

EXTRACTION OF TOCOTRIENOLS FROM ANNATTO (*Bixa orellana*) SEEDS USING SUPERCRITICAL CARBON DIOXIDE

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Annatto (*Bixa orellana*) seeds are well known as a source of carotenoids such as bixin and norbixin that are commonly used for food coloring and flavoring. The seeds are also a rich source of tocotrienols, which are components of vitamin E. Tocotrienols from annatto and other sources are the subject of intense medical and nutritional research, as the literature reports many beneficial activities of these compounds, in particular when no tocopherols are present. Unlike other natural sources of vitamin E, annatto seeds do not contain large percentages of tocopherols and thus, this raw material is interesting for developing added-value products for the nutraceutical and food industries.

In this work we report on our experiments about the separation of tocotrienols from annatto seeds by supercritical fluid extraction. Seeds obtained from a local supplier were freeze dried, milled, sieved, and then subjected to extraction with supercritical carbon dioxide in a lab-scale apparatus. The experiments were arranged according to a central composite design in which temperatures from 35 to 50 °C, and carbon dioxide densities from 0.7 to 0.9 g/mL were considered. At each set of temperature and density, we used the Bender equation of state to find the pressure at which the experiment was to be run. The so-planned factorial experiment was augmented by several runs at 42.5 °C and 0.8 g/mL to determine the experimental error. Yield of extracts were obtained gravimetrically at different extraction times. Samples of the extracts were analyzed by HPLC to determine the percentage of tocotrienols obtained. The antioxidant capacity of the extracts was determined by their ability to react with the 1,1-biphenyl-2-picryl-hydrazyl (DPPH) free radical, as measured by spectrophotometry at 550 nm, and was reported as the concentration of each extract required to reduce in a 50% the concentration of the free radical (i.e., the so-called EC50 value).

The results show that extraction yields up to 2.96 wt% are obtained at 50 °C and 0.9 g/mL CO₂ density (350 bar). A large percentage of the active compounds extracted is composed by δ -tocotrienol, followed by γ -tocotrienol and small amounts of other tocotrienols and α -tocopherol. Antioxidant capacities corresponding to values of EC50 as small as 50 μ g/mL were obtained, which is about ten times that of butylhydroxytoluene (BHT) a common, pure synthetic antioxidant. This indicates that the extracted fractions might be interesting for using them directly as antioxidants in food industries, and also as a rich source of δ -tocotrienol for pharmaceutical or nutraceutical industries.

Keywords: annatto, tocotrienols, vitamin E.

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