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CHANGES IN VIRGIN OLIVE OIL ANTIOXIDANTS, POLYPHENOL OXIDASE AND PEROXIDASE ACTIVITIES DURING FRUIT RIPENING

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Abstract

One of the current challenges in olive oil quality is to assure that olive antioxidant compounds are preserved in order to maintain their role on oxidative degradation protection.

Olive endogenous oxidoredutases, mainly polyphenol oxidases (PPO) and peroxidases (POD) are suggested to play an important role by promoting oxidation of phenolic compounds. POD oxidize phenols and PPO catalyses the hydroxylation of monophenols or *o*-diphenols to yield *o*-quinones, which is followed by condensation or polymerization reactions. Moreover, both enzymes seem to act synergistically on the changing of phenolic compounds.

The aim of this study was to evaluate the olive fruit PPO and POD activities in two Portuguese cultivars (*Olea europaea*, cv 'Cobrançosa' and cv 'Galega Vulgar'), along fruit ripening. PPO and POD activities in mesocarp and seed were assayed 20 weeks after flowering and along two months thereafter. Polyphenols and tocopherols in the olive oils extracted from these samples were determined. An increase in PPO activity in early stages of ripening was observed for both cultivars. PPO activity measured in olive mesocarp of Cobrançosa fruits was always higher than in Galega fruits. On the contrary, POD activity in seeds was higher in Galega than in Cobrançosa seeds. Cobrançosa olive oil is richer in total phenols and also in tocopherols than Galega oil. This is in agreement with sensory properties of these olive oils.

Keywords: *Olea europaea* L., Cobrançosa, Galega, enzymes, maturation, phenols

Introduction

In the frame of modern concepts, extra virgin olive oil (EVOO) quality is mainly differentiated by sensory characteristics. While volatile compounds have a key role in developing flavours, olive phenolic compounds play an important function in organoleptic evaluation namely in attributes related to bitterness and pungency. Moreover, phenolic compounds contribute for nutritional and healthy properties of EVOO (Boskou et al., 2006).

In the oxidative degradation of phenolic compounds there are two enzymes – polyphenol oxidases (PPO) and peroxidases (POD) – that are believed to be very relevant in terms of olive oil quality, not only in sensory properties but also by decreasing the level of antioxidants (Servili et al., 2000; Servili et al., 2007). PPO (EC 1.14.18.1) is the main enzyme involved in the oxidation of phenols, which is performed by two different reactions in the presence of oxygen: the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones, followed by condensation and polymerization

reactions (Yoruk and Marshal, 2003). POD (EC 1.11.1.7) performs single-electron oxidation on a wide variety of compounds in the presence of hydrogen peroxide. Although the contribution of POD to the oxidation of phenols is limited by the low internal level of hydrogen peroxide, it has been proposed that PPO could act as promoter of POD activity, which could be due to the generation of H_2O_2 during the oxidation of phenolic compounds (Tomás-Barberan and Espin, 2001; Garcia-Rodriguez et al., 2011). Therefore, the study of the influence of enzymatic activity on EVOO phenolic compounds changes is a current research challenge. The present work aims to evaluate PPO and POD activities during the 2010 harvest season in two of the main olive cultivars produced in Portugal: Galega and Cobrançosa. The first one is well known as a producer of soft oils, with a relatively low content of phenolic compounds whereas with Cobrançosa olives, bitter and pungent EVOO are produced. It is expected that changes of phenolic compounds and enzymatic activity levels can occur during ripening (Ortega-Garcia et al., 2008, Ortega-Gracia and Peragón, 2009). So, the effect of harvest time on enzymatic activities and on antioxidant levels in EVOO, was investigated.

Material and Methods

In 2010, a trial was conducted in a non irrigated olive grove, under integrated production, located in Beira Baixa, an inland region in the centre of Portugal. Olive fruits (*Olea europaea* L.) cvs Galega vulgar and Cobrançosa were picked in the beginning of October (20 weeks after flowering – WAF) till the 2nd fortnight of November. Their ripening indices (RI) were determined following the guidelines of Estación de Olivicultura y Elaiotecnia, Jaén, Spain (Hermoso et al., 1997); humidity and fat content (by Soxtec) of the fruits were also evaluated. Only healthy fruits were selected for enzyme activity assays and for olive oil extraction. Acetone powders were prepared by homogenizing olive destoned fruits with frozen acetone (-20°C) in an ultraturrax (2 min), followed by filtration in fiber glass filters, washing the pellet with acetone (-20°C) until total removal of pigments, followed by drying at room temperature with N_2 (Saraiva et al., 2007; Lester et al., 2004). POD and PPO extracts were prepared from 0.4 g of acetone powder suspended in 5 mL of extraction buffer (0.05 M potassium phosphate, pH 6.2 containing 1M KCl) (Servili et al., 2007) and 2% (m/m) of PVP and stirred for 30 min, 4°C, 400 rpm; the suspension was centrifuged at 12,000 rpm for 30 min and filtered (0.45 µm). Enzymatic activity assays were performed using continuous spectrophotometric methodologies. PPO was evaluated using catechol (30 mM) as substrate based on Oktay et al. (1995) methodology, following the increase in absorbance at 420 nm, during 1 min. POD activity was performed using the procedure described by Gajewska et al. (2006), by the increase in absorbance at 470 nm (2 min) using 30 mM guaiacol and 4 mM H_2O_2 as substrates. Protein evaluation was performed by Bradford method (1976).

Olive oils were extracted in Abencor equipment. Oil extraction was performed by thermobeating at 27-30°C, for 30 min. European Union chemical quality criteria (acidity value, peroxide index (IP) and UV light absorption (K_{232} and K_{270})) was carried out following the analytical methods described in EEC/2568/91 EU Regulation. Total phenolic compounds were determined employing Folin-Ciocalteau reactive with solid phase extraction (Favati et al., 1994) and quantification by VIS spectroscopy (results expressed as gallic acid equivalents (mg GAE kg^{-1})). Tocopherols were determined by high-performance liquid chromatography in an Agilent 1100 Series chromatograph (NP-HPLC), fluorescence detection with excitation set at 290 nm and emission set at 330 nm. A Lichrosorb Si 60 column (5µm) and a flow rate of 1.2 ml min^{-1} were used at room temperature. Sensory analysis of olive oils was performed using a quantitative descriptive analysis (QDA) for

positive attributes adapted from Angerosa (2000). Quantification of attributes was carried out using an unstructured scale.

Results and Discussion

Extraction of olive oils was performed with olives with humidity between 53 and 56%; ripening indices began in 0.6 and finished at 4.4, with fat contents ranging from 27 to 37% (DW). Results of quality criteria showed that all the olive oils were classified as EVOO (acidity < 0.3% oleic acid; IP < 12 meq O_2 kg^{-1}; K_{270} < 0.19; K_{232} < 1.60). POD activity is detected predominantly in the seed while PPO is located mainly in the fruit mesocarp, which is in agreement with other authors (Servili et al., 2000; Garcia-Rodriguez et al., 2011). At the beginning of October (nearly 20 WAF) Galega cultivar showed almost undetectable PPO activity, while about 70 U g^{-1} FW was detected in Cobrançosa cultivar (Fig. 1). At this date, the RI was 0.8 for Galega cultivar, and 0.6 for Cobrançosa cultivar, meaning that PPO enzyme activity is more influenced by the cultivar than by the ripening stage. Cobrançosa cultivar showed higher mesocarp polyphenol oxidase activity than Galega. PPO activity rises in the 2nd fortnight of October, decreasing thereafter, as well as total phenol content for both cultivars (Fig. 1 and 2).

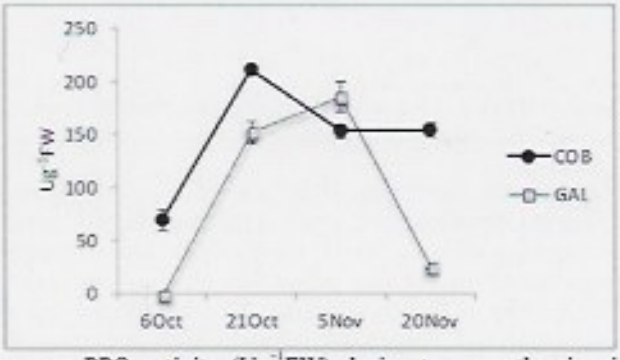

Fig. 1. Olive mesocarp PPO activity ($Ug^{-1}FW$) during two months ripening (mean and SD). (COB - Cobrançosa; GAL - Galega).

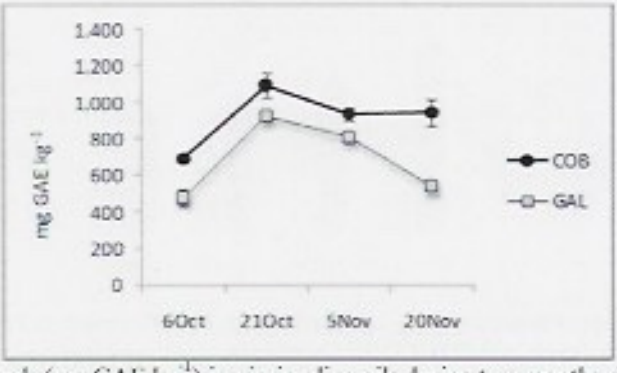

Fig. 2. Total phenols (mg GAE kg^{-1}) in virgin olive oils during two months ripening (mean and SD) (COB - Cobrançosa; GAL - Galega).

This behaviour was also observed by Garcia-Rodriguez et al. (2011) for Picual and Arbequina cultivars. However, higher PPO activities were reported. These differences

could be explained by the use of 4-*tert*-butylcatechol (TBC) as substrate instead of catechol (Garcia- Molina et al., 2007). The contribution of phenolic compounds to bitterness of olive oil is well established (Angerosa, 2000). Galega olive oils are always referred as having very low contents of phenols (Peres et al., 2000; 2009) and consequently low bitter oils are usually obtained. Sensory analysis was performed in olive oils obtained in the last harvest date (November 20th). Cobrançosa olive oils presented strongly bitter and pungent notes in levels considered as "intense" according to EC Reg. N° 640/08, 4 July. On the contrary, sweeter oils were obtained from Galega olives in the same date, although with bitter notes. This may be due to the fact that olives were picked from non irrigated trees (Stefanoudaki et al., 2009).

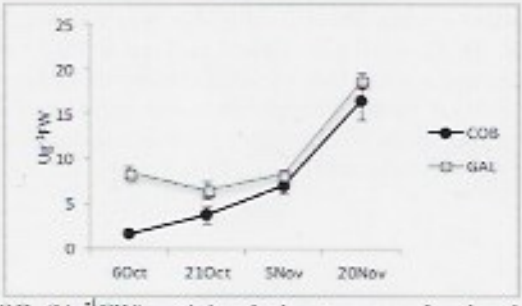

Fig. 3. Olive seed POD ($Ug^{-1}FW$) activity during two months ripening (mean and SD) (COB - Cobrançosa; GAL - Galega).

This preliminary study shows that after October 20th total phenols in Galega oils decrease faster than in Cobrançosa oils, which is richer in phenolic compounds. Also PPO activity in fruit mesocarp shows a similar trend as total phenol content. An increase in seed POD activity during the ripening period was observed for both cultivars (Fig. 3). Higher seed POD activity of Galega olives can contribute to the degradation of phenols during olive oil extraction. For Picual olives a high mesocarp PPO activity was accompanied by a high seed POD activity (Garcia-Rodriguez et al., 2011).

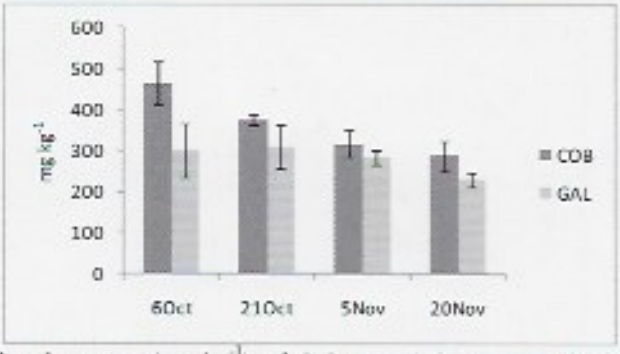

Fig. 4. α-Tocopherol content (mg kg^{-1}) of Galega and Cobrançosa EVOO during two months ripening (mean and SD) (COB - Cobrançosa; GAL - Galega).

In what concerns tocopherols, Fig. 4 shows the results of α-tocopherol content during fruit ripening. Alpha-tocopherol is the most important lipophilic phenol in EVOO, contributing for more than 90% of all tocopherols present in olive oils (Boskou et al, 2006). Cobrançosa oils have always higher contents of α-tocopherol than Galega oils.

Along fruit ripening a decrease in this compound was observed in both oils, conversely to the observed POD activity in seed. A low content of β-tocopherol was observed in both oils (3.7 ± 0.77 mg kg^{-1}); Galega oils had a γ-tocopherol content of 11.9 ± 0.48 mg kg^{-1} while Cobrançosa oils had 8.4 ± 0.60 mg kg^{-1}, which seems to be a good parameter to discriminate these oils.

In conclusion, harvest time related with the ripening stage of each cultivar, is one of the most important parameters that influence EVOO quality. The knowledge about the enzymatic behaviour during ripening may be an indicator if it is possible to have a long harvest time or if it is better to harvest as soon as possible, in order to have high quality EVOO, for a specific cultivar.

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References

Angerosa F., 2000. Sensory quality of olive oils. In: R. Aparicio and J. Harwood (eds). Handbook of Olive Oil. Aspen Publishers, Inc. Maryland. pp 355-392.

Boskou D., Blekas G., and Tsimidou M., 2006. Olive oil composition. In: D. Boskou. Olive Oil Chemistry and Technology. 2nd ed. AOCS Press, Champaign. pp 41-72.

Bradford M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding. Anal Bioch. 72: 248-254

European Commission, Off. J. European Communities, July 4,Commission Regulation (EC) N° 640/2008 of 4 July 2008 amending Regulation (EEC) N° 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis.

Favati F., Caponale G., and Bertuccioli M., 1994. Rapid determination of phenol content in virgin olive oil. Grasas y Aceites. 45: 68-70

Gajewska E., Skłodowska M., Słaba M. and Mazur J., 2006. Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. Biologia Plantarum 50: 653-659.

Garcia-Molina F., Muñoz J.L., Varón R., Rodríguez-López J.N., Garcia-Cánovas F. and Tudela J., 2007. A Review on spectrophotometric methods for measuring the monophenolase and diphenolase activities of tyrosinase. J. Agric. Food Chem. 55: 9739-9749.

García-Rodríguez R., Romero-Segura C., Sanz C., Sánchez-Ortiz A. and Pérez A.G., 2011. Role of polyphenol oxidase and peroxidase in shaping the phenolic profile of virgin olive oil. Food Res. Intern. 44: 629-635.

Hermoso M., Uceda M., Frias L. and Beltran G. 1997. Maduración. In: El Cultivo del Olivo. D. Barranco, D. Fernandez-Escobar, L. Rallo (Ed.), Ediciones Mundi-Prensa, Madrid, pp. 139-153.

Lester G.E., Hodges D.M., Meyer R.D. and Munro K., 2004. Pre-extraction preparation (fresh, frozen, freeze-dried, or acetone powdered) and long-term storage of fruit and vegetable tissues: effects on antioxidant enzyme activity. J. Agric. Food Chem. 52: 2167-2173

Oktay M., Kufrevioglu I., Kocacaliskan I. and Sakiroglu H., 1995. Polyphenoloxidase from Amasya Apple. J. Food Sci. 60: 494-496.

Ortega-Garcia F., Blanco S., Peinado M.A. and Peragón J., 2008. Polyphenol oxidase and its relationship with oleuropein concentration in fruits and leaves of olive (*Olea europaea*) cv 'Picual' trees during fruit ripening. Tree Physiology. 28: 45-54.

Ortega-Garcia F. and Peragón J., 2009. Phenylalanine ammonia-lyase, polyphenol oxidase and phenol concentration in fruits of *Olea europaea* L. cv Picual, Verdeal, Arbequina, and Frantoio during ripening. J. Agric. Food Chem. 57: 10331-10340.

Peres M.F., Henriques L.R., Pinheiro-Alves M.C. and Simões P., 2000. Characterization of three monovariety olive oils of Beira Baixa (Portugal). Acta Hort. 586: 641-643.

Peres M.F., Henriques L.R., Simões-Lopes P. and Pinheiro-Alves M.C., 2009. Azeites da Galega Vulgar – Efeito do loteamento e do armazenamento. Actas Portuguesas de Horticultura.13: 186 (in cd-rom).

Saraiva J.A., Nunes C.S. and Coimbra M.A., 2007. Purification and characterization of olive (*Olea europaea* L.) peroxidase – Evidence for the occurrence of a pectin binding peroxidase. Food Chem. 101: 1571-1579.

Servili M., Baldioli M., Begliomini A.L. Selvaggini R. and Montedoro G.F., 2000. The phenolic and volatile compounds of virgin olive oil: Relationships with the endogenous oxidoreductases during the mechanical oil extraction process. In: Flavour and Fragrance Chemistry; Proceedings of the Phytochemical Society of Europe, Lanzotti, V., Taglialatela-Scafati, O., Eds.; Kluwer Academic: Dordrecht, pp 163-173.

Servili M., Taticchi A., Esposto S., Urbani S., Selvaggini R. and Montedoro G.F., 2007. Effect of olive stoning on the volatile and phenolic composition of virgin olive oil. J. Agric. Food Chem. 55: 7028-7035.

Stefanoudaki E., Williams M., Chartzoulakis K. and Harwood J., 2009. Effect of irrigation on quality attributes of olive oil. J. Agric. Food Chem. 57: 7048-7055.

Tomás-Barberan F.A. and Espin J.C., 2001. Phenolic compounds and related enzymes as determinants of quality of fruits and vegetables. J. Sci. Food Agric. 81: 853-876.

Yoruk R. and Marshal R., 2003. Physicochemical properties and function of plant polyphenol oxidase: a review. J. Food Biochem. 27: 361-422.