

Extraction of carotenoids from tomato processing waste using Deep Eutectic Solvent

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Over the past few years, sustainability has gained immense importance in every aspect of human daily life, to the extent that there has been a need to become a sustainable society. One of the fundamental principles on which it is based, also an objective of the Agenda 2030, is to reduce waste, especially that of raw products. To achieve this, it is important to consider by-products and waste from industrial processes as raw materials for other industrial processes or applications. The agri-food industry, through the processing of raw plant materials, generates a large amount of waste that still holds commercial potential as they are rich in bioactive molecules. This study has focused on the waste generated during tomato processing, namely peels, seeds, and discarded fruit collectively referred to as pomace.

Tomatoes are fruits rich in carotenoids, which are still found in large quantities in the pomace from which they can be extracted. Traditionally, organic solvents are used, which are toxic and polluting (Lazarini et al., 2022). The aim of this work is to develop an extraction method based on the extraction of carotenoids from tomato pomace using sustainable solvents such as Deep Eutectic Solvents (DES). The carotenoids extracted from tomatoes have numerous applications, such as ingredients in pharmaceutical, cosmetic, and nutraceutical products, due to their coloring and antioxidant capacities. Among them, lycopene stands out in importance; it is an excellent antioxidant highly demanded at an industrial level, to the extent that its synthesis is necessary to increase the supply (Silva et al. 2019).

The selected DES are composed of menthol and various fatty acids (butyric, hexanoic, caprylic, lauric, and palmitic). The synthesis involves blending the components on a heated magnetic stirrer at different molar ratios. Some of these were found to be solids and were consequently discarded as they were unsuitable for extractions. Subsequently, the properties of the liquid solvents, such as density, viscosity, and IR spectra, were analyzed. To assess the extractive capacities of the synthesized DES towards carotenoids, numerous extractions were performed under the same operating conditions (25°C for 24 hours at 200 rpm in an incubator). Anova and Tukey tests were then conducted to validate the DES screening. Once the best DES was identified, an extraction optimization was carried out. The Design Of Experiment (DOE) was utilized to maximize efficiency and reduce the number of experiments required to determine the optimal conditions. Initially, a model varying 3 parameters simultaneously (temperature 25-65°C, time 10-50 minutes, and biomass/solvent ratio 1:10-1:30) was selected, and subsequently, a DOE with only the 2 most influential parameters (temperature and time) was chosen. The CAT (Chemometric Agile Tool) software was used for result analysis. The response surface methodology (RSM) provided by CAT enabled the identification of the optimal point. The yield of carotenoids under the best extraction conditions obtained with organic solvents (acetone and petroleum ether) and the DES was then compared. A kinetic study of the process was conducted at the optimal extraction temperature. Finally, the experimental work concluded by quantifying lycopene and β -carotene contents in the optimal extract using HPLC. From a theoretical perspective, methods proposed in literature for separating DES from extracted carotenoids and for assessing antioxidant power via the DPPH assay were analyzed.

The results showed that temperature and time are the most influential factors on the carotenoid content. In this regard, the optimal extraction values identified by the DOE are 65°C and 50 minutes, with a biomass/solvent ratio set at 1:10. Under these conditions, the DES is capable of extracting $204.11 \mu\text{g/g} \pm 6.43$ of carotenoids on a dry weight basis. The initial characterization of the matrix and the subsequently obtained extract highlighted significant levels of bioactive compounds. Through HPLC analysis, the lycopene content in the extract was evaluated, showing a result twice that of β -carotene. Specifically, $23.65 \mu\text{g/g} \pm 4.87$ of β -carotene and $52.38 \mu\text{g/g} \pm 12.05$ of lycopene were identified. The high lycopene content and its excellent antioxidant properties promote the reuse of this waste product in substantial quantities for cosmetic, pharmaceutical, and nutraceutical products. Furthermore, the easy availability and low cost of tomato pomace favor the recovery of carotenoids from them. Considering sustainability in the choice of extraction solvent, the menthol-butyric acid mixture proved to be more efficient than conventional organic solvents. In fact, under the same operating conditions, the DES extracted 204.11

$\mu\text{g/g} \pm 6.43$ of carotenoids, whereas acetone obtained $112.79 \mu\text{g/g} \pm 0.06$ and petroleum ether only $73.05 \mu\text{g/g} \pm 6.58$.

DES proves to be the best choice for these extractions, ensuring sustainability, biodegradability, and high yields. The use of DES avoids the need for extract purification, significantly reducing process costs. The extract containing DES is of natural origin and can thus be used directly in subsequent applications. In cosmetics and nutraceutical products, the extract is used as is. However, in pharmaceutical applications, further investigation is necessary to understand if separating DES from carotenoids is obligatory.

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