

Optimization of ultrasonic and microwave assisted extraction of antioxidant compounds from cherry stems using NADES

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Cherry stems have been used in traditional medicine, commonly as decoctions, for the treatment of a broad variety of conditions, due to their antioxidant and anti-inflammatory properties. (Rubegeta, 2023, García-Villegas, 2024). Nevertheless, large quantities of cherry stems are generated and wasted as byproducts during fruit processing, without any major use and it is only recently that attention is drawn to their characterization and the assessment of the antioxidant potential of their extracts. (Mateus, 2023, Afonso, 2020)

The aim of this study was to develop a green method for the extraction of bioactive molecules from dried cherry stems, using natural deep eutectic solvent (NADES) along with the use of high energy techniques such as microwave irradiation and ultrasonic heating for the first time. NADES are composed of naturally occurring compounds and among their several advantages is that they can be tailored for specific applications through the appropriate selection of their constituents. This allows them to exhibit desired physicochemical properties and, in many cases, to be directly incorporated into end-use products without further processing. This contributes to the overall sustainability of the process and also often may add value to the final products. (Tzani, 2023, Karadendrou, 2025)

Three different extraction techniques combined with two solvents were employed in this study and were assessed for the optimization of the extraction process. A NADES of betaine:glycerol:water at a molar ratio of 1:3:1 was synthesized and used for microwave assisted extraction (MAE) and ultrasonic assisted extraction (UAE) following an experimental design. A 24-hour mechanical magnetic stirring extraction using a conventional ethanol-water solvent system at different ratios was also carried out and compared to the NADES extraction techniques.

The extraction process was optimized by a 4-factor 3-level Taguchi Experimental Design to study the effect of temperature (30-60°C), extraction time (15-45 min), solid-to-liquid ratio (S/L) (1-7g/100mL) and the %water content in the NADES-water system (10-40%). Mechanical magnetic stirring extractions were also conducted using hydroethanolic solvents with different ethanol concentrations, ranging from 0-100% v/v at a solid-liquid ratio of 3g/100mL.

All the extracts were characterized using as response factors, the total phenolic content (TPC) expressed as mg of gallic acid equivalent (GAE)/g of plant material and the total flavonoid content (TFC) expressed as mg of catechin equivalent (CE)/g of plant material. Optimal extracts were evaluated for their antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay.

Statistical analysis performed on MAE and UAE showed that all parameters had a positive impact on total content with the most significant one being the solid to liquid ratio for S/N ratios as well as data means as depicted in Figure 1.

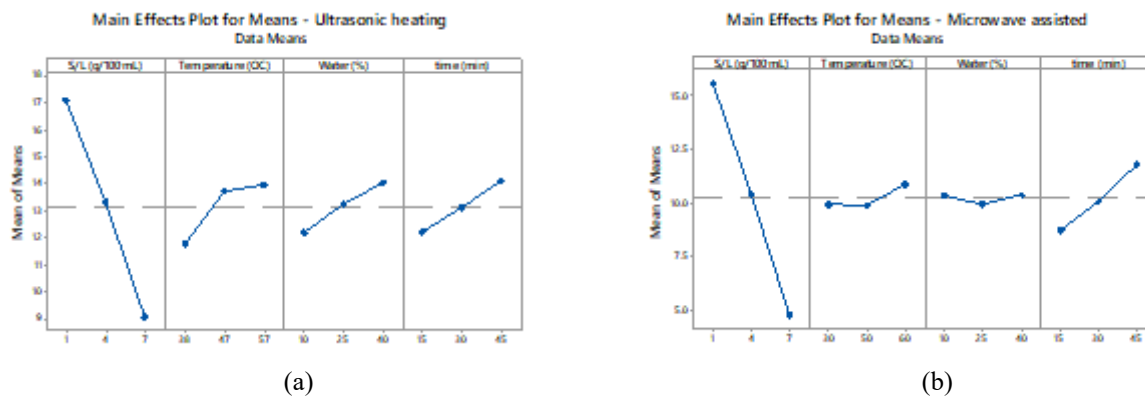


Figure 1. Main effects plot for means for ultrasonic heating (a) and microwave assisted extraction (b)

The optimal conditions deduced for both techniques resulted in a 45 min extraction time, 1g/100mL S/L, 40% water content, and a temperature of 57°C for ultrasonic bath and 60°C for microwave. Confirmation experiments were conducted and the RSD of predicted and measured values varied from 2.2 to 5.1%. The maximum content determined for microwave-assisted extraction was 14.7±0.3 mg GAE/g for TPC and 21.5±1.18 mg CE/g for TFC. Ultrasonic bath extraction led to a 28.0±1.0 mg GAE/g and 12.2±0.3 mg CE/g, respectively. Mechanical magnetic stirring yield obtained with 50% v/v ethanol, was up to 24.4 ±0.8 mg GAE/g for TPC and 16.8 ±0.3 mg CE/g for TFC. Antioxidant activity resulted in an IC50 of 3.4 µL and 5.5 µL of NADES-water extract/mL for UAE and MAE respectively.

In conclusion, the use of NADES in combination with green extraction techniques enhances the extraction of bioactive compounds achieving high contents in short extraction time. The results obtained are comparable to many traditional plants with antioxidant properties (Kähkönen et al 1999), rendering the extract to a promising ingredient for cosmeceutical products.

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