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Flavonoid-rich Extracts from Bitter Orange Industrialisation By-products with Sustainable Green Tech: NADES/UAE Extraction

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Abstract

The agro-industrial processing of citrus fruits generates large volumes of byproducts that remain underutilized despite their high content of bioactive compounds. Bitter orange (Citrus aurantium) byproducts, particularly peels, are recognized as rich sources of flavonoids with antioxidant and functional properties, making them suitable candidates for valorisation within circular economy frameworks (Maqbool et al., 2023, Ren et al., 2024). The aim of this study is to maximise the extraction of flavonoids from C. aurantium by-products through the application of green and innovative extraction strategies based on natural deep eutectic solvents (NADES) combined with ultrasound-assisted extraction (UAE). Several NADES systems formulated with different hydrogen bond donors were evaluated as alternatives to conventional organic solvents, with the objective of improving extraction efficiency and selectivity while reducing environmental impact, as previously reported for citrus-derived polyphenols (Fernández-Cabal et al., 2025). UAE was employed to enhance mass transfer, shorten extraction times and increase the recovery of bioactive compounds. While the primary focus remained on the quantification of flavonoid content, a phytochemical characterisation was performed to evaluate the extracts functional potential (Pires et al., 2025). This included the assessment of the polyphenolic profile and antioxidant properties, alongside the determination of key micronutrients such as ascorbic acid. Furthermore, ultra-high-performance liquid chromatography (UPCL) was utilised to obtain a detailed profiling of individual flavonoids, providing deeper insights into solvent selectivity and the specific chemical composition of the extracts. Total flavonoid content, showed marked variability among treatments, ranging from 338 ± 4.92 to 1,684 ± 3.19 mg EQ/ 100 g dry matter. The nature of the hydrogen bond donor was the main factor influencing flavonoid recovery, followed by its interaction with water content, while water proportion alone had a minor effect, as supported by statistical evidence. Fructose-based systems with higher hydration achieved the highest yields, highlighting the combined role of donor type and water content in enhancing mass transfer and flavonoid solubilisation. UPLC profiling revealed a characteristic fingerprint of flavanones and flavonols, with naringenin identified as the predominant compound, exceeding 900 mg/ 100 g dry matter in fructose-based formulations, followed by hesperidin (304.26 ± 7.68 mg EQ/ 100 g) and neo-hesperidin (203.77 ± 4.28 mg EQ/ 100 g). Additional flavonoids, including catechin, rutin, quercetin/luteolin and kaempferol were detected at lower concentrations and exhibited pronounced hydrogen bond donor dependent variability, highlighting the capacity of tailored NADES systems to modulate extraction selectivity and target specific flavonoid profiles. Overall, this study demonstrates that coupling NADES and UAE is an effective and sustainable

strategy for the valorisation of bitter orange by-products, enabling the development of novel bioactive materials from food resources with potential food and pharmaceutical applications.

Keywords: Bitter orange byproducts, Natural deep eutectic solvents, Flavonoids, Circular economy

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