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Chlorophyll catabolism in olive fruits (var. Arbequina and Hojiblanca) during maturation

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ABSTRACT

The central reaction of chlorophyll (chl) breakdown pathway occurring during olive fruits maturation is the cleavage of the macrocycle pheophorbide *a* to a primary fluorescent chl catabolite (pFCC) and it is catalyzed by two enzymes: pheophorbide *a* oxygenase (PaO) and red chl catabolite reductase (RCCR). In subsequent steps, pFCC is converted to different fluorescent chlorophyll catabolites (FCCs) and nonfluorescent chlorophyll catabolites (NCCs). This work demonstrated that RCCR activity of olive fruits is type II. During the study of evolution of PaO and RCCR activities through the olive fruits maturation in two varieties: Hojiblanca and Arbequina, a significant increase in PaO and RCCR activity was found in ripening stage. In addition, the profile and structure of NCCs present in epicarp of this fruit was studied using HPLC/ESI-TOF-MS. Five different NCCs were defined and for the first time the enzymatic reactions implied in chlorophyll degradations in olive fruits elucidated.

1. Introduction

Chlorophyll pigments are highly appreciated as functional components in fruits and vegetables both for their green colouring properties as its health benefits for the human consumption derived from their biological properties (Ferruzzi & Blakeslee, 2007). In addition, the ripening process of fruits for food production is associated with chemical and/or enzymatic specific transformations of these pigments making them quality indicators of end products and demonstrating a potential applicability as a tool for traceability the processing (Gandul-Rojas, Roca, & Mínguez-Mosquera, 2000). The beginning of the maturation and senescence in fruits and leaves involves a cessation in the photosynthetic activity, the dismantling of the photosynthetic apparatus and the beginning of catabolism of the pigments responsible for the photosynthesis: chlorophylls. The biochemical pathway involved in the degradation of chlorophylls during the senescence and maturation is delineated. Nevertheless, there remain yet big questions that are stressed in the case of fruit maturation, which apart from differing from foliar senescence, is the least studied case. Therefore, a larger amount of researches dealing with the reactions involved in the degradation of chlorophylls in fruits are necessary. In addition, specifically in the case of olive fruit, it is essential to know the mechanisms responsible for the pigmentation in the fruit, since this composition leads to the coloration of the corresponding olive oils (Roca & Mínguez-Mosquera, 2001). Coloration is a critical parameter in the product marketing. Further, it is a quality and authenticity parameter (Gandul-Rojas et al., 2000) of the product.

The first enzymatic reaction implicated in the catabolism of the chlorophyll *a* (chl) molecule which takes place during leaf senescence is the removal of Mg by Mg-dechelating substance (MCS) (Suzuki & Shioi, 2002), producing pheophytin *a* followed by the removal of phytol and the formation of pheophorbide *a* in a reaction catalyzed by pheophytin pheophorbide hydrolase (PPH) (Krautler & Hörtensteiner, 2013). However, the occurrence of these reactions during the fruit ripening is not clear, mainly because there is much less research regarding the degradation systems which are involved in the chlorophyllic catabolism in fruits. In fact, there are contradictory results: Guyer et al., 2014 have proved PPH functionality in tomatoes (*Solanum lycopersicum*). Nonetheless, other authors (Shemer et al., 2008) in citrus fruits have checked that the pathway starts with sequential action of chlorophyllase (CHLASE) catalyzing the phytol hydrolysis in chl *a* resulting chlorophyllide *a* (Amir-Shapira, Goldschmidt, & Altman, 1987)

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followed by the removal of Mg by Mg-dechelating substance (MCS) (Suzuki & Shioi, 2002).

Independently of the pathway, pheophorbide *a* is degraded to primarily fluorescent chl catabolites (pFCCs), via the enzymatic system pheophorbide *a* oxygenase (PaO) in conjunction with red chl catabolite reductase (RCCR) (Fig. 1). This reaction constitutes the cleavage of the tetrapyrrole and up to now, the only reaction regulated, being consequently the central step in the chlorophyll catabolism pathway (Krautler & Hörtensteiner, 2013). In thylakoid membranes, PaO and RCCR work as a coupled enzymatic system that depends on the presence of ferredoxin (Fd) reduced as an electron donor to sequentially transform pheophorbide *a* into RCC and this into pFCC (Rodoni, Vicentini, Schellenberg, Matile, & Hörtensteiner, 1997). *In vivo*, Fd is reduced in the presence of light by means of Photosystem I (PSI). However, *in vitro*, a reducing power in the form of NADPH is needed for the formation of pFCC from pheophorbide *a* (Rodoni et al., 1997). PaO is a monooxygenase dependent on oxygen, cloned in 2003 (Pružinská, Tanner, Anders, Roca, & Hörtensteiner, 2003), which is located in the inner membrane of chloroplasts (Krautler & Hörtensteiner, 2013). Regarding RCCR, it is a soluble protein located in the stroma of chloroplast and it can produce two pFCC stereoisomers at C-1, (pFCC-1 and pFCC-2) (Mühlecker, Ongania, Kräutler, Matile, & Hörtensteiner, 1997; Mühlecker & Krautler, 2000) depending on the plant species. The replacement of phenylalanine by valine in the amino acid sequence of RCCR is responsible for the stereospecificity (Pružinská et al., 2007).

Following, pFCCs are exported to the cytosol where they are modified with specific functional groups in three fixed positions generating fluorescent chl catabolites (FCCs). The first position is at $C8^2$ (Fig. 1), where hydroxylation, glucosylation and/or malonylation could take place. The second modifiable position is $O13^4$ where a reaction of demethylation is possible, allowing the occurrence of esterified or de-esterified possible structures.

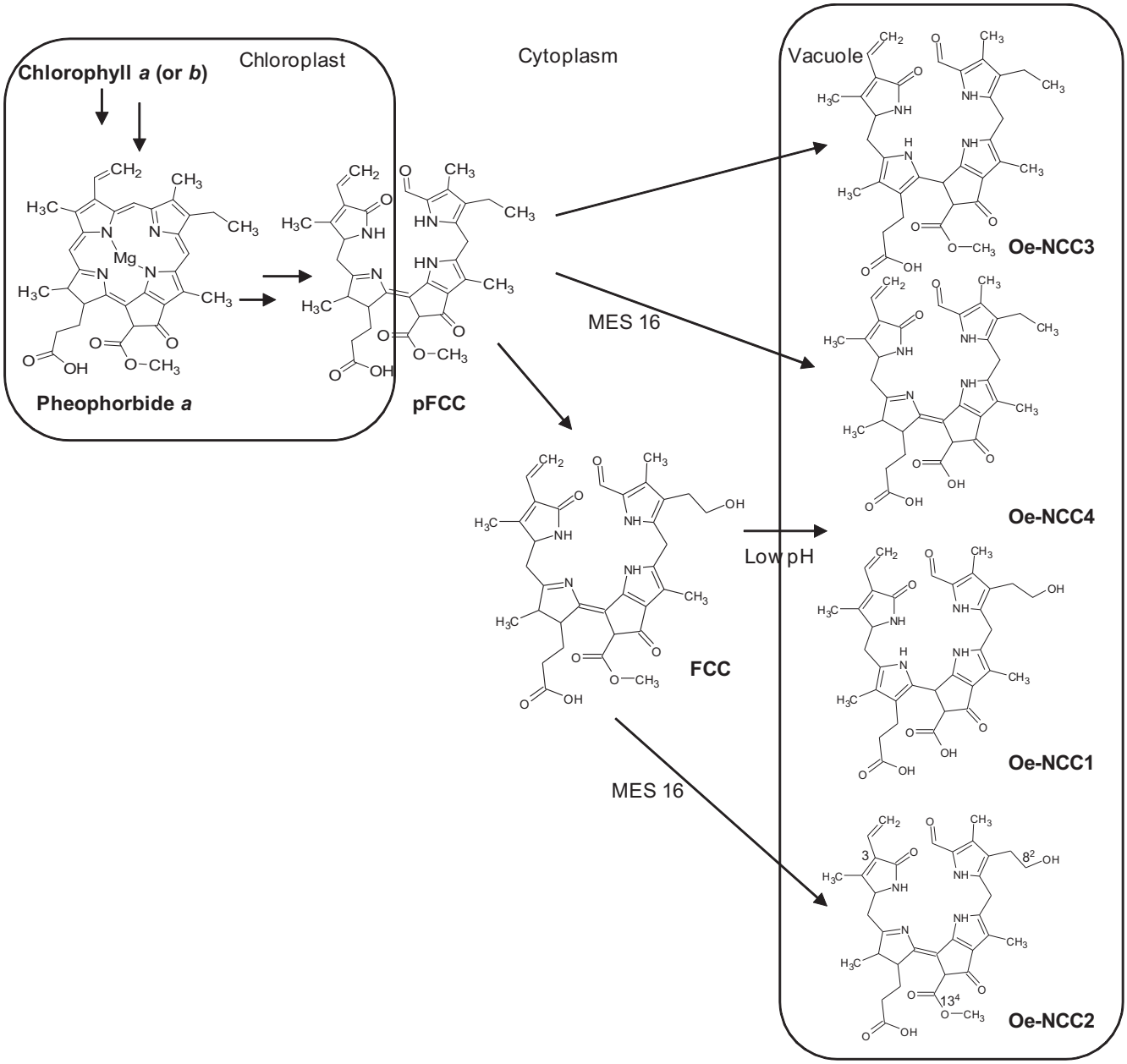

Fig. 1. Proposed chlorophyll degradation pathway in ripe olive fruits.