

# 5º Encontro Nacional de Cromatografia

Universidade de Aveiro  
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## P.79 Determination of tocopherols in *Agaricus sp.* edible mushrooms by a normal phase liquid chromatographic method

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There is a growing interest in natural antioxidants for their potential role in the prevention of oxidative stress-related diseases, since synthetic antioxidants are being questioned due to their potential carcinogenic activity. Therefore, natural antioxidants such as tocopherol, polyphenols and carotenoid pigments are having a greater relevance in the protection against lipid oxidation. The use of mushrooms extracts as antioxidants is becoming increasingly popular<sup>1-3</sup> generating a great need for a fast and efficient technique for separating and quantifying the individual antioxidant components such as tocopherols.

In this study, individual tocopherol profile of five *Agaricus* mushroom species, widely consumed in Portugal, was obtained by a normal-phase high-performance liquid chromatography (NP-HPLC). It was used a simple solid-liquid extraction procedure without saponification step and the chromatographic separation was achieved using a YMC-Pack Polyamine II column using a isocratic elution with hexane/ethyl acetate (70:30, v/v) at a flow rate of 1,0mL/min. The effluent was monitored by a series arrangement of a UV followed by a fluorescence detector. All compounds were separated in a short period of time (30 min). The method proved to be sensitive, precise, reproducible, accurate and fast, allowing routine determinations of tocopherols.

Although several reports have been published on the tocopherols content of mushrooms,<sup>4-6</sup> all reported the same methodology including saponification. Herein, we selected an extraction method that claimed to exhibit the minimum loss of Vitamin E which did not include saponification, then further modified to minimize oxidative losses. The accuracy, precision and robustness of the method were improved by incorporating an internal standard from the extraction step through to the chromatographic analysis. As well, the use of fluorescence rather than UV as the detection mode provided the sensitivity and the selectivity required for the accurate determination of low levels of these homologs in mushrooms.

In all the samples  $\beta$ -tocopherol was the major compound, while  $\gamma$ -tocopherol was not detected in none of the samples. The results obtained in the analysis of mushroom samples point to the existence of apparent differences in what concerns tocopherols composition among different species.

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