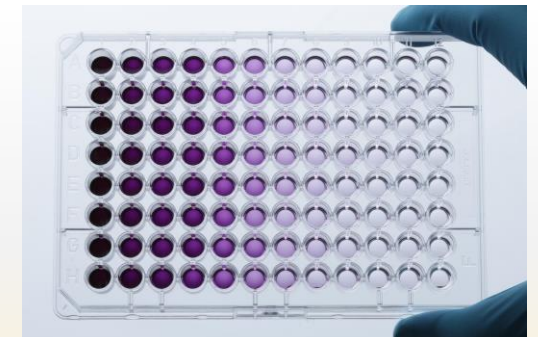
4. Biological activity



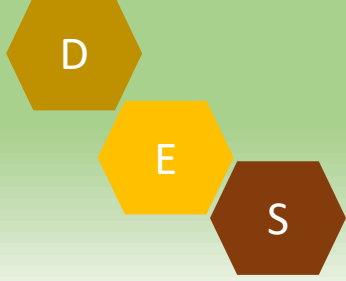
In vitro antiproliferative assay HeLa cells



MTS assay



RESULTS AND DISCUSSION



Predicted $\ln \gamma_{\text{solute}}$ for β -carotene and β -cryptoxanthin in DES using COSMO-RS.

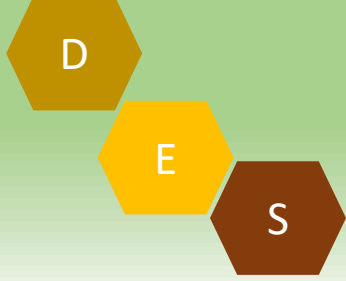
| NADES | Abb | Ratio | β -carotene $\ln \gamma_{\text{solute}}$ | β -cryptoxanthin $\ln \gamma_{\text{solute}}$ |
|------------------------------------|------------|-------|---|--|
| Hexane | Hex | 1 | -0.27 | 2.06 |
| L-Menthol: D.L- Camphor | Me: Cam | 1:1 | 0.56 | -0.59 |
| L-Menthol: Eucalyptol | Me: EU | 1:1 | 0.65 | -0.37 |
| L-Menthol: Thymol | Me: Ty | 3:2 | 1.28 | 0.07 |
| L-Menthol: Linoleic acid | Me: C18:2 | 1:1 | 1.48 | 0.19 |
| L-Menthol: Decanoic acid | Me: C10 | 1:1 | 1.81 | 0.41 |
| L-Menthol: Octanoic acid | Me: C8 | 1:1 | 1.94 | 0.03 |
| Thymol: Coumarin | Ty: Cou | 3:2 | 2.01 | 0.20 |
| L-Menthol: Peryllic acid | Me: PA | 1:1 | 2.07 | 0.40 |
| Thymol: Octanoic acid | Ty: C8 | 1:3 | 2.12 | -0.20 |
| Lauric acid: Decanoic acid | C12: C10 | 1:3 | 2.55 | 0.31 |
| Lauric acid: Octanoic acid | C12: C8 | 1:3 | 2.72 | 0.42 |
| Proline: Malic acid | Pro: Ma | 1:1 | 16.80 | 13.59 |
| Betaine: Ethylene glycol | B: EG | 1:2 | 17.74 | 14.84 |
| Choline Chloride: Lactic acid | ChChl: LA | 1:3 | 17.87 | 14.86 |
| Sorbose: Ethylene glycol | Sor: EG | 1:2 | 17.90 | 17.59 |
| Betaine: Lysine | B: Lys | 1:1 | 18.25 | 15.37 |
| Betaine: Malic acid: Proline | B: Ma: Pro | 1:1:1 | 18.53 | 15.60 |
| Fructose: Ethylene glicol | Fru: EG | 1:2 | 19.20 | 18.90 |
| Choline: Chloride: Ethylene glycol | ChChl: EG | 1:2 | 19.28 | 16.40 |
| Betaine: Sucrose | B: Suc | 4:1 | 19.55 | 16.57 |
| Water | | 1 | 36.6 | 33.554 |

HYDROPHOBIC
DES

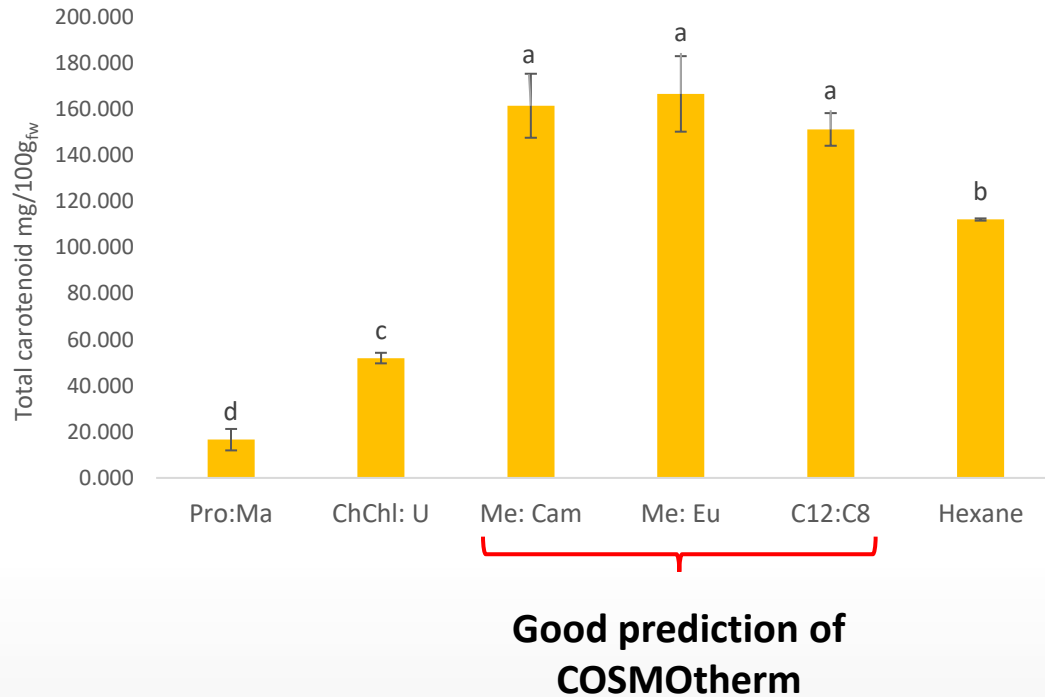
HYDROPHILLIC
DES

| | |
|--|---------------|
| | High |
| | Medium |
| | Low |

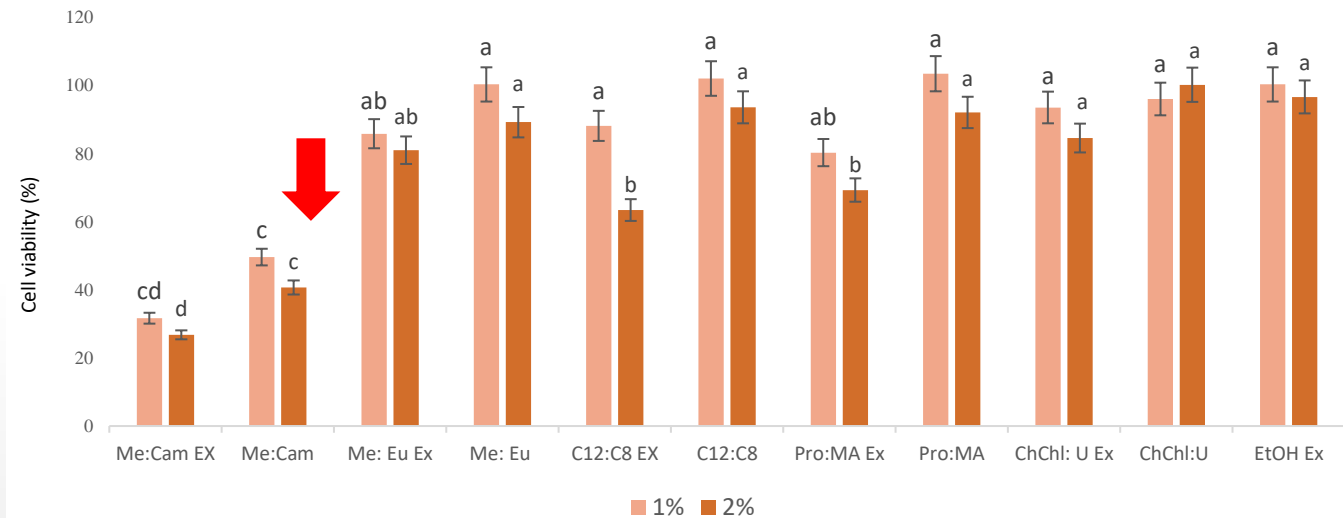
RESULTS AND DISCUSSION



Total carotenoid content (mg/100g_{fw}) in hydrophilic and hydrophobic DES selected for model validation.



Effect of DES extracts on HeLa cell viability determined by the MTS assay, it was assessed in volume ratio 1% - 2% (v/v).



HeLa cells showed a 26.70% of cell viability in Menthol: Camphor extract

CONCLUSION AND FUTURE TRENDS

We follow the principles of green chemistry, using green solvents, by-products sources, and emerging technologies.

COSMOtherm demonstrated great accuracy in predicting suitable solvents for carotenoid extraction.

Novel hydrophilic and hydrophobic DES were evaluated to extract carotenoids.

The extracts obtained with hydrophobic deep eutectic solvents demonstrated to reduce the cell viability in tumor HeLa cells.

In this work

The fundamental benefit of the obtained extract is that the whole formulation (DES and bioactive compound) can be included in the final products.

DES are expected to replace other conventional solvents in the short future; however, more research should be directed to the study of their application in food formulations.

D

E

S

ACKNOWLEDGEMENTS

- This work was financially supported by the Ministry of Science and Innovation (Spain) -State Research Agency (PID-2019-111331RB-I00/AEI/10.13039/501100011033)
- “Generación Bicentenario” scholarship from the Ministry of Education of the Republic of Peru (PRONABEC).
- European Union through the European regional development fund, Competitive, ness and Cohesion 2014-2020 (KK.01.1.1.07.0007.) and by the Croatian Science Foundation (Grant No. 7712 and 9550).
- Agricultural Cooperative Sant Bernat from Carlet, Spain, donated the raw materials.



D

E

S