

SUPERCritical CHROMATOGRAPHIC SEPARATION OF PEPPER CAROTENOIDS

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Carotenoid analysis are most commonly performed by high-performance liquid chromatography (HPLC), particularly reversed phase liquid chromatography (RP-LC) with usually C30 stationary phases employing high amount of organic solvents as mobile phase with long analysis time^[1]. The separation of carotenoids with supercritical fluid chromatography (SFC) can provide a „greener” and at the same time faster and more efficient alternative^[2]. Using carbon dioxide (CO₂) and eventually co-solvent (e.g. ethanol) as mobile phase, separation and the elution velocity can be controlled by the density of the mobile phase and the concentration of co-solvent. The high solubility of carotenoids in supercritical CO₂ (scCO₂), highly reduces retention times while the mild analysis conditions applied avoid their degradation.

In our study the separation of carotenoids extracted from different maturation stages peppers was investigated with SFC in an Ultra Performance Convergence Chromatography (UPC²) system. SFC analysis was optimised in solvent gradient mode using a scCO₂ and ethanol as mobile phase with a small particle size bonded-phase octadecylsilyl column. Good separation of very rich carotenoid profiles, including native xanthophylls, carotenes and xanthophyll esters, was obtained in 11 min run time. During the analysis four fractions were collected and further analysed by a conventional RP-LC combined with photodiode array detector and mass spectrometry. High orthogonality was achieved between the two separation techniques, despite the similarity of the stationary phases. Using the proposed offline two-dimensional chromatographic system, identification of the carotenoid esters in red pepper was more accurate respect to the results obtained only with conventional RP-LC.

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References:

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