Analysis of phenolic compounds in fruits and brines of portuguese olive tree cultivars by HPLC with different detectors and mass spectrometry

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Olea europaea L. is one of the most important and widespread crop of the Mediterranean basin. Most of the olive production is used for olive oil; however, a considerable part is processed for human consumption as table olives. In the traditional process, olives are handled in order to favour the growth of *Lactobacillus* spp. in the fermentation brines, essential to provide the amount of lactic acid needed for preservation as well as for its characteristic flavour (Leal-Sánchez *et al.*, 2003). However, lactic acid is not always produced in the amounts needed for the adequate preservation of olives, and spoilage can occur. The use of “lactobacilli starter cultures” may improve the microbiological control of the process, increasing the lactic acid yield and, therefore, providing a product with a consistent higher quality (Leal-Sánchez *et al.*, 2003).

The main phenolic compounds in olives are oleuropein, hydroxytyrosol and tyrosol (Bianchi G., 2003). During table olive processing, phenolic compounds undergo chemical transformations, diffuse from olive pulp to the surrounding solution and their acid hydrolyses occur (Brenes M. and Castro A., 1998).

In this work we intended to characterize phenolic compounds present in the fermentation process of table olives from olive tree cultivars: ‘Galega de

Portalegre', 'Galega de Castelo Branco' and 'Cordovil de Castelo Branco'. Two types of fermentation processes were compared: traditional and with inoculation of a "starter culture of lactobacilli".

Fruits and brines at different stages of fermentation were analysed. Fruit pulps were extracted with a mixture of methanol:water. Brines were analysed without pretreatment. The samples were analysed using reverse phase HPLC with diode array (200-600 nm), fluorescence (280 nm/320 nm) and electrochemical detection. Identification of phenolic compounds was done by comparison with standards and literature data. Mass spectrometry was also used for compound identity confirmation. Total phenolic content using Folin-Denis reagent was also determined in samples. Quantification of the major compounds detected in pulp extracts and brines was also achieved using UV detection at 280 nm. Tyrosol and hydroxytyrosol were also quantified by fluorescence and electrochemical detection, respectively.

The methods used allowed us to obtain the profile of the phenolic components of different samples and to compare both processes of fermentation. Hydroxytyrosol and oleuropein were the main compounds detected in fruits. Hydroxytyrosol, tyrosol, *p*-coumaric and vanillic acids were the main compounds detected in brines.

The fruits obtained with traditional fermentation showed higher total phenolic content than the inoculated ones; the same results were obtained for brines and can be explained by a higher rate of consumption of phenolic compounds by inoculated bacteria. Samples from 'Cordovil de Castelo Branco' cultivar showed higher level of most phenolic compounds detected.

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