

Individualization and characterization of oxidative degradation products of secoiridoids in virgin olive oil by innovative analytic techniques

Ilona Di Maio (email: ilonadimaio@libero.it)
Dept. of Economic and Food Sciences, University of Perugia
Tutor: Prof. Maurizio Servili

The aim of this PhD thesis research project is to individualize and characterize oxidative degradation products of the most important secoiridoids present in virgin olive oil. The work is aimed at finding some analytical indicators which can be used both as molecular markers of virgin olive oil “freshness” and in the evaluation of its shelf-life.

Individuazione e Caratterizzazione dei Prodotti di Degradazione Ossidativa dei secoiridoidi contenuti nell’ Olio Vergine di Oliva mediante tecniche analitiche innovative

Questo progetto di tesi di dottorato ha lo scopo di individuare e caratterizzare i prodotti di degradazione ossidativa dei principali secoiridoidi presenti nell’olio vergine di oliva. L’obiettivo è quello di definire degli indici utilizzabili sia come markers di “freschezza” dell’olio vergine di oliva, sia nella valutazione della sua shelf-life.

1. State-of-the-Art

Hydrophilic phenols like phenolic alcohols and acids, flavonoids, lignans and secoiridoids are the most important class of natural antioxidants found in virgin olive oil. Secoiridoids are the prevalent compounds of virgin olive oil. They are present in a greater quantity in virgin olive oil and they are represented in particular by the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA or *p*-HPEA-EDA), an isomer of oleuropein aglycone (3,4-DHPEA-EA) and the ligstroside aglycone (*p*-HPEA-EA) (Montedoro *et al.* 1992a; 1992b). These substances are aglycone derivatives of secoiridoid glucosides contained in the olive fruit, originating during oil mechanical extraction process, by hydrolysis of oleuropein, demethylpleuropein and ligstroside. These reactions are catalysed by endogenous β -glucosidases (Servili *et al.*, 2002). Secoiridoids are involved in the virgin olive oil oxidation process as primary antioxidants to inhibit oxidation in virgin olive oil acting as chain breakers by a donation of a radical hydrogen to alkylperoxyl radicals generated by the lipid oxidation and formation of stable derivatives. It has been proved in literature that there is a relationship between concentration and antioxidant activity of simple phenols, lignans and secoiridoids found in virgin olive oil. Several papers report that the amount of these phenols is strongly associated to the virgin olive oil shelf-life (Ninfali *et al.*, 2001; Gordon *et al.*, 1990; Papadopoulus and Boskou, 1991). The effectiveness as antioxidant of these molecules is demonstrated also by structure-activity relationships. It has been shown that 3,4-DHPEA and the other secoiridoids containing this compound in their molecular structures (namely 3,4-DHPEA-EDA and 3,4-DHPEA-EA) are the natural antioxidants of VOO with the highest antioxidant efficacy (Baldioli *et al.*, 1996; Servili *et al.* 2004). The oxidation process has negative effects on the virgin olive oil sensory behaviour and on its shelf-life, therefore on the virgin olive oil quality. Polyphenols play their role as antioxidants in nonenzymatic oxidative reactions; hypothesis have been advanced about the mechanism of stabilization and neutralization of alkylperoxyl radicals generated by the lipid oxidation, but the relative oxidation products are still little known. Individualization and characterization of oxidation products of phenolic antioxidants present in virgin olive oil give the possibility to identify some analytical indicators which can be used both as markers of the virgin olive oil “freshness” and in the evaluation of its shelf-life.

2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

- A1) Selected References** on the oxidation reaction of phenolic compounds and relative oxidation products;
- A2) Enzymatic oxidation and chemical oxidation** of standard phenolic compounds;
- A3) Setting up analytic methods for LC-MS analysis of oxidation products** of standard phenolic compounds;
- A4) Individualization and characterization of oxidation products by LC-MS system** and probably comparison/confirmation by NMR;
- A5) Individualization of oxidation products** of phenolic antioxidants in virgin olive oil during the oxidation process;

A6) Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	Research																		
	1) Selected References																		
A2)	Oxidation Systems																		
	1) Enzymatic Oxidation Reaction																		
	2) Chemical Oxidation Reaction																		
A3)	LC-MS Investigation																		
	1) Setting up Analytic Methods																		
	2) LC-MS Analysis																		
A4)	Structural Analysis																		
	1) Characterization of Oxidation Products by LC-MS																		
	2) Acquisition of NMR Spectrum of Oxidation Products																		
A5)	1) LC-MS Analysis of Oil during the Autoxidation Process																		
	2) Individualization of Oxidation Products																		
A6)	Thesis and Paper Preparation																		

3. Selected References

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