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CICLO XXVIII

Methods and Indices for Monitoring and Optimization of Extra Virgin Olive Oil Production

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1. INTRODUCTION

In recent years we assisted to an increasing interest in the field of olive-oil supply chain by consumers, producers and the world of scientific research. Despite this renewed attention there is a lack of support guidelines to check and optimizate extra virgin olive oil (EVOO) extraction. A process control implies a selection of significant process steps (i.e., critical control points CCPs) and parameters (i.e., markers) to follow the history of quality characteristics from raw materials to end products. This entails a thorough knowledge of both positive and negative transformation phenomena, which may affect raw material components and, as a result, have positive and negative effects on the quality of end product.

Based on literature data, transformation pathways of the main components of olive oil will be described in detail in the next paragraphs. They will also be attributed to olive oil chain processing, which is expressed by operating modules. These include the following unit operations having specific homogeneous properties and optimization requirements:

- *The Growing of Olive Tree Module*. It contains all activities from selection of olive cultivar to harvesting of fully-ripe, undamaged, healthy olives.
- *The Management of Ripened Olives Module*. It contains all process operations from olive transport to washing step; these are simple operations, which however, when carried out badly, may have a negative effect on the final quality of oil.
- *The Oil Extraction Module*. This is the basic module of the production process. It contains all operations from olive crushing to olive oil separation.
- *The Oil Storage and Distribution Module*. It aims at maintaining extra virgin olive oil characteristics as much unchanged as possible over time. It contains all operations from oil clarification by decanting to product distribution on the market.

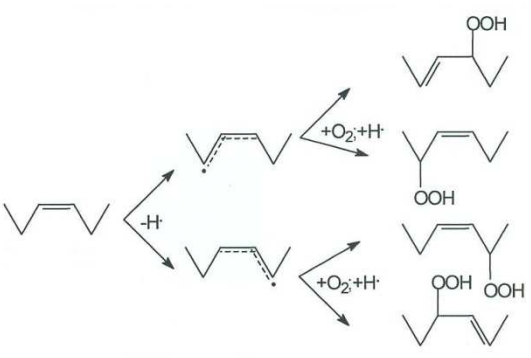
1.1 Transformation pathways of triglycerides

Olive oil triglycerides are biosynthesized from the simple sugars of olives. The precursor of biosynthesis of fatty acids is acetyl-CoA, derived from catabolism of glucose and fructose (Lavee, 1986).

Triglycerides can be degraded to free radicals and hydroperoxides by auto-oxidation and photo-oxidation (i.e., *radical oxidation*). Hydroperoxides, being very unstable, break down to alkoxy free radicals, which decompose to aldehydes, alcohols and ketones from 6 to 11 atoms of carbon (Fig. 1.1). These compounds are volatile and responsible for oil rancid defect; hexanal and nonanal are often considered significant compounds to contribute to the typical rancid odor for oxidized oil (Aparicio *et al.*, 2012; Frankel, 1991; Hamilton, 1983).

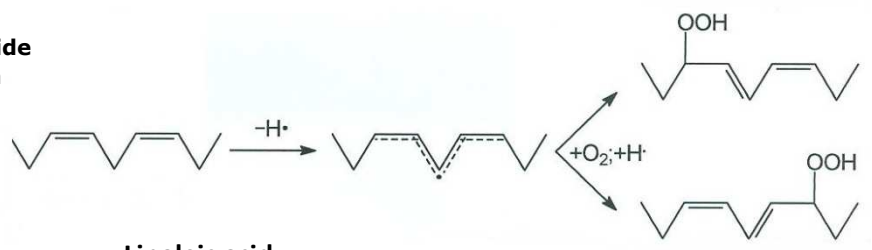
Triglycerides can also be degraded to free fatty acids by enzymatic hydrolysis (i.e., lipase activity). Free fatty acids can either be subjected to radical oxidation or be transformed into aldehydes, alcohols and esters with 6 atoms of carbon by an enzyme oxidation, called the lipoxygenase pathway (i.e., *LOX pathway*) (Angerosa *et al.*, 2000). These volatile compounds from LOX pathway are responsible for pleasant sensory descriptors, such as fruity and grassy (Fig. 1.2).

All the above phenomena may occur at different steps along the olive oil chain. Presence of water, fruit surface scratches and olive crushing are known to favour enzymatic reactions. These are typically defined as fast reactions when optimal temperature conditions are available and, for oxidative reactions, also in the presence of oxygen (i.e, high redox potential values). Enzymes have both an endogenous and exogenous nature as a result of contamination by moulds, bacteria and yeasts (Vichi *et al.*, 2011).

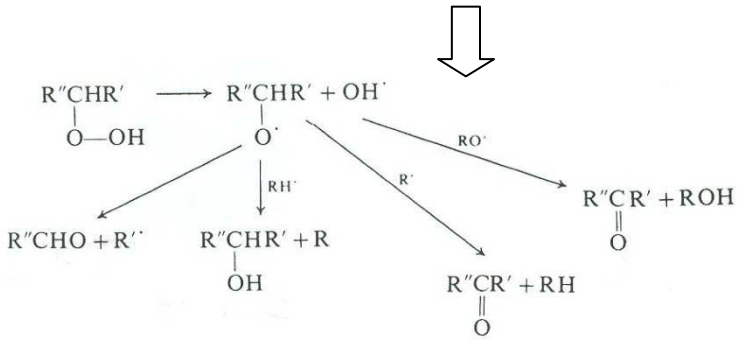


Oleic acid *OR*

Hydroperoxide formation

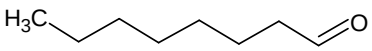


Linoleic acid

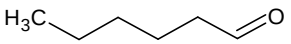


Hydroperoxide decomposition

For example:



Nonanal



Hexanal

Figure 1.1. Radical oxidation of triglycerides (Zanoni, 2014).

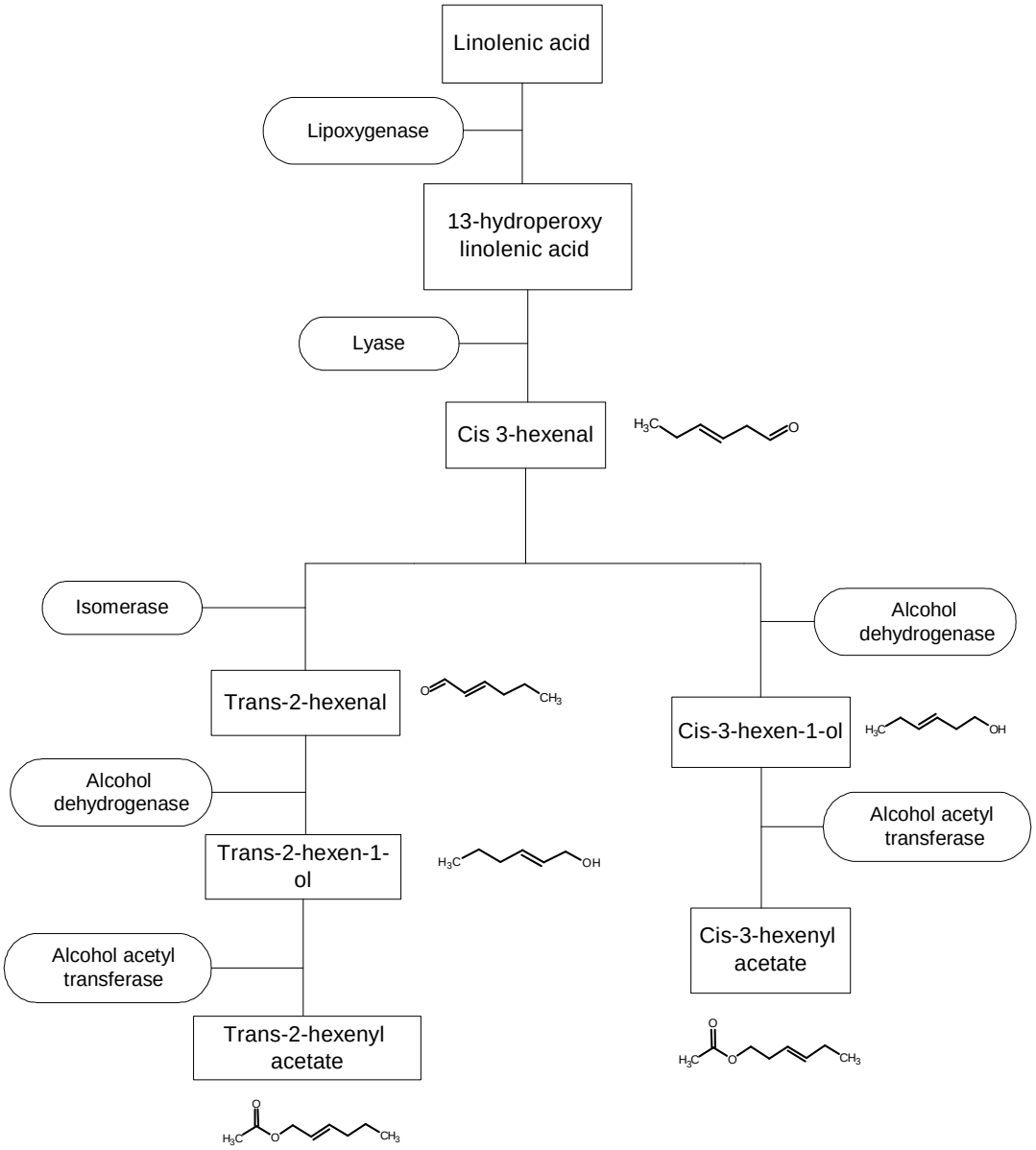


Figure 1.2. LOX pathway from linolenic acid (LnA). LOX has a preferential action on linolenic acid compared to linoleic acid.

Instead, both absence of water and long holding times at critical temperatures are known to favor non-enzymatic auto-oxidation and photo-oxidation, which are typically defined as slow reactions. Therefore, transformation phenomena of triglycerides can be related to olive oil chain steps as follows (Fig. 1.3): (i) biosynthesis of triglycerides occurring during growing of olive tree module; (ii) lipase activity occurring during the management of ripened olives module; (iii) enzyme oxidation occurring during the oil extraction module; (iv) radical oxidation occurring during the oil storage and distribution module.

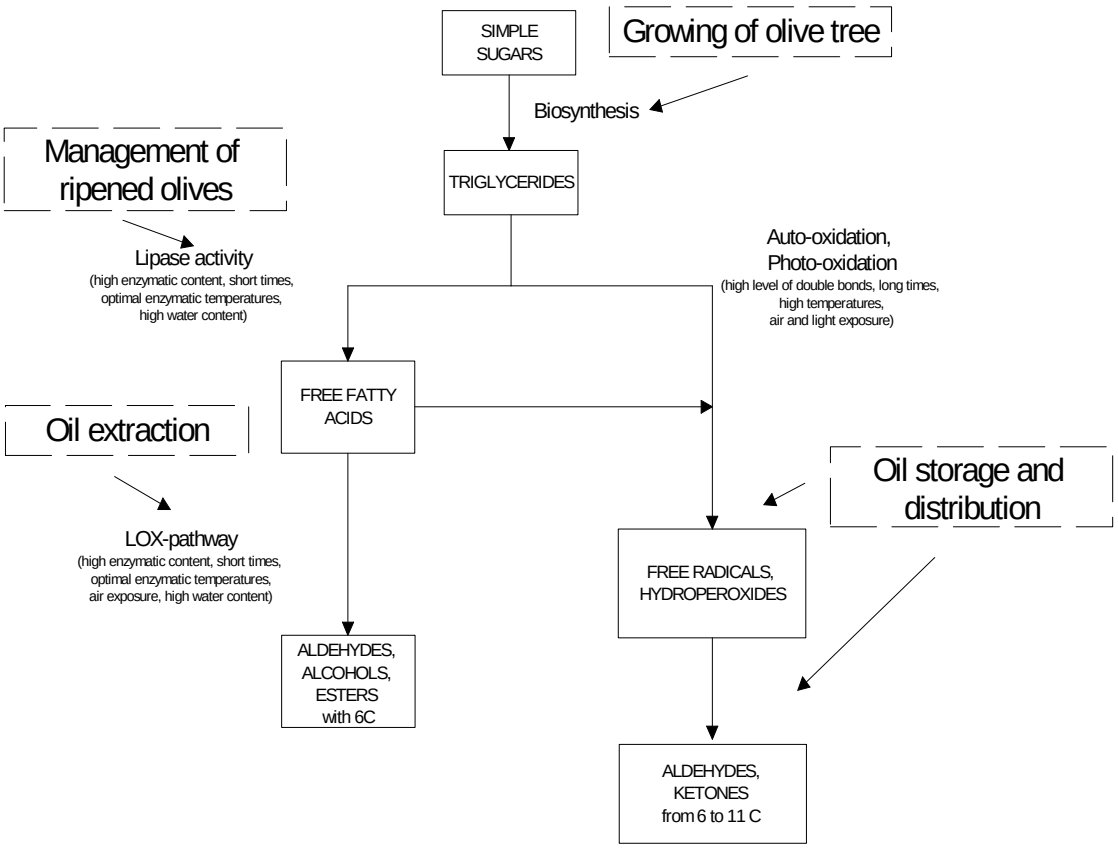
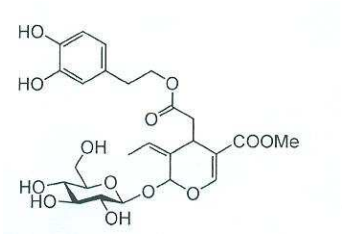


Figure 1.3. Transformation pathway of triglycerides (bracketed text explains operating conditions that favour relevant phenomena)(Zanoni, 2014).

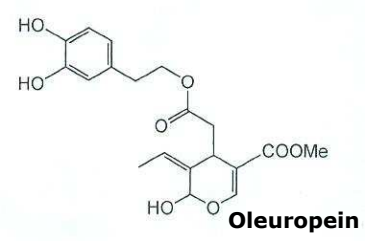
1.2 Transformation patways of phenolic compounds

Simple sugars are also precursors of olive oil phenolic compounds. Oleuropein and ligstroside are the most important secoiridoids in olives. In the proposed biosynthetic pathway for secoiridoid production (Ryan *et al.*, 2002), simple sugars give rise to amino acids, phenylalanine and tyrosine, which in turn are changed into phenolic acids and then secoiridoids. During olive ripening the typical browning of ripe fruits occurs. This colour change is due to the decrease in clorophylls and secoiridoids and formation of anthocyanins (El Riachy *et al.*, 2011). Oleuropein and ligstroside are thought to be subjected to degradation, resulting in hydrolytic and oxidative changes of both an enzymatic and non-enzymatic nature (Artajo *et al.*, 2007; Gutierrez-Rosales *et al.*, 2010; Kalua *et al.*, 2006, Servili *et al.*, 2004). Hydrolytic degradation pathway (Fig. 1.4) causes fast formation of aglycons (3,4-DHPEA-EA - oleuropein aglycone; p-HPEA-EA - ligstroside aglycone), as a result of the loss of a sugar molecule, which can be caused by β-glucosidase activity. Aglycons thus obtained can undergo isomerization, which involves opening of the secoiridoidic ring, followed by rearrangement into open dialdehydic forms. Dialdehydic forms in turn decarboxylate into respective aglycons (3,4-DHPEA-EDA - dialdehydic form of decarboximethyl oleuropein aglycone; p-HPEA-EDA - dialdehydic form of decarboximethyl ligstroside aglycone). Finally hydroxytyrosol (3,4-DHPEA) and tyrosol (p-HPEA) compounds are slowly formed by hydrolysis of the ester linkage. Literature data show relationships both between 3,4-DHPEA-EA and bitter intensity and between p-HPEA-EDA and pungency intensity. Oleuropein, 3,4 DHPEA-EA (i.e., oleocanthal), 3,4-DHPEA and p-HPEA compounds have been demonstrated to have human healthy effects. Oxidative degradation of secoiridoids may be both an enzymatic degradation pathway and a non-enzymatic degradation pathway. In the former pathway, polyphenoloxidases (PPO) and peroxidases (POD) in olive paste catalyze, in the presence of oxygen and high water content, the oxidation of phenolic compounds to corresponding quinones; evidence for this oxidation is provided by browning of olive paste being processed. In the latter pathway, which is connected to termination reactions of radical auto-oxidation of triglycerides to peroxides and derivatives, the release of hydrogen atoms by phenolic compounds can inhibit the formation of hydroperoxide radicals. The above-mentioned phenomena cause a degradation of phenolic compounds and can bring about changes in both intensity of sensory descriptors such as bitter and pungent and degree of antioxidant power of oil, resulting in decreased shelf-life and nutritional value of product. An exception is represented by β-

glucosidase activity that increases the amount of phenolic compounds in olive oil, as aglicons are more soluble in oil than oleuropein and ligstroside. Phenomena on secoiridoids have been shown to occur at different steps of extra virgin olive oil chain (Fig. 1.5). Both biosynthesis of secoiridoids and their first enzymatic hydrolysis to form aglicons occur during growing of olive tree module. A remarkable presence of 3,4-DHPEA and P-HPEA also in ripe olives (Vinha et al., 2005) should be regarded as a result of biosynthetic pathway of secoiridoids as well as formation of anthocyanins (El Riachy *et al.*, 2011). During oil extraction module, especially during olive crushing and malaxation, enzymatic degradation prevails, as well as the first steps of hydrolytic degradation, leading to the formation of dialdehydic forms of decarboxymethyl aglycons. Instead, it is non-enzymatic oxidative degradation as well as 3,4-DHPEA and P-HPEA forming hydrolytic degradation that prevail during oil storage and distribution module. It should be noted that enzymatic activities have both an endogenous and exogenous nature as a result of contamination by moulds, bacteria and yeasts (Vichi *et al.*, 2011).

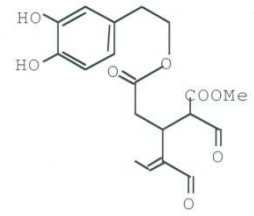


Oleuropein



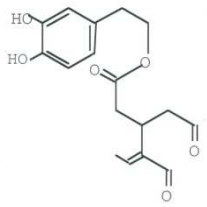
Oleuropein aglycone

3,4-DHPEA-EA

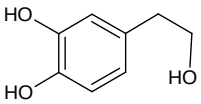


Dialdehydic form of

3,4-DHPEA-EA



3,4-DHPEA-EDA



3,4-DHPEA

Figure 1.4. Hydrolytic degradation pathway of oleuropein.

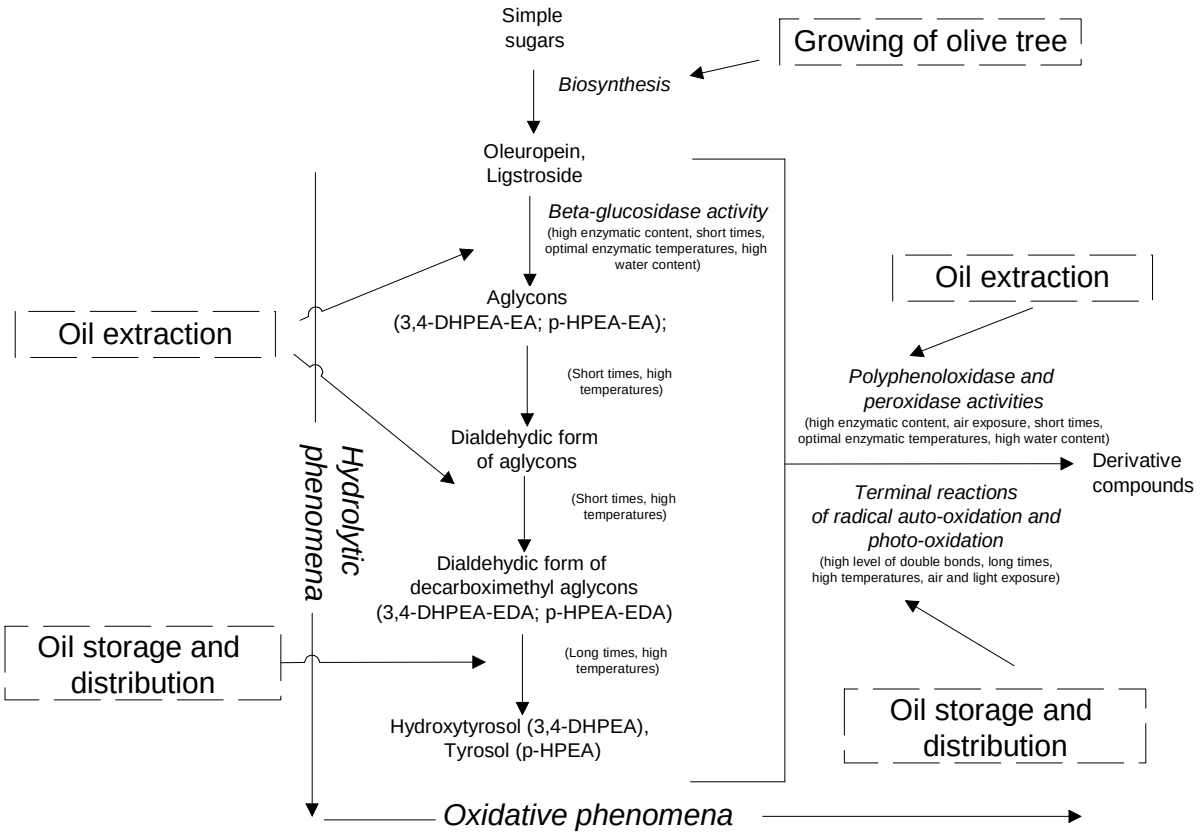


Figure 1.5. Transformation pathway of secoiridoids (Zanoni, 2014)(bracketed text explains operating conditions that favour relevant phenomena).

1.3 Extra virgin olive oil volatile compounds

Even though they are minor components of extra virgin olive oil, volatile compounds play a critical role on liking and legal conformity of product. They are so numerous that no clear pathways of biosynthesis and transformation have been so far evidenced. However, volatile compounds can be correlated with common sensory attributes, both positive and negative (Di Giacinto *et al.*, 2010, Morales *et al.*, 2005), as shown in Table 1.1 and Table 1.2.

Taking into consideration the involved phenomena reported in Table 1.1 and 1.2, fusty, musty and winey-vinegary defects have been shown to be influenced by operating conditions performed during both the management of ripened olives module and the oil extraction module. Microbial contamination and activity are very important to develop the above negative attributes. Instead, rancid negative attribute may significantly occur during the oil storage and distribution module. Fruity attribute is expressed by LOX pathway, it is then influenced by the oil extraction module condition. All above-mentioned phenomena of synthesis and transformation are also limited by component mass transfer to oil matrix during processing. Triglycerides content depends on the operating conditions for olive oil extraction. Malaxation has to create a continuous mass of oil in the olive paste; in order to disrupt the interfacial films, olive paste has to be mixed modifying the dispersion degree of oil droplets and promoting the coalescence phenomena. Separation, for example by "decanter" centrifugation, has to maximize olive oil yield separation, minimizing retention times to maintain the positive effects of malaxation on olive oil yield. Secoiridoids content depends on their solubility in oil and water. They are amphipathic molecules, but their polarity makes them more soluble in water than in oil; therefore, their concentration in oil is always very low. However, solubility of secoiridoids may change as a result of their transformation pathway. Elimination of glucose by secoiridoids determines a change of polarity, and the relevant aglycones are more soluble in oil than in water. Therefore, malaxation and separation are critical conditions; they have to aid mass transfer of secoiridoids to oil and to minimize secoiridoids removal with vegetation water.

Table 1.1. Correlation between volatile compounds and fruity , fusty, musty and winey sensory attributes.

Volatile compounds	Sensory attributes	Involved phenomena
Hexan-1-ol	Fruity and other positive attributes	LOX pathway
1-Penten-3-ol		
cis-2-Penten-1-ol		
cis-3-Hexen-1-ol		
trans-2-Hexen-1-ol		
Hexanal		
cis-3-Hexenal		
trans-2-Hexenal		
2,4-Hexadienal		
1-Penten-3-one		
Hexyl acetate		
cis-3-Hexenyl acetate		
trans-2-Hexenyl acetate		
Butyric acid	Negative attribute: Fusty or muddy sediment	Microbial anaerobic fermentation
Butan-2-ol		
Butyl acetate		
Ethyl butirate		
Ethyl propionate		
Methyl propionate		
Benzene-ethanol	Negative attribute: Musty or humid	Moulds and yeasts development
Phenol		
4-Ethylphenol		
Guaiacol		
4-Ethyl guaiacol		
cis-1-Otten-3-ol		
Pentanal		
Octane		
Ethyl acetate	Negative attribute: Winey-vinegary or acid-sour	*Lactobacillus* and yeasts development
Methyl acetate		
Cis-2-esenil acetate		

Table 1.2. Correlation between volatile compounds and rancid sensory attribute.

Volatile compounds	Sensory attributes	Involved phenomena
Benzaldehyde		
Pentanoic acid		
1-Pentanol		
trans-2-Pentenal		
Hexanoic acid		
Hexanal		
Heptane		
1-Heptanol		
2-Heptanol		
Heptanal		
2,4-Heptadienal		
trans-2-Heptanal		
6-Methyl-5-epten-2-one	Negative attribute: Rancid	Radical oxidation of triglycerides
2-Heptanone		
Octanoic acid		
1-Octanol		
cis-1-Octen-3-ol		
trans-2-Octanal		
Octanal		
2-Octanone		
1-Octen-3-one		
1-Nonanol		
Nonanal		
2,4-Nonadienal		
trans-2-Nonenal		
trans-2-Decenal		
2,4-Decadienal		

1.4 CCPs and markers of extra virgin olive oil process control

Process control is focused on monitoring, which is the act of conducting a planned sequence of measurements of parameters to assess if process is under control.

Monitoring of extra virgin olive oil production may be planned at two levels.

The first level aims at controlling planned operating conditions (i.e., residence times, temperatures, rotational speeds, clarity levels, etc...). It differs according to the equipment and plants used, and general indications cannot be given on this level of monitoring.

The second level aims at controlling the effects of operating conditions on product during processing. It is not dependent upon machines used, and therefore, monitoring may be generally performed. Table 1.3 shows an example of this approach (Zanoni, 2014).

The above-mentioned transformation phenomena of the main components of olive oil are planned to be monitored by critical steps of process (i.e., CCPs). Some significant parameters (i.e., markers) are chosen to be measured in olive oil samples. Two levels of control are identified: a basic and a high level. They require increasing analytical work, and, however, allow for increasing efficacy in terms of control. High level control markers are a more direct expression of monitoring phenomena than basic level control markers.

Effects of monitored phenomena on extra virgin olive oil quality are shown in the last column in Table 1.3.

Basic level control includes chemical and sensory markers, which are commonly measured to control conformity of both process extraction and extra virgin olive oil. Some table cells are blank, as the relevant simple markers have not yet been identified in the literature.

With reference to high level control, some overall markers are chosen for LOX-pathway, radical oxidation of triglycerides and activities of spoilage microorganisms such as "Sum of C6 volatile compounds content from free linolenic acid", "Sum of hexanal and nonanal volatile compounds content" and "Sum of volatile compounds correlated with negative attributes", respectively.

3,4-DHPEA-EDA may be considered a direct marker of both extraction yield and integrity of phenolic compounds during olive oil processing; whereas 3,4-DHPEA/hydroxytyrosol may be considered a marker of degradation of phenolic compounds during olive oil storage.

Microbial counts are also included to monitor mould and yeast contamination in olives and oil.

Material balances are finally suggested to measure efficacy of component mass transfer to oil during malaxation and separation.

Table 1.3. Process control of extra virgin olive oil (Zanoni, 2014).

What? **Monitoring phenomena**	**Where?** **Critical Control Points (CCPs)**	**How?** **Basic level control markers**	**How?** **High level control markers**	**Why?** **Effects on EVOO quality**
Biosynthesis of TGs	Olive harvesting	Visual inspection for olive ripening indices	Olive oil content	Yield
Enzymatic hydrolysis of TGs	Olive storage	Visual inspection for olive sanitary conditions	Olive mould count	Acidity level Shelf-life stability
	Olive oil separation	Olive oil acidity		
LOX-pathway of TGs	Olive oil separation	Olive oil taste	Sum of C6 volatile compounds content from C18:3 free fatty acid	Fruity level
Radical Oxidation of TGs	Olive oil storage	Olive oil taste Peroxide value UV spectroscopic indices	Sum of hexanal and nonanal volatile compounds content	Rancid level Peroxide value level UV spectroscopic indices level Antioxidant power
Mass transfer of TGs	Olive paste malaxation	Visual inspection for olive oil coalescence	Microscopic or rheological measurements of olive oil coalescence	Yield
	Olive oil separation	Visual inspection for efficacy of separation	Material balances	
Biosynthesis of PhCs	Olive harvesting	Visual inspection for olive ripening indices	Total phenolic compounds content Oleuropein content	Bitter/Pungency level Antioxidant power Shelf-life stability
Hydrolytic phenomena of PhCs	Olive oil separation	-	3,4-DHPEA-EDA content	Antioxidant power Shelf-life stability
	Olive oil storage	-	3,4-DHPEA content	
Oxidative phenomena of PhCs	Olive paste malaxation	Olive paste color	3,4-DHPEA-EDA content	Bitter/Pungency level Antioxidant power Shelf-life stability
	Olive oil storage	Total phenolic compounds content		
Mass transfer of PhCs	Olive paste malaxation	-	Total phenolic compounds content 3,4-DHPEA-EDA content	Bitter/Pungency level Antioxidant power Shelf-life stability
	Olive oil separation	Olive oil taste Total phenolic compounds content	3,4-DHPEA-EDA content Material balances	
Microbial contamination and activity	Olive storage	Visual inspection for olive sanitary conditions	Olive mould and yeast count	Fusty, Musty and Winey-vinegary level
	Olive oil separation	Olive oil taste	Sum of volatile compounds correlated with negative attributes	

1.5 Main issues

The following conclusive remarks can be drawn from the foregoing observations.

- Extra virgin olive oil can be regarded not just as a product obtained from simple mechanical extraction from olives; rather, it should be considered as a result of complex changes in fruit components. These changes affect, on the one hand, both free and esterified fatty acids, and on the other hand, secoiridoid phenolic compounds, resulting in formation of derivatives, which impact qualitative characteristics for both sensory acceptability and nutritional value of extra virgin olive oil.
- Health claims related to polyphenols in olive oil (Anonymous, 2011) attribute positive effects (i.e., antioxidant properties and positive effect on lipid metabolism) on human health, when the following conditions of use are applied: "*20 g of an olive oil with a polyphenol content of 200 mg/kg / Min 2 mg per day of hydroxytyrosol*". From a nutritional point of view, a reference to hydroxytyrosol appears to be appropriate, as it is one of the most powerful antioxidants. This reference, however, appears inappropriate from a production point of view, as hydroxytyrosol (3,4 DHPEA) is a direct marker of hydrolytic degradation of phenol compounds.
- Microorganisms take on a significant role in transformation pathways of extra virgin olive oil. Mould, bacteria and yeast contamination is especially associated with sensory defects and enzymatic degradation activities.
- Although transformation pathways of the main components of olive oil during processing are known, no data on relevant kinetics (i.e., pseudo-*n* order reactions, K_0 and E_a) are reported in the literature. It would be useful and recommended to collect a large number of data on this subject. This would avoid incomplete production recommendations such as the customary rule that temperature of malaxation has not to exceed 27°C. Nothing confirms that 27°C is a temperature tied to some particular phenomenon; in addition, indication of

temperature without indication of time is incomplete and misleading recommendation.

- In view of numerous complex transformations that occur in olive oil components during the extraction process, the current approach to optimize operating conditions of oil mill is still based on the "*trial and error*" principle, using a limited number of results from numerous literature data. On the other hand, obtaining useful information of wide application on the effect of either olive or extraction processing on the quality of extra virgin olive oil may appear to be difficult and misleading. This may derive from the fact that most experimental designs have been developed on the basis of industrial oil mills and mini oil mill plants, which are characterized by poor reproducibility and laborious process control. An experimental approach based on lab-scale processing under controlled conditions (Table 1.3) appears a convenient alternative.

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2. AIM AND EXPERIMENTAL DESIGN

Quality characteristics of virgin olive oil depend on several transformation phenomena of oil composition during processing.

First of all, the harvesting time of drupes is a CCP and could compromise extra virgin olive oil quality. It would also be acceptable to the producers to control the state of ripeness of the olives in a quick and simple way. Vis/NIR spectroscopy is one of the most promising technologies for the analysis of technological and nutraceutical features of fruit and vegetables.

On the other hand, the malaxation is the critical operation to control the transformation phenomena such as enzyme reactions. Its monitoring is combination time- temperature taken during process. The role of time- temperature conditions for malaxation was investigated but contradictory results were often obtained.
So this PhD thesis research project focuses on two different topics:

1) Study on the biochemical maturation of the fruit.

Since the traditional methods of visual assessment of the degree of ripeness of the olives have evident application limits, it would be appropriate to identify indicators directly related to the biochemical maturation of the fruit, which are able to be measured "in the field" and that they are potentially object to predictive models of ripening.
Furthermore the 2014 crop season was characterized by a very intense attack of the olives fruit fly in Tuscany. So in this thesis research project was evaluate also the impact of the olive fruit fly attack in the 2014 crop season in Tuscany, with particular attention to the chemical characteristics of the olive fruits and the extracted extra virgin olive oil.

2) Kinetics study during malaxation

The aim was to carry out a kinetic study time-temperature of extraction yield, coalescence and phenolic compounds. Actually the extraction yield was measured indirectly. Little is known about the chemical and physical phenomena during malaxation. For this reason there was a finalization of the detection method of the oil droplets and get clear information from the literature to identify indices strictly related to critical phenomena and optimal conditions during malaxation.

A schematic vision of the thesis with aim, methods and process, is given in Figure 2.1 where to simplify is associated the Topic 1 (T1) to "OLIVES" and Topic 2 (T2) to "MALAXATION".

For each topic the results will be presented in the form of articles and final conclusions will be following.

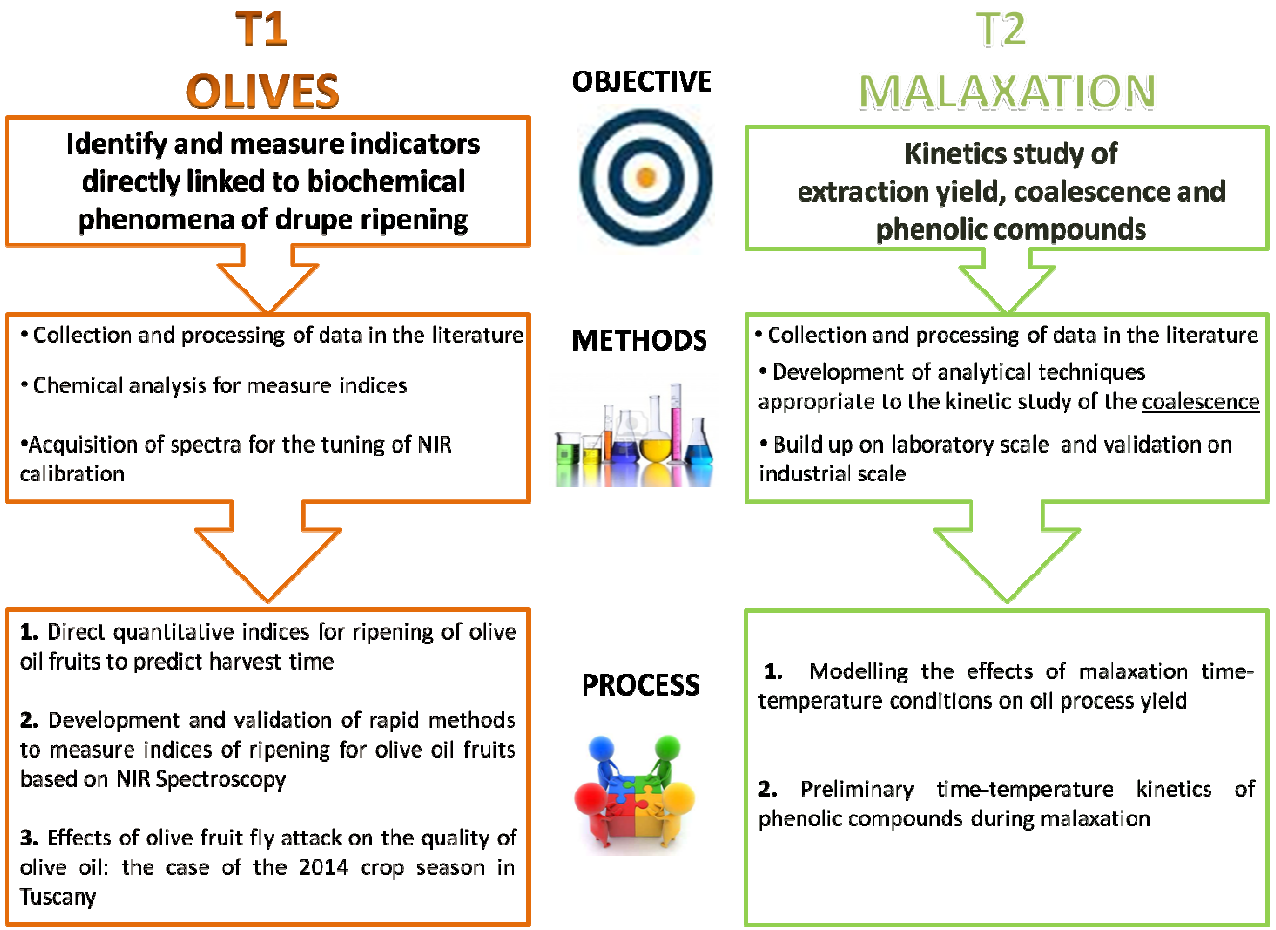


Figure 2.1. Flow chart of the topics reported in this Ph.D. thesis.

3. RESULTS AND DISCUSSION

T.1 OLIVES

3.1 Direct quantitative indices for ripening of olive oil fruits to predict harvest time

3.1.1 Introduction

Optimal time for harvesting of olive oil fruits is generally considered to be when the highest oil concentration has been achieved. Once extracted, oil should also have the desired qualitative level, which, in most cases, corresponds to extra virgin olive oil.

In view of the high cost of olive picking operations, careful planning of olive harvest may be crucial to maximize olive oil production yields while minimizing quality losses in the oil-mill (Bertolotti, 2014). This aim may be hard to achieve, as both physical and chemical transformation phenomena of olive oil fruits during ripening are affected by several agronomical factors, such as olive cultivar, geographical area of production, climatic conditions during crop season and olive health condition. Hence, the monitoring of selected ripening indices may be essential both to define an adequate ripening degree of olive oil fruits and to predict harvest time.

Literature studies have revealed three possible approaches to predict harvest time of olive oil fruits. The first approach is based on monitoring of semi-quantitative indices (i.e. the semi-quantitative approach). The method for olive skin and pulp color measurement (i.e. Maturity or Ripening Index) is the most common tool available to olive growers (Anonymous, 2001; Uceda and Frias, 1975). This method is easy and can be applied in the field by non-specialists too, but it has some application limits. It cannot be applied to all cultivars and is based on a strong indirect correlation with transformation phenomena of the chemical composition of olives during ripening. The second approach consists in evaluating the qualitative characteristics of olive oil, extracted by a laboratory scale mill, as indirect ripening indices (i.e. the lab oil extraction approach). This approach has been frequently used to evaluate the effect of individual cultivars, geographical origin and climatic conditions on the quality of extracted olive oil (Dag *et al.,* 2014; Yorulmaz *et al.,* 2012; Benito *et al.,* 2012; El Riachy *et al.,* 2012; Yousfi *el al.,* 2006; Vinha *et al.,* 2005; Aguilera *et al.,* 2005; Salvador *et al.,* 2001). This approach has the benefit of being a quantitative approach and trying to relate the ripening of olives with the quality of extracted olive oil. Its practical limits include the need to have an equipped laboratory, and mainly the fact that results may be affected

by oil extraction procedures. The quality of extracted olive oil will not only depend upon the ripening level of olives, but it will also be the result of complex changes in olive fruit components, depending on operating conditions of the applied extraction system (Zanoni, 2014). The third approach consists in monitoring those indices that are directly connected to biochemical phenomena of ripening (i.e. the direct approach). The most commonly used index is the oil content of olives, which is measured during the development and ripening of fruits to study the kinetics of oil accumulation (Dag *et al.,* 2014; Breton *et al.,* 2009; Mailer *et al.,* 2007; Beltran *et al.,* 2007; Mickelbart *et al.,* 2003; Dag *et al.,* 2011; Garcia *et al.,* 2013). Biochemical processes result in both accumulation of oil and formation of a characteristic acidic composition of olive oil. The precursor of biosynthesis of fatty acids is acetyl-CoA, derived from catabolism of sugars such as glucose and fructose (Harwood *et al.,* 2013; Salas *et al.,* 2002; Nergiz and Engez, 2000; Rangel *et al.,* 1997; Lavee 1986). For this reason, the olive sugar content, which has been found to be correlated to the oil content (Migliorini *et al.,* 2011; Menz and Vriesekoop 2010; Cherubini *et al.,* 2009), has also been proposed as a ripening index in recent years. This approach has the benefit of directly investigating phenomena that form composition of native olives. As a result, it can then be used to set up kinetics for harvesting time prediction. The direct approach has though some operating limits. A complex experimental design is required to develop it, and a study on ripening should not be restricted to kinetics of variation in oil and sugar contents. It should rather be extended to other olive components, such as phenolic compounds, in consideration of their qualitative relevance to extractable oil (Servili *et al.,* 2004). The aim of this work was to enrich the number of experimental data available in the literature by introducing also the direct monitoring of phenolic compound evolution during ripening of olive oil fruits. In addition, the validity of direct approach was evaluated to predict harvesting time of olive oil fruits, as compared to semi-quantitative measurement of olive skin and pulp color.

3.1.2 Materials and methods

3.1.2.1. Olive oil fruit samples

During 2013 crop season, olive oil fruits (*Olea europea* L. *Frantoio* and *Moraiolo* cv.) were supplied both by a farm (identified as A) located in Fiesole (Florence, Italy) and by a farm (identified as B) located in Pelago (Florence, Italy). Regular irrigation of orchard was only applied to farm A. Full blooming occurred at farm A by 5th June for *Frantoio* and by 10th June for *Moraiolo*; full blooming occurred at farm B by 10th June for *Frantoio* and by 15th June for *Moraiolo*.

Ten *Frantoio* and *Moraiolo* cultivar trees were selected for each farm, as they were quantitatively representative of orchard. Olives were picked by hand once a week at 08:00 a.m. from the beginning of September to the beginning of December; they were picked along plant circumference at approx. 1.7 m from the soil. Olives (300 – 400 g), which presented no infection or physical damage, were selected for each crop date. Olive samples were quickly transported to the laboratory to be analysed.

3.1.2.2. Analysis methods

Olive samples were analysed for average weight, pulp/stone ratio and Maturity Index (Anonymous, 2001; Uceda and Frias, 1975). The Maturity Index was based on the evaluation of olive skin and pulp colours. Values ranged from 0 (skin colour deep green) to 7 (skin colour black with all the flesh purple to the stone).

A homogeneous batch of olives (i.e. approx. 300 g) was crushed in a laboratory crusher (Zeutec, Rendsburg, Germany), and the olive paste was used for the following chemical analyses.

Water Content

The water content was measured on olive paste by heating 60 g of sample in an oven at 105°C until reaching constant weight. Results were expressed as paste moisture content (%).

Oil Content

The total oil content was determined on 5 g of dried olive paste (see the above oven method). Samples were extracted with hexane in an automatic extractor (Randall mod.148, VELP Scientifica, Milan, Italy), following the method of Cherubini *et al.*, 2009. Results were expressed as oil content on dry matter basis (g kg^{-1} dm).

A theoretical estimate of mill processing yield from olive samples (*CPY*) was also calculated as reported by COI (Anonymous, 2001):

$$CPY = FMO \cdot 0.82 - 0.2 \cdot (Mst - 56) \quad [1]$$

Where: *CPY* is the calculated processing yield (%), *FMO* is the oil content of olive paste on fresh matter (%), *Mst* is the paste moisture content (%).

Sugar Content

Eight g of olive paste was cold extracted (6 ± 2°C) with distilled water in a 200 mL flask for 2 hours. The content of the flask was filtered on paper, and 10 mL of the obtained solution was diluted with water in a 20 mL flask. Measurements were performed by analyzing the obtained solution with an enzymatic method using an automatic ChemWell analyzer (Awareness Technology, ChemWell 9210, Palm City, FL). Three enzymatic kits were used to measure, respectively (i) the sum of two monosaccharides contents, namely glucose and fructose; (ii) the sum of disaccharide sucrose content with glucose and fructose contents; (iii) the mannitol content. All kits were purchased from R-Biopharm (Darmstadt, Germany). Measurements were done by means of external calibration standards: fructose and glucose (purity > 99%, Sigma Aldrich SrL, Milan, Italy), and mannitol (purity > 98%, Sigma Aldrich Srl, Milan, Italy). Results provided by the instrument were expressed in g L^{-1}; they were also converted in sugar content on dry matter basis (g kg^{-1} dm) as the average of two readings, carried out for each sample. The sucrose contents were determined by multiplying for 0.95 the difference between the sum of glucose, fructose, sucrose contents and the sum of glucose and fructose contents.

Phenolic Compounds content

Extraction of phenolic compounds from olive paste was carried out by Cecchi *et al.*, 2013 method. This method consisted in phenolic compounds extraction from fresh olive paste (and not from oil extracted from olive paste) and was able to prevent phenolic degradation during analysis procedure.

Immediately after crushing, 8 g of olive paste was added to a plastic test tube together with 0.500 mL of an internal standard (i.e. syringic acid, 1.5 mg mL^{-1} in a MeOH/H_2O 80:20 solution) and together with 30 mL of EtOH/H_2O 80:20 solution. The mixture was homogenized with ULTRA-TURRAX at 11,000 rpm in an ice bath for 4 min, centrifuged at 4,000 rpm at 10°C for 10 min, and the supernatant was added to a 250 mL flask. The extraction procedure was repeated with 30 mL of EtOH/H_2O 80:20 solution, and the obtained supernatant was added into the flask.

The obtained solution was concentrated under vacuum at 35-40°C, washed twice with hexane to remove aqueous solutions and pigments, centrifuged at 7,000 rpm at 10°C and filtered in a 10 mL flask by a 0.45 µm cellulose acetate membrane. Five mL of methanol was added to the solution, and Milli-Q-Water (Millipore SA, Molsheim, France) was adjusted to volume. The solution was immediately used for the chromatographic analysis.

Chromatographic analyses were carried out by an HP1100 Liquid Chromatograph, equipped with an 1100 Autosampler, a column heater module, a quaternary pump, and coupled with DAD and MS detectors, interfaced to an HP1100 MSD API-electrospray (Agilent Technologies, Palo Alto, CA).

A Hypersil Gold QRP-18 column (4.6 mm, internal diameter; 250 mm, length; 3 µm, particle size) (Thermo Electron Corporation, Austin, TX) was used. It was equipped with a 10 x 4 mm pre-column of the same phase and maintained at 30°C.

Elution was performed at a flow rate of 0.8 mL min^{-1} with a multistep linear gradient, using H_2O at pH 3.2 by formic acid (solvent A), acetonitrile (solvent B) and methanol (solvent C). The three-step linear gradient of both solvents B and C changed as follows: from 2.5% to 27.5% in 45 min, then to 50% in 10 min. Isocratic elution with 50% of solvent B and 50% of solvent C was then maintained for 5 min. Total time of analysis was 60 min. All used solvents were of HPLC grade. The detection of phenolic compounds with DAD was carried out simultaneously at 240 nm, 280 nm and 330 nm. The absorption of total phenolic compounds was at 280 nm.

Syringic acid was chosen as an internal standard. Syringic acid and oleuropein were chosen as external calibration standards to evaluate the relative response factor (RRF); values for each phenolic compound were expressed as $mg_{oleur}\,kg^{-1}$ on dry matter (dm).

3.1.2.3. Data processing

The coefficient of variation (CV%) was calculated for all above analytical methods. Olive samples were chosen and used to carry out analyses in quintuplicate. CV% was calculated from the standard deviation and resulted to be as follows: (i) 5% for the water content, the sugar content and the total phenolic compounds content; (ii) 7% for the oil content; (iii) % values for each phenolic compound, as reported by Cecchi *et al.*, 2013.

Kinetics for ripening indices of olive oil fruits was processed using Table Curve 2D Version 4 software (Systos Software Inc., Richmond, CA).

3.1.3. Results and discussion

Our study was purposely carried out on two different olive cultivars in two different olive orchards, as for both microclimate areas and agronomic techniques, in order to have available a large number of experimental data to support the discussion of results. For the sake of brevity, not all results obtained can be reported below, but only those regarded as the most representative results are shown. In order to provide a better comparison of the results obtained, the ripening times for all olive samples tested were expressed as "Day After Full Blooming" (DAFB).

3.1.3.1. Oil and sugar content kinetics during ripening and their modelling

Experimental trends for oil and sugar contents during olive ripening are shown in Figure 3.1.1; the ripening time was approx. 85 DAFB (corresponding to September) to approx. 180 DAFB (corresponding to December). Formally similar kinetics was obtained for both studied cultivars and farms. Oil contents showed an increasing sigmoidal trend tending to an asymptote; sugar contents showed an opposite behaviour with a decreasing sigmoidal trend tending to an asymptote. The sugar contents were expressed by the combination of measured glucose and fructose contents in olives. No significant contribution was obtained from saccharose and mannitol contents (data not shown). As reported in the literature (Migliorini *et al.,* 2011; Cherubini *et al.,* 2009), and known in

the northern hemisphere (Nasini and Proietti, 2014), olive fruits started to accumulate oil at an accelerated rate during September and up to the first half of October. In the second half of October the rate of increase in oil content decreased, until a basically maximum, constant oil content was reached during November.

Since the precursor of biosynthesis of fatty acids is acetyl-CoA, derived from decomposition of sugars such as glucose and fructose, oil and sugar contents of olives during ripening showed similar, though opposite, kinetics.

In agreement with Cherubini *et al.*, 2009, experimental data were statistically processed in order to model the kinetics (Fig. 3.1.1). The kinetics was represented with the following sigmoidal model, optimised by non-linear regression analysis:

$$y = \frac{a+b}{\left\{1+\exp\left[\frac{-(x-c)}{d}\right]\right\}} \qquad [2]$$

Where y is the sugar or oil content (g kg^{-1} dm); x is the time (DAFB); *a, b, c* and *d* terms are proportionality constants. The *a* term relating to the kinetics model for sugar content also has a phenomenological meaning of minimum asymptotic value attained by the sugar content during olive ripening. Table 3.1.1 shows values of the model constants for each farm and each cultivar; determination coefficient r^2 and *F* value of the model are also reported. The sigmoidal model fitted well the experimental data. Frantoio *cultivar* was found to reach a lower constant sugar content than Moraiolo *cultivar*. In addition, olives obtained from Farm B attained a lower constant sugar content than those obtained from Farm A, regardless of the *cultivar* type. The above-mentioned modelling of oil and sugar content kinetics during ripening may improve the prediction approach to harvesting time of olive oil fruits, as compared to the common method used for olive skin and pulp colour measurement (i.e. the Maturity Index). For instance, assuming maximization of the Calculated Processing Yield (*CPY* values) as the harvesting target, the modelling applied to our experimental data would show that this target may be attained on the basis of the following phenomenological evidence: a constant maximum value for oil content corresponds to attainment of a constant minimum value of sugar content (Fig. 3.1.2).

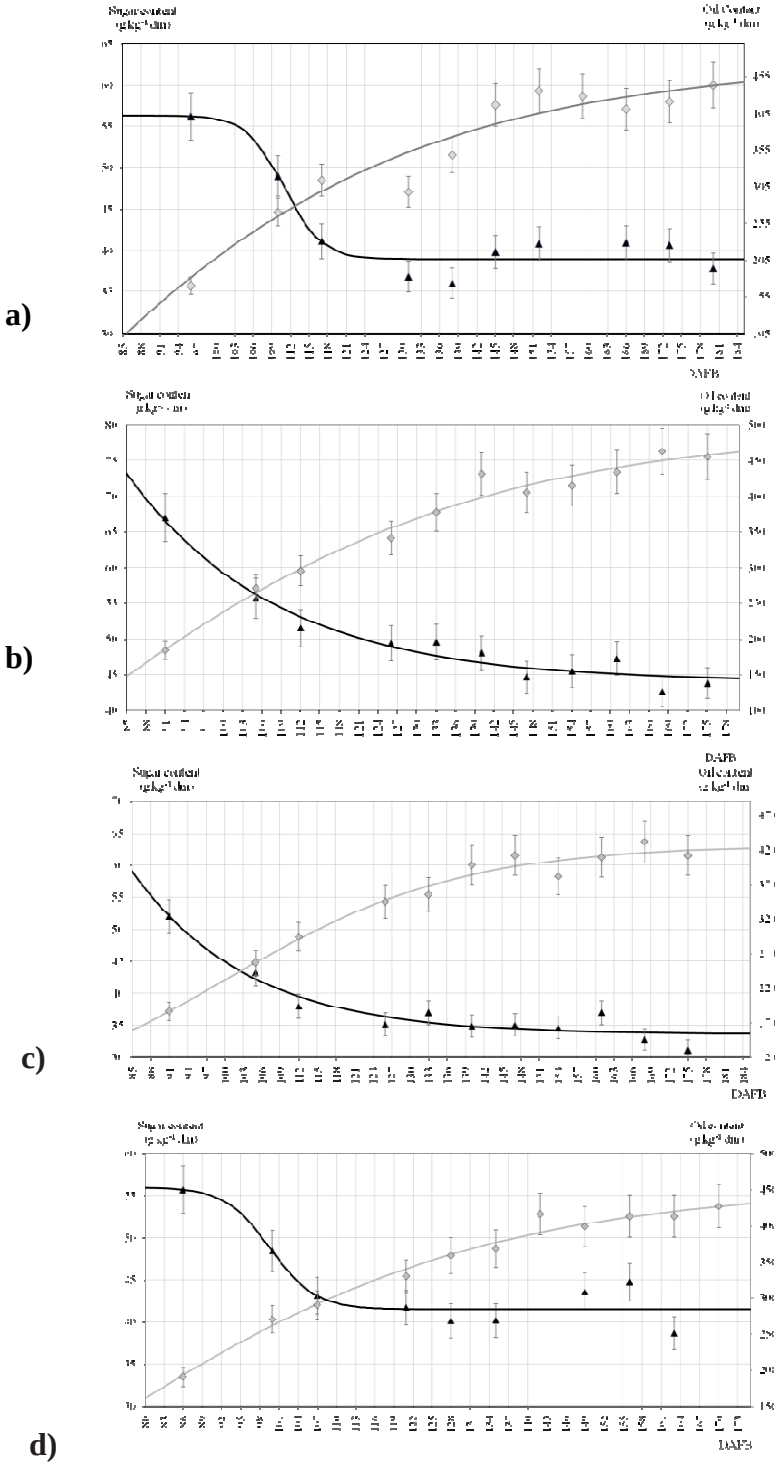


Figure 3.1.1 Evolution of sugar contents during olive oil fruit ripening (▲symbols are experimental data; continuous lines are sigmoidal model) and oil (◆ symbols are experimental data; continuous lines are sigmoidal model). **a)** Farm A, *Frantoio* cv; **b)** Farm A, *Moraiolo* cv; **c)** Farm B, *Frantoio* cv; **d)** Farm B, *Moraiolo* cv. Experimental data are shown with their standard deviation.

Table 3.1.1. Values of sigmoidal model constants (dm:dry matter; DAFB:day after fullblooming).

		Sugar						Oil					
		a g kg^{-1} dm	b g kg^{-1} dm	c DAFB	d DAFB	r^2	F	a g kg^{-1} dm	b g kg^{-1} dm	c DAFB	d DAFB	r^2	F
Farm A	*Frantoio*	**39.0**	17.4	110.9	-3.0	0.93	25.2	-1044.7	1519.9	46.7	35.2	0.92	27.4
	Moraiolo	**44.0**	2567.7	-17.4	-22.9	0.96	52.9	37.1	389.4	100.1	19.6	0.98	94.7
Farm B	*Frantoio*	**33.6**	968.2	19.3	-18.2	0.93	29.2	-394.1	887.7	70.3	32.9	0.97	94.9
	Moraiolo	**41.5**	14.5	99.7	-3.5	0.88	12.2	-268.6	722.9	68.0	31.6	0.98	131.5

Figure 3.1.2 shows that a constant value for the oil content was obtained from approx. 160 DAFB (corresponding to mid-November). This value was correlated to attainment of a *CPY* value of about 20%, which is generally regarded as an optimal target by olive growers and mill managers. Such an indication could not have been obtained by applying the Maturity Index method. Figure 3.1.2 shows that Maturity Index values during ripening of olive fruits remained low (below the threshold values 3 and 4 reported in the literature (Anonymous, 2011) for properly ripened olives), although olives had already reached their highest oil content. Maturity Index values for *Frantoio* cultivar remained low (below 1) at all times during ripening. The Maturity Index value only increased at the end of our trials, reaching highest values as low as approx. 2 (i.e. corresponding to skin colour green with reddish spots on < half the olive fruit surface). Maturity Index values for *Moraiolo* cultivar increased more regularly, but they too showed a highest value as low as approx. 3 (i.e. corresponding to skin colour with > half the olive fruit surface turning reddish or purple). Therefore, harvesting olives at higher Maturity Index values would be of little use, as it would not result in an increase in the oil content of olives.

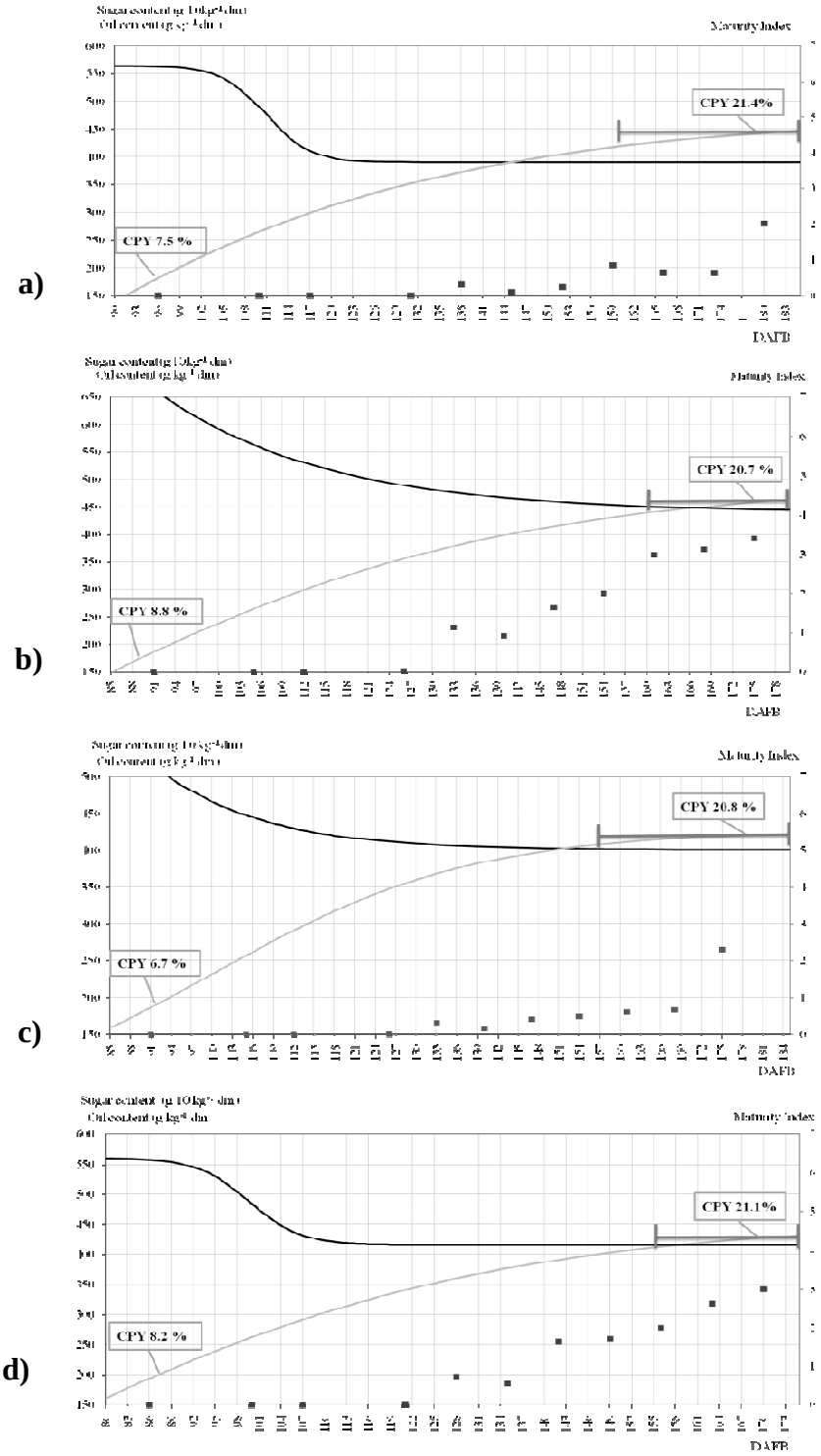


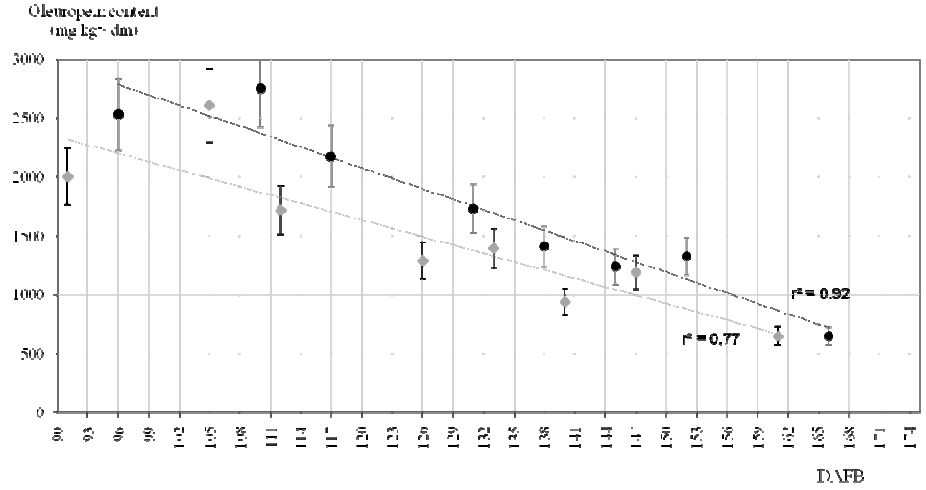
Figure 3.1.2. Sigmoidal kinetics of sugar (black lines) and oil (grey lines) contents, as compared to Maturity Index (■). The sugar content is expressed as g 10 kg^{-1} dm for graphic needs. The following Calculated Processing Yield (*CPY*) values are reported: (i) values at the beginning of olive oil fruit ripening; (ii) values relating to the region at constant maximum value for oil content during ripening. **a)** Farm A, *Frantoio* cv; **b)** Farm A, *Moraiolo* cv; **c)** Farm B, *Frantoio* cv; **d)** Farm B, *Moraiolo* cv.

3.1.3.2. Phenolic compounds content kinetics during ripening and their modelling

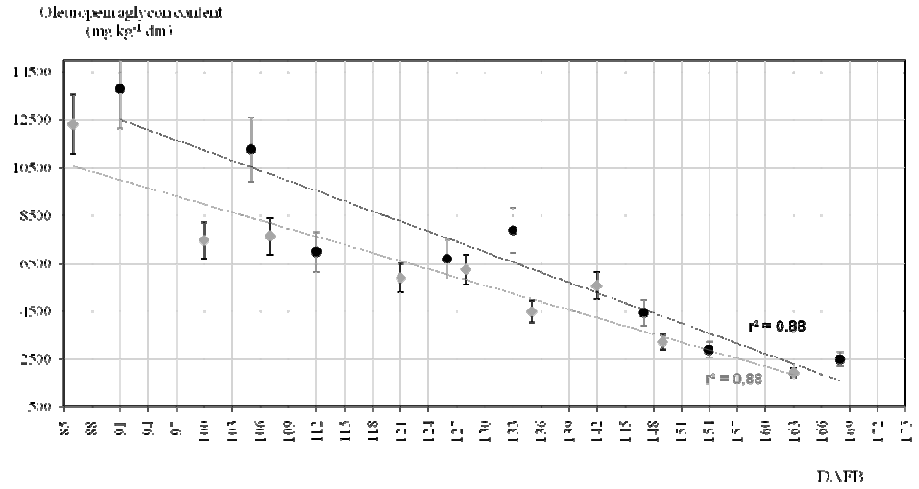
Experimental data collected for total phenolic compounds content during olive ripening are shown in Table 3.1.2. A higher total phenolic compounds content was measured in *Moraiolo* cultivar than that measured in *Frantoio* cultivar for both farm A and B. The total phenolic compounds content showed a decreasing trend during ripening. However, this decrease was irregular and not easy to describe with a kinetic model. The secoiridoids oleuropein aglycon (3,4-DHPEA-EA) and oleuropein were among the most abundant phenolic compounds, in agreement with the literature (Cecchi *et al.,* 2013). The presence of an intense β-glucosidase activity during olive ripening was shown by the higher 3,4-DHPEA-EA content than oleuropein content. The most abundant phenolic compounds found in extra virgin olive oil (Zanoni, 2014; Servili *et al,.* 2004), namely the secoiridoids dialdehydic forms of aglycons, in particular the dialdehydic form of decarboximethyl oleuropein aglycon (3,4-DHPEA-EDA) and the dialdehydic form of decarboximethyl ligstroside aglycon (p-HPEA-EDA) were absent. As a result, the olive oil extraction process had no effect on transformation of phenolic compounds, as the phenolic compounds content was directly measured on olives, and not on oil extracted from olives (Cecchi *et al.,* 2013). Oleuropein and 3,4-DHPEA-EA contents showed a linear decrease during olive ripening; trends were cultivar-dependent and they were similar for both Farm A and Farm B. A modelling was carried out, and significant pseudo zero-order kinetics was set up as follows (Fig. 3.1.3).

Table 3.1.2. Evolution of total phenolic compounds content during ripening (SD: standard deviation; nd: not determined; dm: dry matter; DAFB: day after full blooming).

Farm A						Farm B					
Frantoio			*Moraiolo*			*Frantoio*			*Moraiolo*		
DAFB	Total Phenolic Compounds Content (mg kg^{-1}dm)	SD	DAFB	Total Phenolic Compounds Content (mg kg^{-1}dm)	SD	DAFB	Total Phenolic Compounds Content (mg kg^{-1}dm)	SD	DAFB	Total Phenolic Compounds Content (mg kg^{-1}dm)	SD
96	30037	±1502	91	68333	±3417	91	32350	±1618	86	53720	±2686
110	33244	±1662	105	60997	±3050	105	32194	±1610	100	43475	±2174
117	27861	±1393	112	43529	±2176	112	29834	±1492	107	48475	±2424
131	27467	±1373	126	39314	±1966	126	31927	±1596	121	35322	±1766
138	28512	±1426	133	50313	±2516	133	30417	±1521	128	34233	±1712
145	30264	±1513	140	39162	±1958	140	30455	±1523	135	32438	±1622
152	27427	±1371	147	39602	±1980	147	32113	±1606	142	35702	±1785
159	nd	nd	154	38369	±1918	154	nd	nd	149	30461	±1523
166	23250	±1163	161	nd	nd	161	22400	±1120	156	nd	nd
173	nd	nd	168	31618	±1581	168	nd	nd	163	25308	±1265



a)



b)

Figure 3.1.3. Evolution of phenolic compounds during olive oil fruit ripening for Farm A (● symbols are experimental data; continuous lines are pseudo-zero order kinetic model) and Farm B (◆ symbols are experimental data; continuous lines are model). $\mathbf{r^2}$ is the determination coefficient, and experimental data are shown with their standard deviation. In graph **a)** kinetic data for oleuropein content are reported for Cultivar *Frantoio*; in graph **b)** kinetic data for oleuropein aglycon content are reported for Cultivar *Moraiolo*.

For *Frantoio* cultivar, oleuropein content was modelled by the relationship:

$$(OC) = a - b \cdot t \quad [3]$$

where *OC* is the oleuropein content (mg kg^{-1} dm); *t* is the time expressed as DAFB; *a* and *b* are proportionality constants: (i) *a* is 5615 mg kg^{-1} dm for Farm A and 4464 mg kg^{-1} dm for Farm B; (ii) *b* is 29.5 mg kg^{-1} dm $DAFB^{-1}$ for Farm A and 23.6 mg kg^{-1} dm $DAFB^{-1}$ for Farm B. For *Moraiolo* cultivar 3,4-DHPEA-EA content was modelled by the relationship:

$$(OAC) = a - b \cdot t \quad [4]$$

where *OAC* is the oleuropein aglycon content (mg kg^{-1} dm); *t* is the time expressed as DAFB; *a* and *b* are proportionality constants: (i) *a* is 25400 mg kg^{-1} dm for Farm A and 20327 mg kg^{-1} dm for Farm B; (ii) *b* is 141.6 mg kg^{-1} dm $DAFB^{-1}$ for Farm A and 113.3 mg kg^{-1} dm $DAFB^{-1}$ for Farm B.

Results obtained from this work showed that the direct approach method to measure biochemical indices is useful for monitoring the ripening process of olive oil fruits. This approach can lead to a sensible choice of the most appropriate harvesting time for olives by performing a combined evaluation of kinetics for direct ripening indices selected. An example is given in Figure 3.1.4, where combined kinetic trends of the sugar, oil, and oleuropein contents are shown for *Frantoio* cultivar from one of the orchards studied. The recommended harvesting time for olives can be considered the range around 160 DAFB. This is the time when olives have substantially reached their highest, steady oil content and, consequently, their lowest, steady sugar content.

As shown in Figure 3.1.4, choosing delayed harvesting of olives would result in harvested olives with a low increase in oil content and a considerable decrease in oleuropein content. Conversely, choosing early harvesting of olives would result in harvested olives with a high oleuropein content, but a low oil processing yield and a high risk of fermentation of olive paste due to a high concentration of residual sugars in olives (Figure 3.1.4).

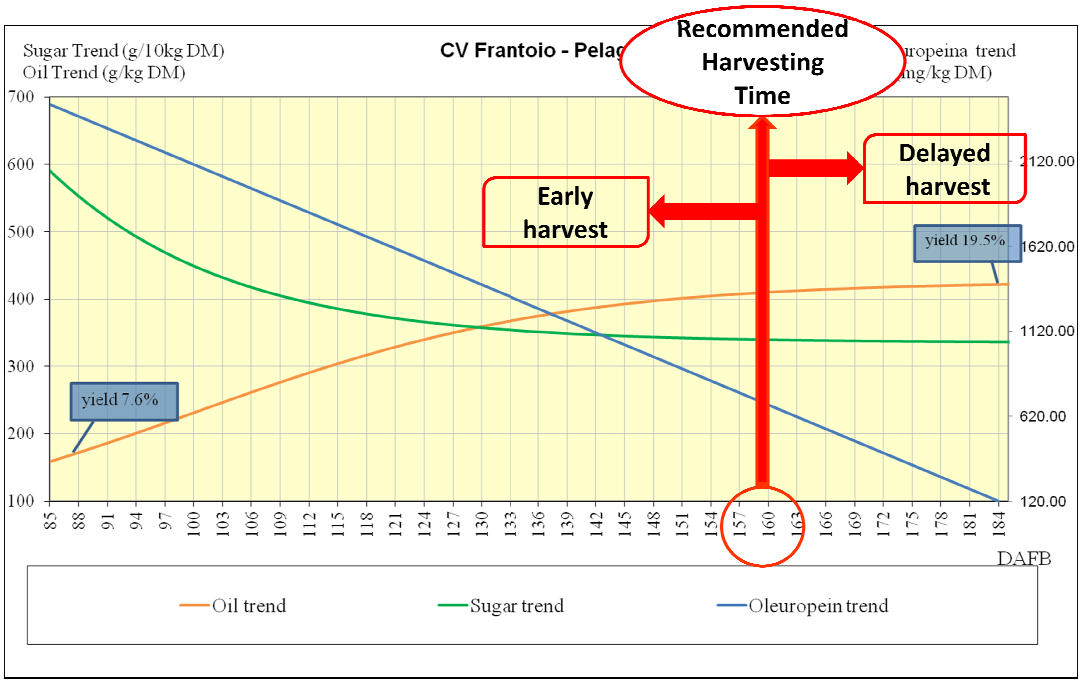


Figure 3.1.4. An example of combined kinetic trends of the sugar, oil, and oleuropein contents to choose the harvesting time of olive oil fruits for *Frantoio* cultivar on one of the orchards studied.

The direct approach method appears to be a useful tool for olive growers to either confirm or refresh their operating choices, which have been applied to olive harvesting over years. It is also a useful tool to understand how a change in harvesting time may affect olive composition and, potentially, the quality of oil to be extracted during either delayed or early harvesting of olives. This type of harvesting may be necessary, for instance, during olive crop seasons in case of both unusual climatic changes and attacks by the olive fruit fly.

The next step of this research activity will include the setting up of rapid methods for evaluating ripening indices.

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3.2 Development and validation of rapid methods to measure indices of ripening for olive oil fruits based on NIR Spectroscopy

3.2.1 Introduction

Bibliographic analysis shows that the quality of olive oil depends primarily on the characteristics of the raw material, defined based on the degree of maturity, olives health and integrity. Since the traditional methods of visual assessment of the degree of ripeness of the olives have strong application limits, indicators directly related to the biochemical maturation of the fruits have been identified. The previous chapter content (3.1) showed that the optimum harvesting time coincides when the sugar and oil become stable in the fruits. However, it is necessary to measure the above indices rapidly and easily. Vis/NIR spectroscopy is one of the most promising technologies for the analysis of technological and nutraceutical features of fruit and vegetables (Berardo and Locatelli, 2009).

The application of NIR spectroscopy on olive oil and products related (olive fruits, paste and pomace) is a well-known analytical tool for the rapid characterization and quantification of characteristic components of these products (Armenta et al., 2010).

Table 3.2.1 shows a schematic representation of the main chronological works from a literature analysis, according to the aim of this thesis, focusing on the application of infrared spectroscopy to whole olives and the paste obtained from olives after the crushing.

Table 3.2.1. Schematic representation of the main chronological works in literature.

	Hermoso *et al.*	**Leon *et al.***	**Garcia-Sanchez *et al.***	**Bendini *et al.***	**Cayuela *et al.***	**Morales-Sillero *et al.***	**Salguero-Chaparro *et al.***	**Maiellaro *et al.***
Year	1999	2003	2005	2007	2009	2011	2013	2014
Aim	Online NIR for evaluating of Moisture and Oil	NIR for evaluating of oil, moisture, acidic composition on whole fruits	Comparative study on NIR, NMR and Soxhlet for oil and moisture determination	FT-NIR *Online* monitoring in mill	NIR for acidity, moisture, yield, in fresh and dried olives	NIR for characterization on “table olives”	Online determination on whole fruits parameters	FT-NIR for moisture and oil determination in paste and pomace
Sample	Olive paste	Whole fruits	Olive paste Pomace	Olives Oil	Whole fruits Olive paste	Whole fruits	Whole fruits Oil	Olive paste Pomace
Sample Number	-	287	2000	287olives lot 161 oils	20 kg olives	448 olives	242 Acidity 252 Moisture 250 Oil	200 paste 180 pomace
Instrument			OliveScan (Foss, Hilleroed, DK) Bruker, NMS 110C/125/40 RTA (Rheinstetten, Alemania)	MPA Multi Purpose Analyzer FT-NIR (Bruker Optics, Milan, Italy)	Portable AOTF-NIR (Luminar 5030, Bimrose., Maryland)	NIR 6500 SY-II (Foss NIRSystems, Silver Spring, MD)	NIR *online*: Vis/NIR Zeiss Corona 45	FT-NIR Buchi NIRFlexN-500
Range IR (nm)	750-2500	400-1700	850-1050	400-2500	1100-2300	400-2498	380-1690	1000-2500
Parameters analyzed	Moisture Oil	Acidic composition Oil Moisture	Oil Moisture	Acidic composition Oil Moisture	Free Acidity Oil Moisture	Acidic composition Oil Moisture Physical parameters	Acidic composition Oil Moisture	Oil Moisture
Statistical Method	PLS	PLS	Artificial neural network	PLS	PLS PCA	MPLSR	PLS PCA	PLS
Software	-	-	-	OPUS r 5.0 (Bruker optics)	Unscrumbler (CAMO Soft. AS, Norway)	WINISI soft.1.5	Matlab soft. package 7.7	NIRCal Buchi 5.2

PLS: Partial Least Squares; MPLSR: Modified Partial Least Squares; PCA: Principal Component Analysis

Bibliographic analysis shows that the NIR application analysis of whole olives and paste concerned in all cases two parameters, the moisture and oil content confirming their recognized role in the oil extraction process.

In some cases (Leon *et al.,* 2003; Bendini *et al.,* 2007; Morales-Sillero *et al.,* 2011) were analyzed the fatty acid composition of the oil extracted in a laboratory or the analysis of free acidity, expressed as oleic acid percentage (Cayuela *et al.,* 2009; Salguero-Chaparro *et al.,* 2013). In only one case it has been rather also taken into account the physical parameters, such as length, color, texture analyzed as single fruit (Sillero-Morales *et al.,* 2011).

NIR instruments adopted differ in the various experiments and have interval IR range from 380 to 2500 nm.

The aim of this work was the development of an innovative system based on near infrared spectroscopy (NIR) with the following requirements:

- beneficial in economical terms as rapid, non-destructive and capable of predicting multiple parameters simultaneously;
- able to determine and monitor the ripening indices, in order to accurately identify optimal harvesting time;
- directly applicable in the mills.

3.2.2 Materials and Methods

3.2.2.1. Olive oil fruit samples

During 2009 - 2014 crop seasons, olive oil fruits (*Olea europaea* L*. Frantoio* and *Moraiolo* cv.) were supplied by different farms located in Florence (Italy).

Ten *Frantoio* and *Moraiolo* cultivar trees were selected on each farm, as they were quantitatively representative of orchard. Olives were picked by hand at approx. 1.7 m from the soil once a week at 08:00 a.m. from the beginning of September to the beginning of December. Olives (300 – 400 g), which presented no infection or physical damage, were selected for each crop date. Olive samples were quickly transported to the laboratory for analysis. Olive samples were analysed for average weight and pulp/stone ratio. A homogeneous batch of olives (i.e. approx. 300 g) was

crushed in a laboratory crusher (Zeutec, Rendsburg, Germany), and the olive paste was used for the following chemical and spectroscopic analyses.

3.2.2.2.Chemical analysis methods

Water Content

The water content was measured on olive paste by heating 60 g of sample in an oven at 105°C until reaching constant weight. Results were expressed as paste moisture content (%).

Oil Content

The total oil content was determined on 5 g of dried olive paste (see the above oven method). Samples were extracted with hexane in an automatic extractor (Randall mod.148, VELP Scientifica, Milan, Italy), following the method of Cherubini *et al.* 2009. Results were expressed as oil content on dry matter basis (g kg^{-1} dm).

Sugar Content

Eight g of olive paste was cold extracted (6 ± 2°C) with distilled water in a 200 mL flask for 2 hours. The content of the flask was filtered on paper, and 10 mL of the obtained solution was diluted with water in a 20 mL flask. Measurements were performed by analyzing the obtained solution with an enzymatic method using an automatic ChemWell analyzer (Awareness Technology, ChemWell 9210, Palm City, FL). Three enzymatic kits were used to measure, respectively (i) the sum of two monosaccharides contents, namely glucose and fructose; (ii) the sum of disaccharide sucrose content with glucose and fructose contents; (iii) the mannitol content. All kits were purchased from R-Biopharm (Darmstadt, Germany). Measurements were done by means of external calibration standards: fructose and glucose (purity > 99%, Sigma Aldrich SrL, Milan, Italy), and mannitol (purity > 98%, Sigma Aldrich Srl, Milan, Italy). Results provided by the instrument were expressed in g L^{-1}; they were also converted in sugar content on dry matter basis (g kg^{-1} dm) as the average of two readings, carried out for each sample. The sucrose contents were determined by multiplying for 0.95 the difference between the sum of glucose, fructose, sucrose contents and the sum of glucose and fructose contents.

Phenolic Compounds content

Extraction of phenolic compounds from olive paste was carried out by Cecchi *et al., 2013* method. This method consisted in phenolic compounds extraction from fresh olive paste (and not from oil extracted from olive paste) and was able to prevent phenolic degradation during analysis procedure.

Syringic acid was chosen as an internal standard. Syringic acid and oleuropein were chosen as external calibration standards to evaluate the relative response factor (RRF); values for each phenolic compound were expressed as $mg_{oleur}\ kg^{-1}$ on dry matter (dm).

3.2.2.3.Spectroscopic analysis

Samples

583 spectra were recorded using filters NIR laboratory operating in the wavelength range 1200-2600 nm. Table 3.2.2 shows the samples recorded for each growing season.

Table 3.2.2 Spectra recorded for each crop season.

Year	Samples
2009	89
2010	90
2011	144
2012	106
2013	74
2014	41
2015	39
Total	**583**

Instruments

The analyzer used for the analysis of the paste of olives was the Spectra Alyzer Zeutec (ZeutecGmbH, Redensburg, Germany), a spectrophotometer with 19 interference filters in the near-infrared (NIR) connected to the software package Application Worx (ZeutecGmbH, Redensburg, Germany).

Each spectra was associated with the chemical results of the following parameters:

• Moisture (%);

• Oil content (%);

• Sugar content (g / kg of dry matter);

• Total phenolic content (mg / kg dry matter);

• Oleuropein content (mg / kg dry matter);

• Hydroxytyrosol content (mg / kg dry matter).

3.2.2.4. Statistical Analysis

The coefficient of variation (CV%) was calculated for chemical methods. Olive samples were chosen and used to carry out analyses in quintuplicate. CV% was calculated from the standard deviation and resulted to be as follows: (i) 5% for the water content, the sugar content and the total phenolic compounds content; (ii) 7% for the oil content; (iii) % values for each phenolic compound, as reported by Cecchi *et al.*, 2010.

The *Application worx* (software NIR SpectraAlyzer Zeutec) was used for calibration set using PLSR (Partial Last Squared Regerssion). SEP *(Standard Error of Prediction)* and RMSEP *(Root Mean Square Error)* were calculated on validation set using Microsoft Excel.

3.2.3 Results and discussion

3.2.3.1 Spectra

Figure 3.2.1 shows the spectra recorded. The spectrum is recorded at 19 selected wavelengths for each wavelength detected on the spectrum (•) corresponds to a reference parameter (water, sugars, etc.) as secondary vibrations of the molecules present (combination bands, overtone I and II, etc.). For each wavelength is recorded the absorbance value corresponding. Figure 3.2.2 shows an example of sample spectrum identifying the parameters corresponding to the characteristic wavelengths.

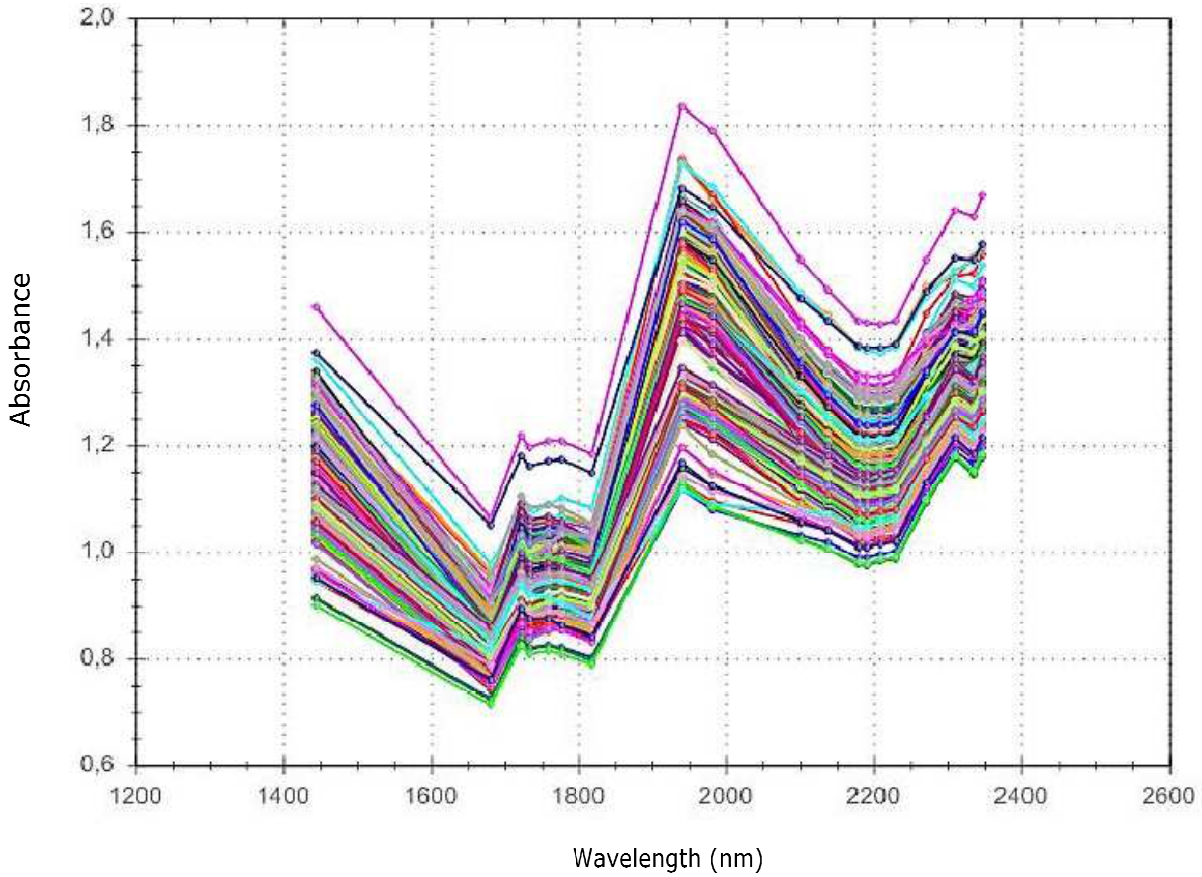

Figure 3.2.1. All spectra recorded with NIR Spectra Alyzer during 2009-2015 crop seasons.

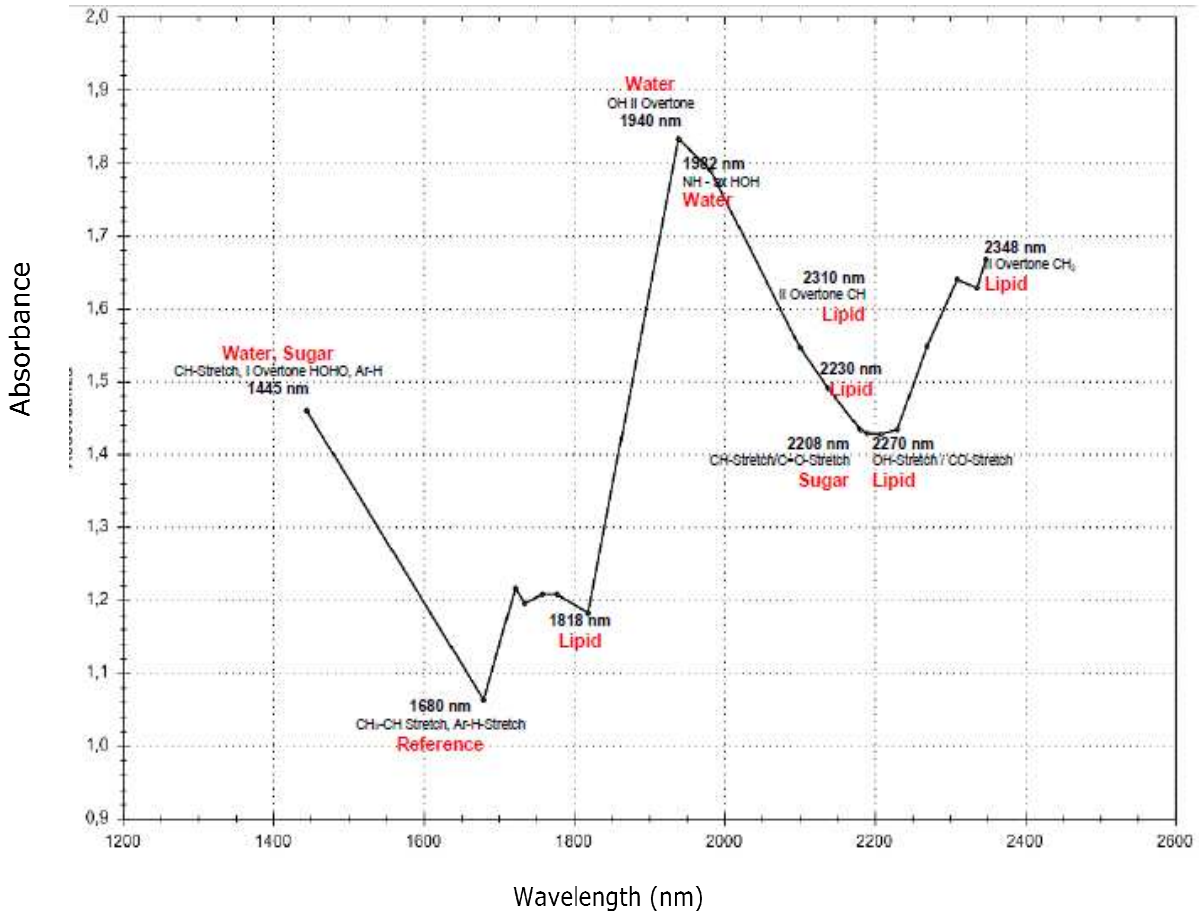

Figure 3.2.2. Example of sample spectrum identifying the parameters corresponding to the characteristic wavelengths.

3.2.3.2 Calibration set

The calibration was carry out on 399 samples for Moisture (%) and Oil (%) 82 samples for sugar (g/kg of dry matter), 291, 167 and 133 for total phenolic content, oleuropein and hydroxytyrosol content (mg/kg of dry matter) respectively.

For each spectra recorded was associated the chemical values. According to PLSR in the instrument software was possible build up the calibration models for each parameters. An example of calibration model for moisture content is given in figure 3.2.3: the green line is calibration line instead the red line is the optimal line where predict values and real values coincides.

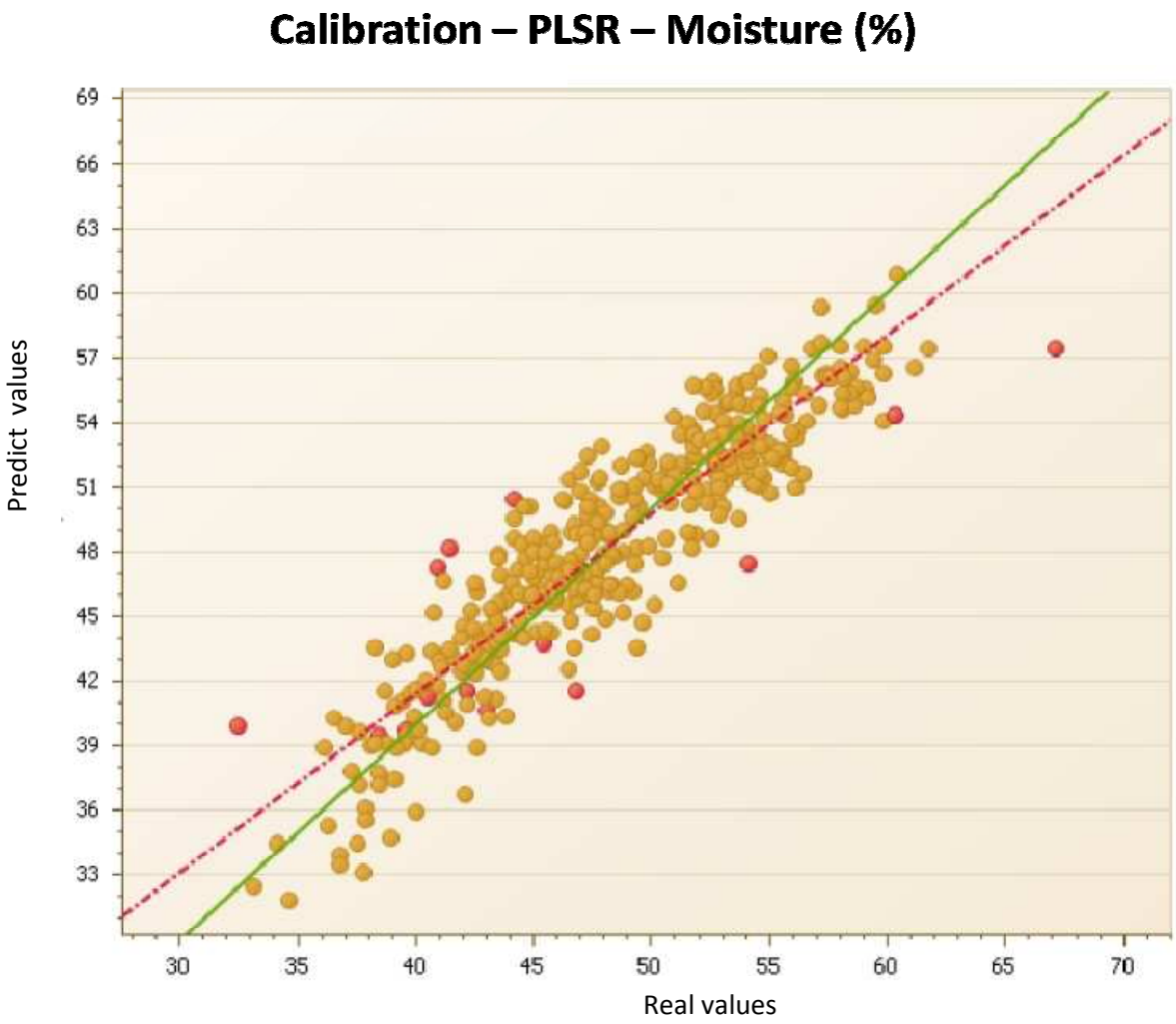

Figure 3.2.3. Calibration model for moisture content.

More details about calibration model are given in figure 3.2.4. For each parameters the software gave bias, the weight for each wavelength and it choose the best regression according to reach minimum error value and higher F statistical value: in this case 7 wavelength (factors) were selected. Table 3.2.3 summarizes the main statistical indicators for each parameter.

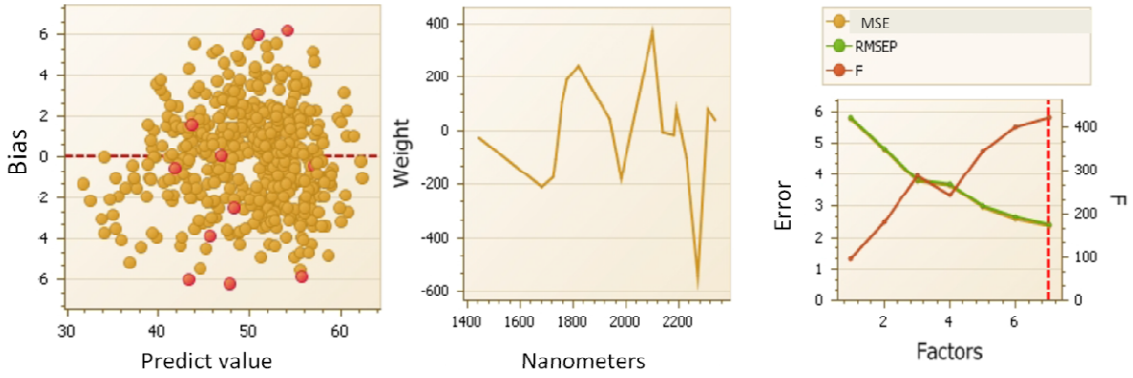

Figure 3.2.4. Calibration model details for moisture content.

Table 3.2.3. Calibration statistical parameters.

Parameters	**Moisture** (%)	**Oil content** (%)	**Sugar content** (g/kg of dry matter)	**Total Phenolic Content** (mg/kg of dry matter)	**Oleuropein content** (mg/kg of dry matter)	**Hydroxy tyrosol content** (mg/kg of dry matter)
Sample number	399	399	82	291	167	133
r^2	0.91	0.90	0.93	0.71	0.73	0.29
Slope	0.83	0.81	0.9	0.5	0.5	0.1
MSE	2.5	3.6	4.0	7384.3	648.4	171.3
RMSEP	2.5	3.6	4.8	7534.8	670.7	174.0
Factors	7	3	6	2	3	1
F	277	446	76	144	64	12

MSE – Mean Squared Error
RMSEP – Root Mean Squared Error %
F – Statistical Index

Calibration results are satisfactory for moisture, oil content and sugar content.

Some problems arose for phenolic compounds where the MSE and correlation index are less encouraging. Probably these results could be attributed to the large number of individual compounds and their different trends during ripening. Thus, it seems more useful to study the performance of individual compounds, in particular oleuropein and hydroxytyrosol. However the calibration parameters aren't encouraging, as well.

3.2.3.3 Validation set

The validation was carry out on 142 samples for Oil (%) and Moisture (%), 66 samples for sugar (g/kg of dry matter), 53, 53 and 22 for total phenolic content, oleuropein and hydroxytyrosol content (mg/kg of dry matter) respectively. Figure 3.2.5 shows the relationship between predicted values and measured values: according to the proximity of the points to the ideal line green, moisture and oil content show a good relationship between real values and predicted values. For other parameters there is variability probably due to the wide range but still satisfactory for sugar content and total phenolic content. The oleuropein content is almost always underestimated by the model. The hydroxityrosol content is always predicted to values between 500 and 600 mg/kg while the real values are in a wider range.

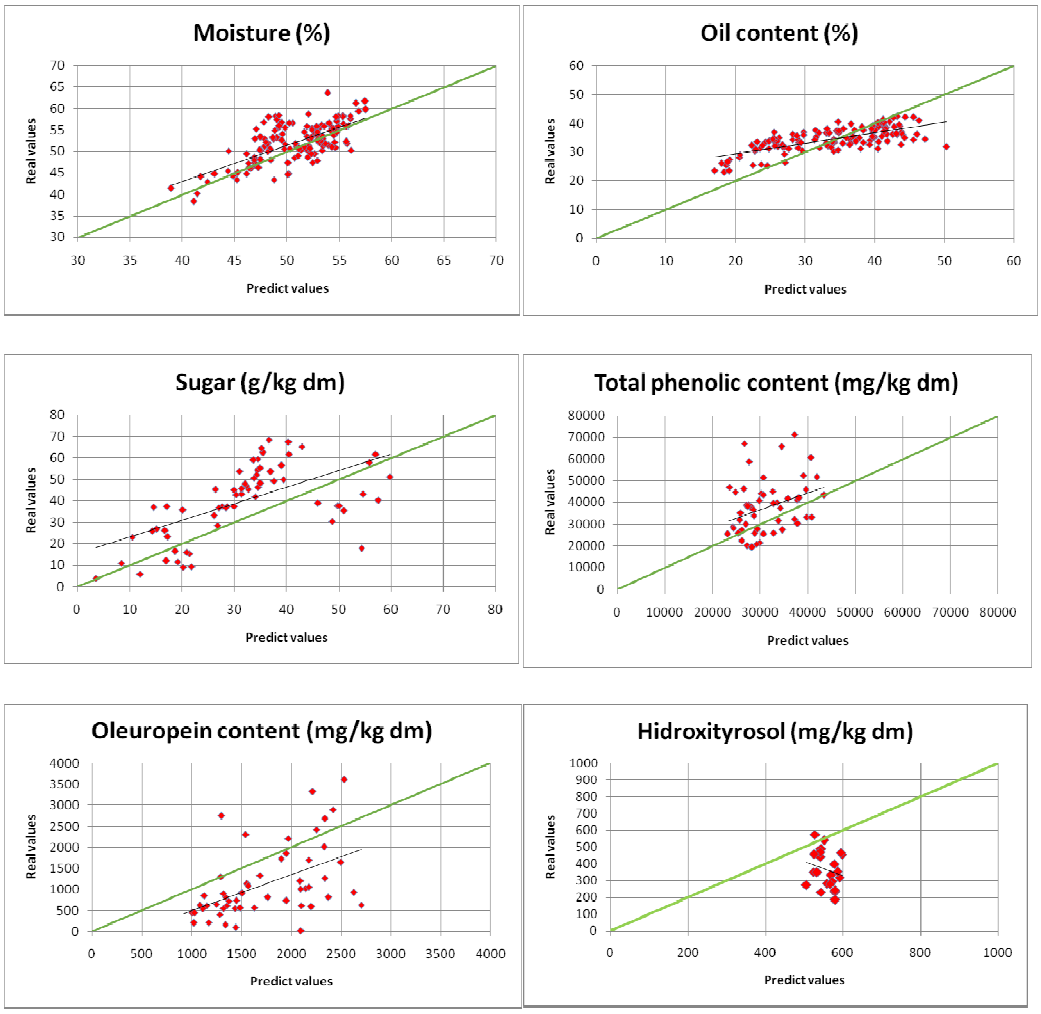

Figure 3.2.5. Validation model (dm= dry matter).

To better understand the predictive ability of the parameters were calculated the Standard error Root Mean of prediction (RMSEP) and the Standard error of prediction (SEP) comparing to coefficient of variation for each chemical analysis (CV%) and the Standard error of laboratory methods (SEL). Table 3.2.4 shows the statistical parameters.

Table 3.2.4. Validation statistical parameters.

PARAMETERS	MSEP%	SEP	CV%	SEL
Moisture (%)	0.004	2.4	5	3.2
Oil content (%)	0.04	6.0	7	4.4
Sugar content (g/kg of dry matter)	0.2	14.8	7	4.7
Total Phenolic Content (mg/kg of dry matter)	0.08	13345	5	3500
Oleuropein content (mg/kg of dry matter)	6.6	899	12	624
Hydroxytyrosol content (mg/kg of dry matter)	0.8	225	8.5	88

The MSEP values are encouraging for all parameters: the oleuropein content value is the highest. Despite MSEP values the real indicator that evaluates the predictive ability is the SEP (Mika *et al.,* 2003; Morales-Silviero *et al.,* 2011) .

Comparing the NIR prediction errors with chemical methods errors the moisture content is well predicted by the system, followed by oil and sugar contents. The moisture content has recorded a lower standard error of prediction value than chemical analysis, oil and sugar contents while although higher than those chemical, showed acceptable error values. Phenolic compounds show high errors, the total content as individual molecules. Presumably the predictive ability increase when the parameter to evaluate is simple and small molecule (i.e water, oil, sugar) and decrease when there are complex molecules. The phenolic compounds are large molecules and probably the NIR system isn't able to identify all the molecular vibrations. However, the results obtained were compared with data in the literature. Table 3.2.5 shows a schematic summary where for each works the samples number, r^2 for calibration and SEP for validation were considered. Moisture and oil contents results are similar to other data and they are better than Cayuela *et al.,* 2009. Reagarding sugar, biophenols, oleuropein and hydroxityrosol content there aren't data in the literature. According to 3.1, Cherubini *et al.,* 2009 and Migliorini *et al.,* 2011, the sugar content is a

crucial parameter to identify the drups ripening: isn't important to evaluate the perfect value but realise if the content reach a stable and minimu value. Moreover the use of NIR allowed to well predict moisture content having some advantages in terms of time whereas the chemical analysis lasts several days. Also the prediction of oil content gave advantages in terms of cost and time since it would economize the use of solvents, sometimes expansive. So, with the purpose to identify the chemical contents on the drupes and optimal harvesting time, NIR spectroscopy should be help for the identification of the indices of ripening and, consequently, optimal harvesting time rapidly, easily and cheaply.

Table 3.2.5. Results compared with data in the literature.

	Garcia-Sanchez *et al.*, 2005			Bendini *et al.*, 2007			Cayuela *et al.*, 2009			Morales-Sillero *et al.*, 2011			Maiellaro *et al.*, 2014			Our results 2015		
Parameter	**N**	**r^2_{cal}**	**SEP $_{val}$**	**N**	**r^2_{cal}**	**SEP $_{val}$**	**N**	**r^2_{cal}**	**SEP $_{val}$**	**N**	**r^2_{cal}**	**SEP $_{val}$**	**N**	**r^2_{cal}**	**SEP $_{val}$**	**N**	**r^2_{cal}**	**SEP_{val}**
Moisture	25	0,97	-	N_C: 423 N_V: 426	0,91	1,29	-	0,91 – 0,99	2,5	332	0,93	2,61	N_C: 170 N_V: 70	>0,9	-	**N_C: 399 N_V: 142**	**0,91**	**2,4**
Oil	25	0,91	-	-	0,92	0,67	-	0,84	21,9	322	0,84-0,97	1,09	N_C: 148 N_V: 52	>0,9	-	**N_C: 399 N_V: 142**	**0,90**	**6,4**
Sugar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**N_C: 82 N_V: 66**	**0,93**	**14,8**
Biophenols	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**N_C: 291 N_V: 53**	**0,71**	**13345**
Oleuropein	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**N_C: 167 N_V: 53**	**0,73**	**899**
Hydroxytyrosol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**N_C: 133 N_V: 22**	**0,29**	**225**

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3.3 Effects of olive fruit fly attack on the quality of olive oil: the case of the 2014 crop season in Tuscany

3.3.1 Introduction

In recent decades, extra virgin olive oil (EVOO) has been attracting the interest of the scientific community, also due to its well-recognized nutraceutical and sensory properties. These properties go to define the quality of EVOO and are affected by various factors, one of which is the olive fruits' health status (Migliorni *et al.*, 2012).

One of the main risk factors for the health of olive fruits is related to the attack of pathogens, the most dangerous of which is the olive fly, *Bactrocera oleae* (Perovic *et al.*, 2007; Wang *et al.*, 2009; Wang *et al.*, 2013).

In most crop seasons, attacks by the olive fly are scarce, so the effect on EVOO quality is low. Instead, in some crop seasons, olive fly attacks can affect up to 100% of the olive fruits in some areas, with very heavy consequences on the sensory and nutritional properties of EVOO.

Various parameters can be used to make a quantitative assessment of olive fly attacks. Among these parameters, active infestation only accounts for the stages of development of the larvae up to the second age and is used to establish the threshold beyond which a chemical treatment is justified (this threshold is 10% of active infestation). The total infestation takes into account all the types of infestation caused by olive fly and, therefore, can be better correlated to the variations in the chemical parameters of the olive fruits (Caleca *et al.*, 2007). In fact, the main risk factors for the fruits' state of health are those that cause the rupture of the skin.

The main consequences of such a rupture are: (i) exposure to oxygen of the olive fruits' chemical constituents; evaporation of water from the fruit no longer protected by the outer waxy layer; (ii) alteration of the metabolic processes of the fruit; (iii) attacks from exogenous and endogenous enzymes. Each of these phenomena affects the olive fruits' chemical composition, which can compromise the quality of EVOO (Servili *et al.*, 2007).

The 2014 crop season was characterized by a very intense attack by the olive fly and, in Tuscany, almost all the olive plantations were totally damaged. One of the main reasons for this attack was

the many climatic anomalies during the Fall 2013 - Fall 2014 period (LaMMA, 2013; LaMMA, 2014a; LaMMA, 2014b; LaMMA, 2015).

The aim of this study was to evaluate the impact of the olive fruit fly attack in the 2014 crop season in Tuscany, with particular attention to the chemical characteristics of the olive fruits and the extracted EVOO.

3.3.2 Materials and methods

3.3.2.1 Data relating to olive fly infestation

Data relating to active infestation and total infestation were collected from the Azienda Agricola Buonamici organic farm (Fiesole, Florence, Italy) by AgroAmbiente (2014).

3.3.2.2 Olive fruit samples

During the 2013 and 2014 crop seasons 10 cv *Moraiolo* olive plants were selected from the Azienda Agricola Buonamici organic farm (Fiesole, Florence, Italy). Olive fruit samples, of approximately 500 g, were collected from these plants on a weekly basis: from September 8 to November 24 in 2013, and from September 1 to October 27 in 2014. All the samples were analyzed as soon as they were delivered to the laboratory.

3.3.2.3 Oil samples

The oil samples consisted of olive oil produced in Tuscany, which was analyzed by the Laboratorio Chimico Merceologico (Promofirenze, Special Agency of the Florence Chamber of Commerce, Florence, Italy) in the 2013 and 2014 seasons.

3.3.2.4 Chemical and sensory analyses

The total phenolic content, water content and oil content in the olive fruit samples were evaluated as previously reported (Cecchi *et al.*, 2013).

One hundred olive fruits were weighed and pitted to calculate the pulp/stone ratio. The stones were weighed, and the pulp weight was calculated as the difference between the weight of the whole olive fruit and the weight of the stones. The pulp/stone ratio was calculated by dividing the pulp weight by the stone weight.

To measure the sugar content of the olive fruits, eight grams of olive paste were cold extracted (6 ± 2°C) with distilled water in a 200 ml flask for 2 hours. The content of the flask was filtered through paper and 10 ml of the solution obtained were diluted with water in a 20 ml flask. The sugar content was analyzed with an enzymatic method using the ChemWell automatic analyzer

(Awareness Technology, ChemWell 9210, Palm City, FL). Three enzymatic kits were used to measure respectively (i) the sum of two monosaccharide contents, namely glucose and fructose; (ii) the disaccharide sucrose content; and (iii) the polyol mannitol content. All the kits were purchased from R-Biopharm (Darmstadt, Germany). Measurements were performed by means of external calibration standards: fructose and glucose (purity > 99%, Sigma Aldrich SrL, Milan, Italy), and mannitol (purity > 98%, Sigma Aldrich Srl, Milan, Italy). The results provided by the instrument were expressed in g/l; they were also converted into sugar content on a dry matter basis (g/kg dm) as the average of two readings carried out for each sample. The sucrose contents were determined by multiplying the difference between the sum of glucose, fructose, and sucrose contents, and the sum of glucose and fructose contents, by 0.95.

The free acidity, peroxide number, and UV spectrophotometric indices (K_{232}, K_{270}, ΔK) of the oil samples were determined according to the analytical methods (Anonymus, 1991).

The tocopherols of the olive oil samples were determined according to the ISO 9936:2006/Corr. 1:2008 analytical method (Anonymus, 2008), while the total phenolic compounds were quantified according to the COI/T.20/Doc. no. 29 analytical method (Anonymus, 2009).

Sensory evaluation of the olive oil samples was performed according to EEC 2568/91 regulations and the following amendments (Anonymus, 1991). The form used for the sensory evaluation was developed according to the EEC 2568/91 regulation (Anonymus, 1991) and the COI/T.20/Doc. No 15/Rev. 8 February 2015 document (Anonymus, 2015).

3.3.3 Results and discussion

Table 3.3.1 compares levels of active and total olive fly infestation in the 2013 and 2014 crop seasons in Tuscany. As shown, in the 2013 crop season, the active infestation never exceeded three attacks per 100 olives, remaining below the threshold for chemical treatment (10 attacks per 100 olive fruits). Instead, in the 2014 crop season, at the end of July, the active infestation had already passed this threshold. Regarding the total infestation, it remained below six attacks per 100 olives throughout the 2013 crop season, while during the 2014 crop season it exceeded 100 attacks per 100 olive fruits.

To understand the effect of the attack on the olive fruits' state of health and on the quality of the extracted EVOO, it is enough to observe that at the peak of attack in the 2014 crop season, there

was an active infestation of approximately 40% while the total infestation even exceeded 100%; this suggests that at least 60% of the fruits had an infestation due to the presence of exit holes or larvae older than the second age, with an obvious detrimental effect on the quality of the fruit itself.

Many factors seem to have contributed to the intense olive fly attack in Tuscany in the 2014 crop season. First, the 2013 crop season was a year of vegetative discharge, as a consequence, in some cases the harvest was not completed; while the 2014 crop season was a year of vegetative charge. Second, Tuscany was affected by major climatic anomalies during the period of Fall 2013 - Fall 2014. The mild winter temperatures meant that the flies could overwinter.

Table 3.3.1. Levels of active and total olive fly infestation in the 2013 and 2014 crop seasons. Data are shown as number of attacks per 100 olive fruits.

	2013		**2014**	
	Active infestation	Total infestation	Active infestation	Total infestation
Jul 17	-	-	4	4
Jul 23	0	0	6	6
Jul 31	0	0	12	12
Aug 07	0	0	15	16
Aug 14	0	0	5	18
Aug 20	0	0	9	22
Aug 27	1	2	13	30
Sep 04	1	2	20	47
Sep 11	1	2	26	72
Sep 18	3	5	32	79
Sep 25	3	3	36	88
Oct 02	3	6	40	95
Oct 09	2	3	42	111

Therefore, they were able to lay their eggs on the olive fruits remaining on the ground, especially in the olive groves where the harvest in 2013 had not been completed; in addition, the June "heat wave" limited the olives' productivity, due to the concomitant start of the fruit set. Finally, the mild

temperatures combined with the high summer rainfall created the perfect precondition for a wide and rapid spread of the fly from generation to generation (LaMMA, 2014b).

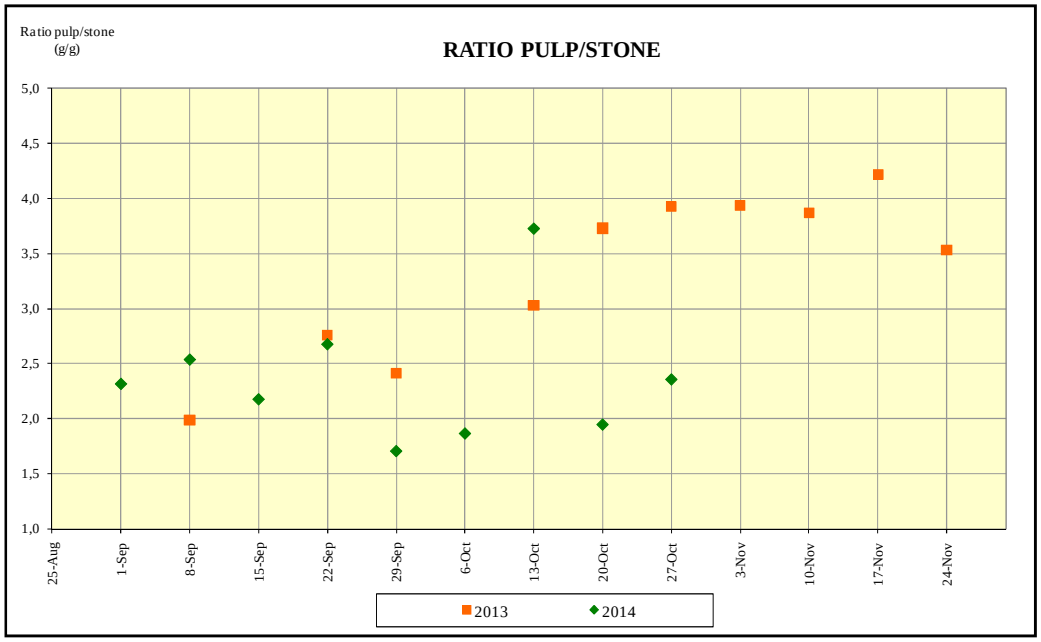
Figures 3.3.1 and 3.3.2 show the chemical contents trends in cv *Moraiolo* olive fruits during ripening in the two crop seasons.

In the 2014 crop season the pulp/stone ratio did not increase during the ripening period, in contrast to what was observed in 2013 (Fig. 3.3.1). This may be due to several effects of the olive fly attack, such as the modification or impairment of the growth processes of the fruit and decrease in pulp owing to the trophic effect caused by the parasite. The natural consequence of the lower contribution of the pulp to the total weight of the olive fruit was a lower moisture content in the olives (Fig. 3.3.1). The moisture contents were comparable until September 22, when the pulp/stone ratio in the two olive oil seasons was not very different. After that date, the moisture in the 2014 crop season plummeted dramatically to values of up to 30% less than in 2013.

The sugar content of the olives was also affected by the olive fly attack (Fig. 3.3.2). In the 2013 crop season we can observe a sugar content of 40-45 g/kg of dry matter at the optimal ripening time (Migliorini *et al.*, 2011; Cecchi *et al.*, 2013). Instead, in the 2014 crop season (October 22) the sugar content was already less than 10-15 g/kg of the dry matter. This drastic reduction may be due to both to the parasite compromising the biochemical maturation process and the evident loss of pulp.

A similar effect was seen for the oil content of the olive fruits (Fig. 3.3.2) and, consequently, the theoretical yields in the mill. In October we observed a break in the biochemical phenomenon of oil accumulation, together with a removal of pulp due to the fly's trophic activity.

Finally, the effect of the olive fly attack on the chemical content of the olive fruits was also confirmed by the data on the phenolic content (Fig. 3.3.2). In the 2014 crop season the phenolic content was always lower than the 2013 crop season, reaching a difference of 35% at the end of October.



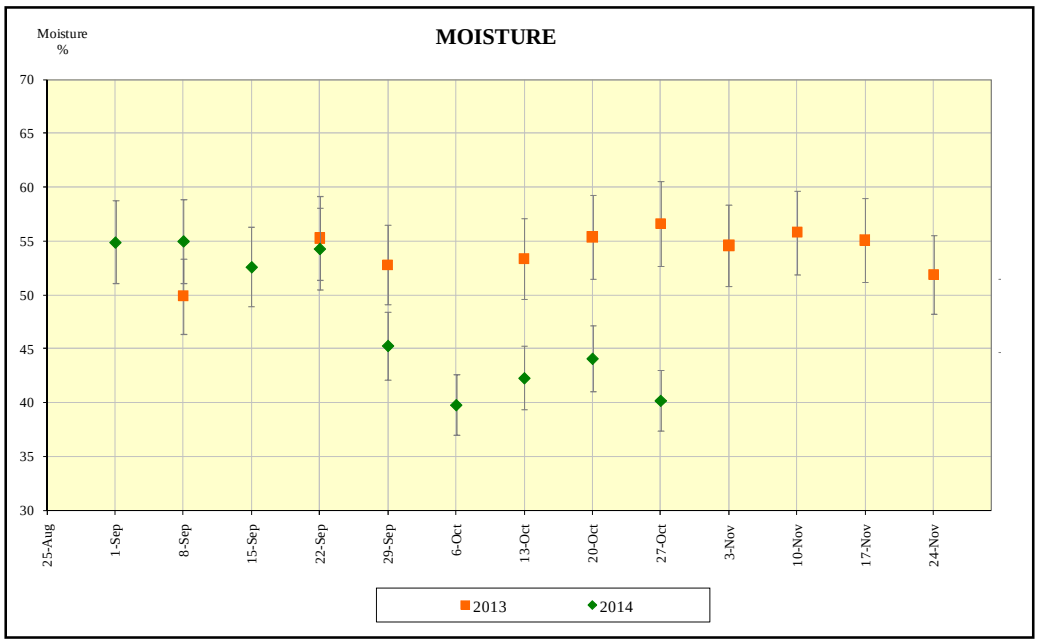
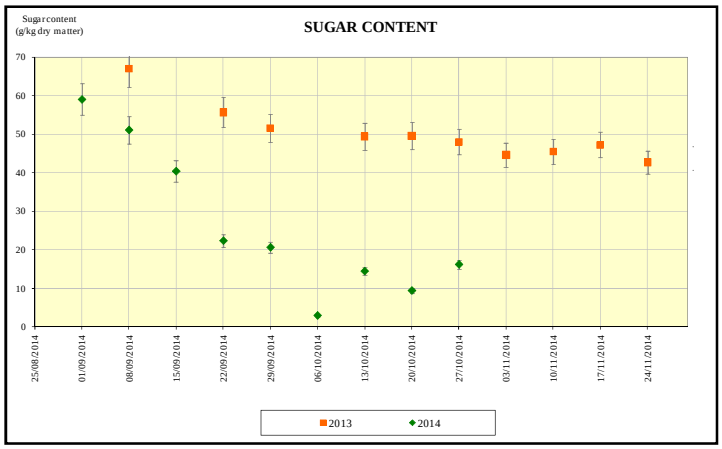
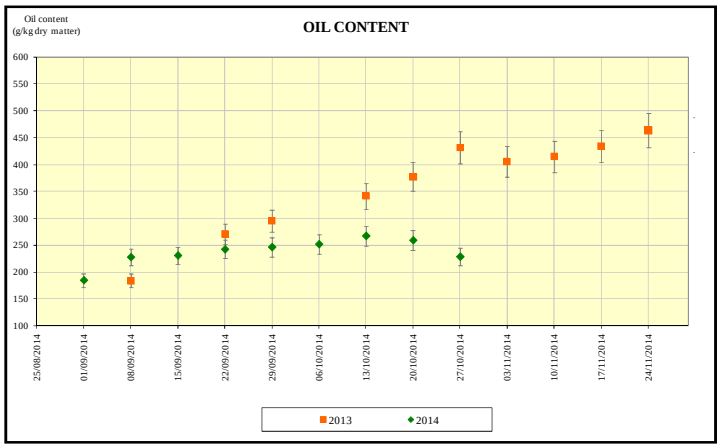


Figure 3.3.1. The chemical contents trends in cv *Moraiolo* olive fruits during ripening in the 2013 and 2014 crop seasons.





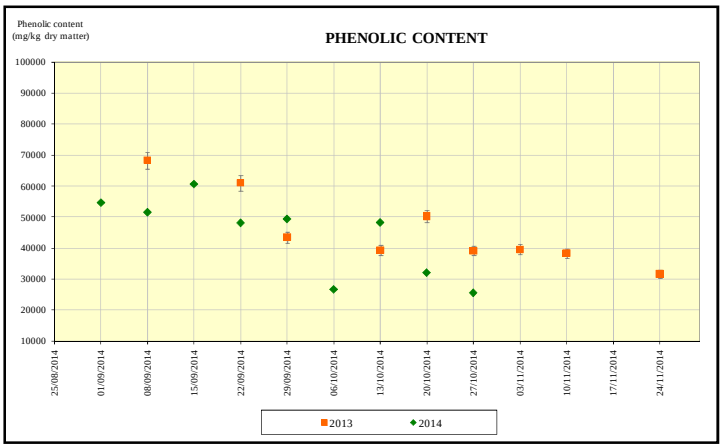


Figure 3.3.2. The chemical contents trends in cv *Moraiolo* olive fruits during ripening in the 2013 and 2014 crop seasons.

The olive fly attack had a strong negative impact on the oil quality. Table 3.3.2 shows the results of the parameters used to describe oil quality. In the 2014 crop season, the olive fly attack, causing the oil to come into contact with the water in the olive fruits and then with the exogenous and endogenous lipases, may have induced the hydrolytic degradation of the triglycerides, with a clear increase in free acidity. Consequently, in the 2014 crop season, as much as 16% of the analyzed oils was found to be outside the legal limits which oil must respect to be classified as extra virgin (Anonymus, 1991), while in the 2013 crop season only one oil sample was found to be outside the legal limit of 0.80% free acidity.

The attack of the olive fly may have caused the fatty acids' exposure to strong oxidative conditions; hence, a sharp increase in both the peroxide number and the value of K_{232} occurred.

Table 3.3.2. Results of the parameters used to describe oil quality in the 2013 and 2014 crop seasons.

	Free Acidity (%)	Peroxide number ($meqO_2/kg$)	Tocopherol content (mg/kg)	Phenolic content (mg/kg)	K232	K270	ΔK
2013 Crop Season							
Number of samples	356	354	202	212	235	235	235
Mean value	0.21	5.2	277	376	1.74	0.14	0.00
Maximum value	0.91	19.8	616	817	2.30	0.83	0.09
Minimum value	0.05	1.4	173	122	1.24	0.08	-0.01
2014 Crop Season							
Number of samples	221	212	52	35	76	76	76
Mean value	0.51	7.8	202	330	1.90	0.15	0.00
Maximum value	4.21	29.2	269	637	2.62	0.53	0.05
Minimum value	0.11	2.6	120	53	1.56	0.08	0.00

The antioxidant components of the oil (i.e. tocopherols and phenolic compounds) were also compromised by the infestation.

Regarding the oil sensory analysis, during the 2013 crop season all of the oil samples were free from defects, so they fell into the extra virgin olive oil category. In the 2014 crop season, instead, due to the major olive fly attack, 41% of the analyzed oils were defective, so they did not fall into the extra virgin olive oil category. Among these, 29% could be categorized as virgin olive oils, while the remaining 12% fell into the "*lampante*" olive oil category.

In conclusion, this research confirmed that the olive fruit fly is a strong risk factor for the quality of olive oil. A strong infestation by the olive fly causes a significant degradation in the chemical properties of the olive fruits and, as a result, poor oil quality.

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4. RESULTS AND DISCUSSION

T.2 MALAXATION

4.1 Modelling the effects of malaxation time-temperature conditions on oil process yield

4.1.1 Introduction

Extra virgin olive oil (EVOO) production is the main income source of olive growers, despite many strategies have been developed to make a profit from olive oil by-products (Masella *et al*., 2015; Parenti *et al*., 2014). Oil content of ripened olive oil fruits is variable between 15 and 22% on fresh weight basis (Nasini and Proietti, 2014). Oil is considered well mechanically extracted when the "Extractability Index – EI" (i.e. the percentage of olive oil extracted on the fruit oil content) is in the range of 80-90% (Boskou, 1996). The EI depends on both olive oil fruits quality and operating conditions in olive mill (Espinola *et al*., 2011); the step during extraction process that mainly affects yield is malaxation in mills equipped with a "decanter" centrifuge (Clodoveo, 2012). Malaxation consists of olive paste slow and continuous kneading that induces oil coalescence phenomena (Ranalli *et al*., 2003): minute oil droplets merge into large oil droplets, thus creating a continuous liquid phase, which can be mechanically separated from olive paste (Kalua *et al.*, 2006). After malaxation more than 80% of oil drops in olive paste should have a diameter greater than 30 μm (Martinez Moreno *et al*., 1957). Moreover, malaxation reduces emulsions formed during olive crushing allowing the recovery of another oil fraction (Ranalli *et al*., 2003; Di Giovacchino *et al*., 2002). Time and temperature are the main operating conditions of malaxation that control the above phenomena, affecting oil process yield. Generally, literature data indicated an increase in yield with increasing time-temperature values of olive paste malaxation (Di Giovacchino *et al*., 2002). Table 4.1.1 resumes consistent literature data. Several enzymatic phenomena also occur during malaxation, modifying quality characteristics of extracted oil. Oil secoiridoids and volatile compounds contents depend by temperature, time and oxygen level during malaxation (Clodoveo *et al*., 2014; Zanoni, 2014). Malaxation optimization time-temperature conditions want a balance between oil yield and oil quality characteristics. EVOO is highly prized for its flavor as well as health characteristics, but an high EVOO recovery has also to be wanted. The aim of this work was the kinetics study time-temperature of both oil coalescence phenomena and modification of processing yield to set up an optimization chart of malaxation operating conditions.

Table 4.1.1. Literature data at different time-temperature condition during malaxation.

Lotsample	Processing equipment	Malaxation conditions	Yield determination	Results	Modelling	Reference
600 kg olives of Leccino, Dritta and Coraleocultivars	Industrial equipment (Rapanelli, Italy)	0, 15, 30, 45, 60 and 75 minat 30°C	Measurement of the EI after oil extraction by a two-phase "decanter" and oil cleaning by a vertical centrifuge	Increase of the EI up to 60 min; from 60-70 min small decrease of the EI	-	Ranalli *et al*. (2003)
1 kg olives of Frantoio cultivar	Lab extraction unit (Abencor, Spain)	30, 60, 90 and 120 min at 15, 30, 45 and 60°C	Measurement of the EI after oil centrifugation, seguita da decanting of oil-water mixture in bottles	Increase of the EI with time and temperature; at 45°C lower EI values than to the other temperatures	-	Kalua *et al*. (2006)
5000 kg olives of Picual cultivar at three different crop seasons	Pilot plant (Pieralisi, Italy)	60 and 90 min at 18, 30 and 40°C	Measurement of pomace oil content on dry weight (PODW)* after oil extraction by a two-phase "decanter" and oil cleaning by a vertical centrifuge	Decreasing trend POWD in increasing time and temperature; differences crop Seasons	-	Aguilera *et al*. (2010)
0.5 kg olives of Picual cultivar at two different crop seasons and at different level of maturity index	Lab extraction unit (Abencor, Spain)	Values selected by an experimental design as follows: - time: 5.5, 20, 55, 90 and 104.5 min - temperature: 15.9, 20, 30, 40 and 44.1°C	Measurement of the EI after oil centrifugation, seguita da decanting of oil-water mixture in a graduated tube	Positive influence on the EI by time-temperature conditions: optimal values of the EI from 20.1 to 32.2 and from 73.3 to 90 min	A quadratic empirical model of the EI as a function of malaxation time and temperature conditions and olive maturity index	Espinola *et al*. (2011)

4.1.2. Materials and methods

Two sets of experiments were carried out. First experiments were used to determine kinetics of both oil coalescence and oil process yield at different time and temperature conditions of olive paste malaxation in lab-scale. Second experiments were carried out to validate the above kinetics during olive paste malaxation in industrial scale.

4.1.2.1 Setting up time-temperature kinetics models

During 2013 crop season, olive oil fruits (*Olea europaea* L.Frantoio cv.) were supplied by a farm located in San Casciano Val di Pesa (Florence, Italy). Ripe olive oil fruits were picked by hand at 08:00 a.m. at the beginning of November along plant circumference at approx. 1.7 m from the soil. Approximately 30 kg of olive oil fruits, which presented no infection or physical damage, were quickly transported to the laboratory.

An Abencor lab equipment (Abencor Analyser, MC2 Ingegneria Y Sistemas S.L., Seville, Spain) was used both for olive crushing and for olive paste malaxation. The equipment consisted of a hammer mill MM-100 (crusher holes5.5 mm in diameter) and a thermostated water bath (Thermo-mixer TB-100) with eight working sites; the working sites consisted of eight mixing jars (velocity of mixing blades: 50 rpm) in stainless steel, in order to simulate parallel several olive paste malaxation treatments (fig. 4.1.1). Oil was extracted from malaxed olive paste by a table centrifuge (type 4235 CWS,Alc Int. s.r.l, Milan, Italy) at 2000 rpm for 2 min or at 5000 rpm for 40 min as reported below in the description of oil process yield measurement. The malaxation trials were carried out at 22, 27, 32 and 37°C for 0, 10, 20, 30, 40 and 50 minutes in triplicate; water and paste temperatures were monitored by a type T thermocouple thermometer (Testo 926, Milan, Italy). Approximately 2.1 kg of olive paste, divided in six mixing jars with 350 g of olive paste, was used for each malaxation trial. Malaxed olive paste samples were partly used to measure oil coalescence and partly to extract oil, which was used to measure oil process yield.

Figure 4.1.1. Abencor thermo-mixer.

4.1.2.2 Validation of time-temperature kinetics models

During 2014 crop season, olive oil fruits (*Olea europea* L.Frantoio cv.) were supplied and processed by Agrofind.o.o. farm (Savudrija, Croatia). Ripe olive oil fruits were mechanically picked at 07:00 a.m. at the end of October. Approximately 500g of olive oil fruits were transported to the laboratory. Homogeneous samples of 350 kg olives were processed, using an industrial continuous plant (Pieralisi Group MAIP, Jesi, Italy) with a two-step "decanter". The plant consisted of two controlled-temperature horizontal malaxation equipments (350 kg capacity for each) with a stainless steel "partition", which allows an olive paste malaxation in series or in parallel way. The malaxation trials were carried out in quadruplicate at 23.5± 0.5°C for 0, 10, 20 and 30 minutes in two consecutive days; paste temperatures were monitored by a type T thermocouple thermometer (Testo 926, Milan, Italy) during malaxation equipmemt. At every tested time and temperature approx. 500g of malaxed olive paste was sampled during malaxation equipment both to measure oil coalescence and to extract oil by a table centrifuge (type 4235 CWS, Alc Int. s.r.l, Milan, Italy). As reported above, oil extraction by centrifuge was carried out to measure oil process yield.

4.1.2.3 Analysis methods

Olives

Olive samples were analysed for average weight, pulp/stone ratio and Maturity Index (Anonymous, 2011). The Maturity Index was based on the evaluation of olive skin and pulp colours. Values ranged from 0 (skin colour deep green) to 7 (skin colour black with all the flesh purple to the stone). A homogeneous batch of olives (i.e. approx. 300 g) was crushed in a laboratory crusher (Zeutec, Rendsburg, Germany), and the olive paste was used for chemical analyses of water and oil contents. The water content was measured on olive paste by heating 60 g of sample in an oven at 105°C until reaching constant weight. The total oil content was determined on 5 g of dried olive paste (see the above oven method). Samples were extracted with hexane in an automatic extractor (Randall mod.148, VELP Scientifica, Milan, Italy), following the method of Cherubini *et al.*, 2009.

Oil droplets coalescence

A microscopic method to measure number and diameter of oil droplets in olive paste samples during malaxation was carried out in triplicate according to Martìnez Moreno *et al.* (1957). For each sample three slides were prepared crawling olive paste, then few drops of a saturated solution of Sudan III in ethanol were added. Eight digital images at different points of each slide were collected at 40x magnification with a microscope (Axiolab, Carl Zeiss AG, Jena, Germany) coupled with a camera. For each image diameter of the drops were measured by comparing drops with are ferenceusing an image acquisition and analysis software. Approximately ninehundred drops were measured for each olive paste sample. The number of oil droplets were subdivided in the following classes of diameter: < 10 µm, 10-30 µm, 30-50 µm, 50-80 µm, 80-150 µm, > 150 µm.

Oil process yield

An Extractability Index (*EI*) of oil during malaxation was measured according to Masella *et al.,*2008 method. This method permitted a simple "on-line" direct measurement of potential extraction performance by centrifugation of an olive paste malaxation treatment. At each

time-temperature conditions of malaxation two aliquots of olive paste (approx. 40 g for each) were immediately centrifuged at 2000 rpm for 2 min; it can be assumed that effect of these operating conditions of centrifugation was similar to a real centrifugation treatment in an oil mill (Masella *et al.*, 2008). Then, extracted oil was decanted in a graduated tube to measure oil volume. Given an oil density value at 20° of 0.911 g/cm^3 (Gila *et al.*, 2015) oil volume was converted to extracted oil mass and a percentage extraction yield (*EY*) was determined for each malaxed olive paste. Two aliquots of olive paste (approx. 40 g for each) were also sampled from olive paste at maximum tested malaxation time (i.e. 50 min in lab-scale trials, 30 min in industrial scale trials) and they were centrifuged at 5000 rpm for 40 min; after these operating conditions of centrifugation no more oil was extractable from olive paste sample (Masella *et al.*, 2008). Then, extracted oil was decanted in a graduated tube to measure oil volume. Given an oil density value at 20°C of 0.911 g/cm^3 (Gila *et al*., 2015) volume was converted to extracted oil mass and a percentage maximum extraction yield of oil (EY_{max}) was determined for each malaxation trial at different temperatures. EY_{max} can be assumed to correspond to the percentage fruit oil content in the processed olives. This is demonstrated in Table 4.1.2, where a non-significant difference between experimental values for fruit oil content and EY_{max} can be observed.

Therefore, the Extractability Index (*EI*) was calculated by the following ratio:

$$EI(\%) = \frac{EY\ (\%)}{EY_{max}\ (\%)} \cdot 100 \qquad [1]$$

where extraction yields are expressed as percentage ratios between mass of extracted oil and mass of centrifuged olive paste.

4.1.2.4 Data processing

Analytical data were processed according to one-way ANOVA followed by Tukey's test (significance level: p = 0.01). Time-temperature models were set up following the common kinetical approach to express the relationships between data and time as pseudo-chemical kinetics and then, to correlate relevant rate constant of reactions with temperature using an

Arrhenius equation. Kinetics data were processed using Table Curve 2D Version 4 software (Systos Software Inc., Richmond, CA).

3. Results and discussion

Characteristics of the processed olive oil fruits are given in Table 4.1.2. Water content and oil content (% on dry basis) of *Frantoio* olive oil fruits processed in industrial scale were higher than relevant contents of *Frantoio* olive oil fruits processed in lab-scale. Maturity Index values of both olive fruits samples was low, but it is known that high oil contents in *Frantoio* cultivar are reached at maturity index values as approx. 2 (Trapani *et al.*, 2015).

Table 4.1.2. Mean values of olive oil fruits characteristics for malaxation trails. Different small letters in the same column indicate significant differences (p = 0.01) for the different samples. Different capitalletters in the same row indicate significant differences (p = 0.01) for the same samples.

Sample	Weight (g)	Pulp/stone ratio	Maturity Index	Water content (%)	Oil content (% on dry basis)	Oil content (% on fresh basis)	EY_{max} (%)
Frantoio olive oil fruits for lab-scale trials	1.9	3.0	1.1	$50.3^{a}\pm$ 2.5	$40.0^{a}\pm$ 2.8	$19.9^{a,A}\pm$ 1.4	$17.6^{a,A}\pm$ 1.4
Frantoio olive oil fruits for industrial scale trials	3.2	4.7	2.1	$58.0^{b}\pm$ 2.9	$46.2^{b}\pm$ 3.2	$19.4^{a,A}\pm$ 1.4	$17.3^{a}\pm$ 0.1

Table 4.1.3 shows yield kinetics data of all time-temperature malaxation trials. Both experimental values of extraction yields and the relevant values of maximum extraction yield were reported; then, Extractability Index values were calculated according the equation [1].

Table 4.1.3. Yield kinetics data of time-temperature malaxation trials; *EY*=mean value of extraction yield, EY_{max} = mean value of maximum extraction yield, *EI* = extractability index, K_{EI} = yield rate constant, r^2 = determination coefficient.

Trial	Temperature (℃)	Time (min)	*EY* (%)	EY_{max} (%)	*EI* (%)	K_{EI} (% min^{-1})	r^2
Malaxation of lab-scale	**22**	10	1.3 ± 0.4	16.5 ± 2.4	7.9 ± 2.6	1.2	0.97
		20	3.6 ± 0.5		21.6 ± 2.4		
		30	6.6 ± 0.5		40.1 ± 0.6		
		40	8.0 ± 0.2		48.7 ± 1.0		
		50	9.6 ± 0.9		58.4 ± 5.8		
	27	10	2.2 ± 0.5	17.7 ± 0.3	12.7 ± 2.4	1.6	0.98
		20	5.6 ± 0.4		31.7 ± 2.1		
		30	9.1 ± 0.1		51.5 ± 0.9		
		40	11.8 ± 0.9		66.4 ± 4.5		
		50	13.6 ± 0.6		76.8 ± 3.9		
	32	10	4.0 ± 1.2	18.0 ± 0.6	22.3 ± 6.9	2.1	0.94
		20	9.1 ± 0.9		50.8 ± 4.6		
		30	12.7 ± 1.0		70.4 ± 5.1		
		40	13.9 ± 0.3		77.0 ± 1.8		
		50	18.0 ± 0.6		99.8 ± 2.0		
	37	10	3.9 ± 0.4	18.0 ± 0.3	20.5 ± 0.8	2.3	0.94
		20	10.5 ± 2.0		51.0 ± 1.6		
		30	14.2 ± 1.6		73.3 ± 2.0		
		40	18.4 ± 0.6		100.3 ± 1.9		
		50	18.2 ± 0.3		101.6 ± 2.0		
Malaxation of industrial scale	**23.5**	10	0.2 ± 0.1	17.3 ± 0.1	1.4 ± 0.3	1.5	0.82
		20	3.9 ± 0.3		27.9 ± 3.4		
		30	6.0 ± 0.2		49.9 ± 0.1		

4.1.3.1 Oil process yield kinetics models

Considering the malaxation trials in lab-scale, evolution of oil process yield expressed as Extractability Index (*EI*) was significantly modelled at every malaxation temperature by the following pseudo zero-order kinetics, assuming reasonably that for $t = 0$ $EI=0$:

$$EI = k_{EI} \cdot t \qquad [2]$$

where:

EI = Extractability Index (%);

k_{EI} = yield rate constant (% min^{-1}), as reported in Table 4.2.3;

t = malaxation time (min).

The rate constant,k_{EI}, was significantly temperature dependent through the Arrhenius equation:

$$k_{EI} = k_0 \cdot e^{-E_a/RT} \qquad r^2 = 0.96 \qquad [3]$$

where:

k_0= frequency factor value pari a 984609 % min^{-1};

E_a = activation energy pari a 33323 J/mol;

R = gas constant pari a 8.314 J/mol K;

T = temperature (K).

Comparison between kinetic data in lab-scale and industrial scale showed a good agreement. The yield kinetics in industrial scale followed the above pseudo-zero order equation (Tab. 4.1.3); value of the relevant rate constant,k_{EI}, was consistent with equation [3] (Fig. 4.1.2).

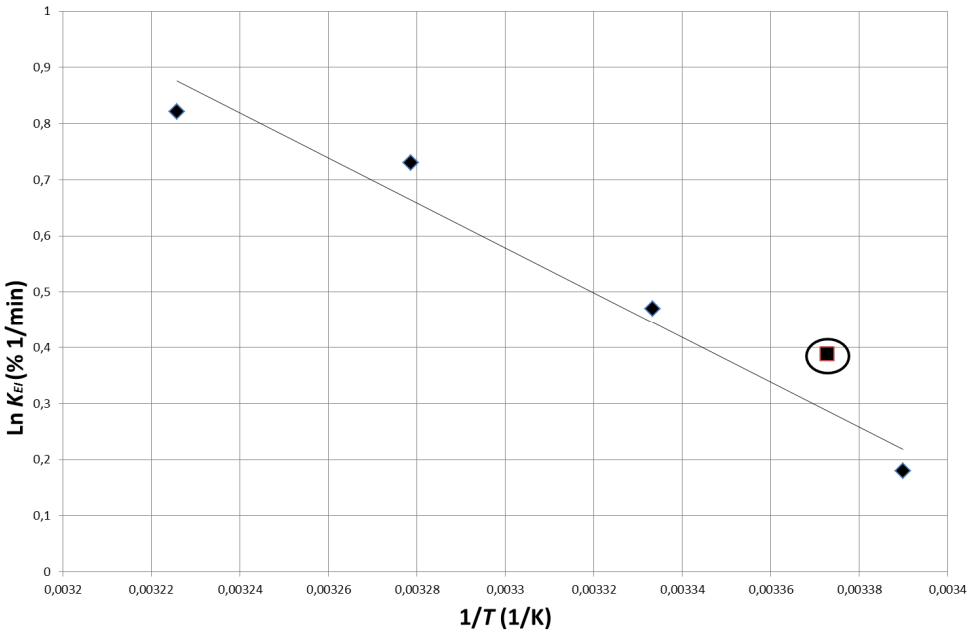

Figure 4.1.2. Arrhenius plot of rate constants, K_{EI}, and comparison between rate constants calculated both by lab-scale trials (◆) and industrial scale trials (■).

In reference to the literature data (Tab. 4.2.1) our kinetic data showed a similar phenomenological relationship where there is a positive influence on extraction yield by increase time and temperature during malaxation.

EI data were consistent with Ranalli *et al.,* 2003 and Espinola *et al.,* 2011; instead, it was not possible to compare our data with those of Kalua *et al.*, 2006, due to their abnormally low value, approx. EI = 40%.

4.1.3.2 Oil droplets coalescence kinetics models

Figure 4.1.3 shows oil droplets coalescence phenomenon during a malaxation trial, as an example. It is clearly highlighted as an olive paste devoid of malaxation in which there are only small and almost invisible drops, and arrives at the end of the malaxation in olive

pastes in which large drops occupy almost the whole of the slide. The coalescence phenomenon was also reported into a variation of the percentages of oil drops divided by diameter classes (Fig. 4.1.4). In particular, according to Martinez Moreno *et al.*, 1957, it has been highlighted as a threshold of diameter of the oil droplets equal to 30 micron could characterize the coalescence phenomenon; by a prevalence of drops with a diameter of <30 micron top of the malaxation it has gone in a progressive manner to a head of drops with a diameter> 30 micron at the end of the malaxation.

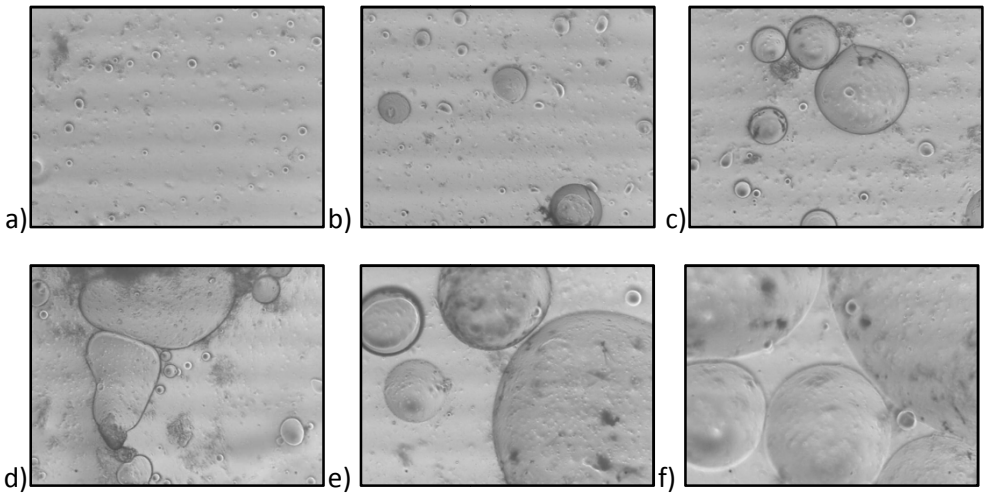

Figure 4.1.3. Evolution of oil drops size during malaxation at 22°C in a slide: a) 0 min; b) 10 min; c) 20 min; d) 30 min; e) 40 min; f) 50 min.

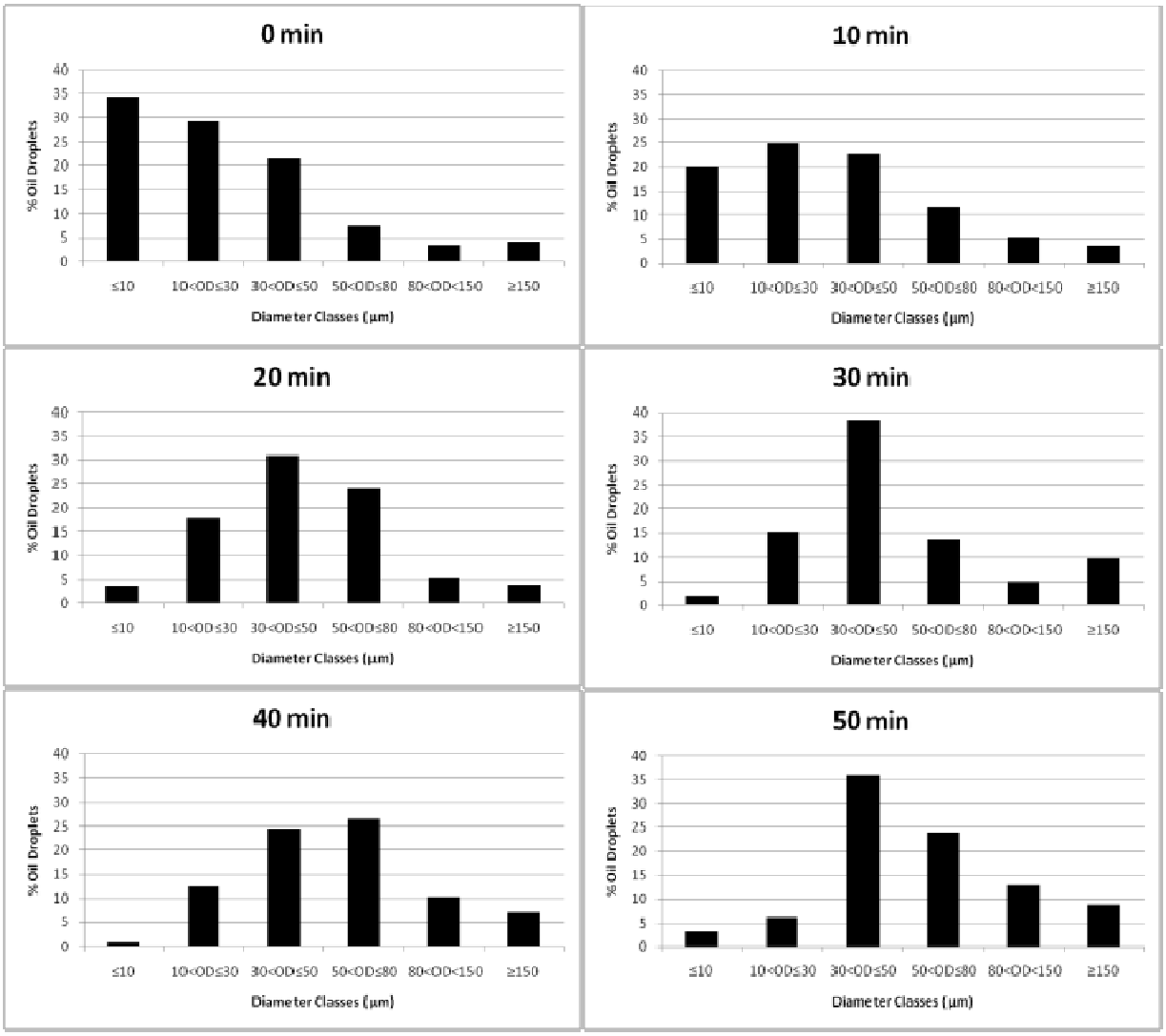

Figure 4.1.4. Evolution of oil droplets (OD) percentage for diameter classes during malaxation at 22°C.

Table 4.1.4 shows oil droplets coalescence kinetics data of all time-temperature malaxation trials. Considering the malaxation trials in lab-scale, the percentage of oil droplets with diameter > 30 μm (*OD*) started at approx. 40 % in olive paste without malaxation and reached approx. 100% in olive paste at the end of malaxation. In order to minimize matrix effects (i.e. different *OD* values between samples at time t = 0), a relative variation of percentage of oil droplets with diameter > 30 μm ($OD/OD_{t=0}$) was also determined. No significant differences of both *OD* and $OD/OD_{t=0}$ occurred between samples at different temperatures for the same time of malaxation; then, coalescence resulted an only time dependent phenomenon. $OD/OD_{t=0}$ was significantly modelled by the following pseudo zero-order kinetics (Fig. 4.1.5):

$$\frac{OD}{OD_{t=0}} = 1 + k_{OD} \cdot t \qquad r^2 = 0.87 \qquad [3]$$

where:

k_{OD} = coalescence rate constant (min^{-1}), which was equal to 0.033 (min^{-1});

t = malaxation time (min).

From equation [3] and Tab. 4.1.4, $OD/OD_{t=0}$ values from 2.0 to 2.6 corresponded to a percentage of oil droplets with diameter > 30 μm from 80 to 100%. Comparison between kinetic data in lab-scale and industrial scale showed a good agreement (Tab. 4.1.4) and the $OD/OD_{t=0}$ values were consistent with equation [3] (Fig. 4.1.5).

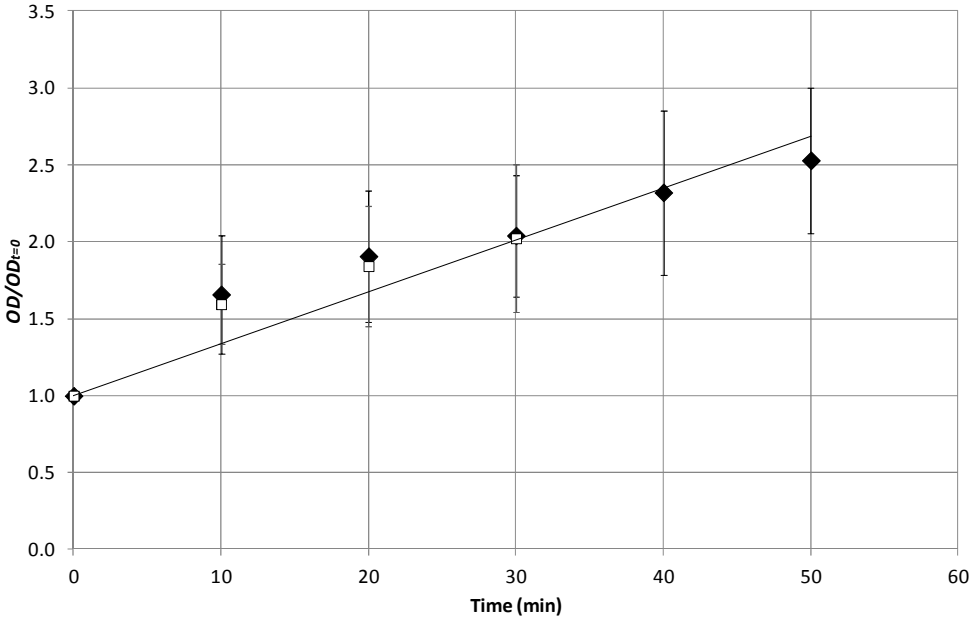

Figure 4.1.5. Oil droplets coalescence kinetics model (———) and comparison between experimental data from lab-scale trials (◆) and industrial scale trials (□).

Table 4.1.4. Oil droplets coalescence kinetics data of time-temperature malaxation trials; *OD* = percentage of oil droplets with diameter > 30 µm, $OD/OD_{t=0}$ = relative variation of percentage of oil droplets with diameter > 30 µm on time=0; different letters in the same row indicate significant differences (p = 0.05) for the different samples.

		Malaxation in lab-scale								*Malaxation in industrial scale*	
		22℃		27℃		32℃		37℃		23.5℃	
	Time (min)										
OD (%)	0	33c	± 8	33.5c	±0.7	48a	± 1	45ab	± 6	38bc	± 6
	10	52.0b	±0.5	72a	± 2	62ab	± 6	72a	±10	60ab	± 5
	20	74a	± 7	75a	± 6	71a	± 7	73a	± 7	70a	± 5
	30	74b	± 6	82a	± 3	79ab	± 2	82a	± 5	76ab	± 6
	40	82b	± 4	96.9a	±0.6	83b	± 10	97a	± 4	n.d.	-
	50	96a	± 6	97a	± 1	99.4a	± 0.5	99a	± 1	n.d.	-
$OD/OD_{t=0}$	0	1.0	-	1.0	-	1.0	-	1.0	-	1.0	-
	10	1.6ab	± 0.3	2.2a	±0.6	1.3b	± 0.1	1.6ab	±0.1	1.6ab	±0.3
	20	2.2a	± 0.1	2.3a	±0.5	1.5b	± 0.1	1.6b	±0.1	1.8ab	±0.4
	30	2.2ab	± 0.7	2.5a	±0.6	1.6b	± 0.1	1.8ab	±0.4	2.0ab	±0.5
	40	2.4ab	± 0.5	3.0a	±0.5	1.7b	± 0.3	2.1b	±0.2	n.d.	-
	50	2.9ab	± 0.6	3.0a	±0.7	2.1c	± 0.1	2.2bc	±0.3	n.d.	-

n.d. not determined

4.1.3.3 Optimization chart

In order to discuss on optimal time-temperature conditions of olive paste malaxation to improve oil process yield, it was useful to compare the kinetics of the above phenomena by an optimization chart. The chart was defined by a logarithmic scale on the y-axis, where malaxation time was shown, and a linear scale on the x-axis, where malaxation temperature was shown (Fig. 4.1.6). On the optimization chart it was possible to plot, the lines, different relationships between times and temperatures of malaxation, corresponding to defined quantitative levels of oil process yield (i.e. *EI* values) and oil droplets coalescence (i.e. $OD/OD_{t=0}$ values). The relationships for oil process yield referred to the time (t_{EI}) to reach in olive paste defined reference levels of Extractability Index (EI_{ref}) as a function of olive paste malaxation temperature (ϑ). This time was calculated according to equation 2 as follows:

$$t_{EI} = \frac{EI_{ref}}{k_{EI_{f(\vartheta)}}} \qquad [4]$$

where $k_{EI_{f(\vartheta)}}$ is the yield rate constant value, which was calculated at different malaxation temperature according to equation [3]. In the following discussion were considered as EI_{ref} values of 60% and 80%, respectively, expression of insufficient and satisfactory process oil yield (Boskou, 1996). The relationships for oil droplets coalescence referred to the time ($t_{OD/ODt=0}$) to reach in olive paste defined reference levels of relative variation of percentage of oil droplets with diameter > 30 μm ($OD/OD_{t=0_{ref}}$). This time was calculated according to equation 3 as follows:

$$t_{OD/OD_{t=0}} = \left(\frac{OD}{OD_{t=0}} - 1\right) \cdot \frac{1}{k_{OD}} \qquad [5]$$

where k_{OD} is the coalescence rate constant value, which was always equal to 0.033 (min^{-1}) at every malaxation temperatures as discussed in the section 4.3.2.

In the following discussion were considered as $OD/OD_{t=0_{ref}}$ as 2.0 and 2.6 respectively the expression of a minimum and maximum coalescence to be achieved to improve process yield oil (Di Giovacchino *et al*., 2002; Martinez Moreno *et al*., 1957). A proposal of a chart of optimization of malaxation time-temperature conditions to improve oil process yield is shown in Figure 4.1.6. This chart can lend to some considerations on the malaxation. First of all, by the evolution of EI it is confirmed the relation between the time of malaxation with the temperature: for increasing temperature decreases the time needed to obtain a desired process oil yield. This behavior appears in part conditioned by the oil droplets coalescence. For example, at 29°C a percentage of oil droplets with diameter> 30 micron by 100% ($OD/OD_{t=0_{ref}} = 2.6$) is obtained to a malaxation time of about 48 min (see the point shown in Figure 4.1.6) corresponding to a value of EI equal to 80%. Instead, a percentage of oil droplets with diameter> 30 micron, 80% ($OD/OD_{t=0_{ref}} = 2.0$) is obtained to a malaxation time of about 30 min corresponding to a value of EI to below 60%. A similar effect of the coalescence is found substantially throughout temperature range from 27 to 31°C. The only coalescence is not however able to explain what is evident at both high (> 31°C) that at low (<27°C) temperatures during malaxation. At high temperatures are obtained high values of EI to below the threshold of maximum oil droplets coalescence (see the marked area in Figure 4.1.6); however, at low temperatures is not sufficient maximum coalescence to have high values of EI, but are necessary malaxation time longer (see the marked area in Figure 4.1.6). When the temperature changes the viscosity of the oil varies exponentially: for example from 20 to 38°C the viscosity is reduced by about 50%, from 84.2 cP 39.2 cP (Bonnet *et al.,* 2011). It is common that during malaxation has already start a physical separation of the oil to effect of floating of a part of oil towards the surface of the olive paste. Since the speed of the displacement of the oil to the surface of the olive paste depends on the viscosity of the oil, as previously seen on the optimization chart leads to identify as between the phenomena that affect the process yield oil to be regarded not only the oil droplets coalescence but also the separation of the oil for outcrop, a phenomenon that appeared in our experimentation is particularly important at temperatures > 31 °C and <27 °C.

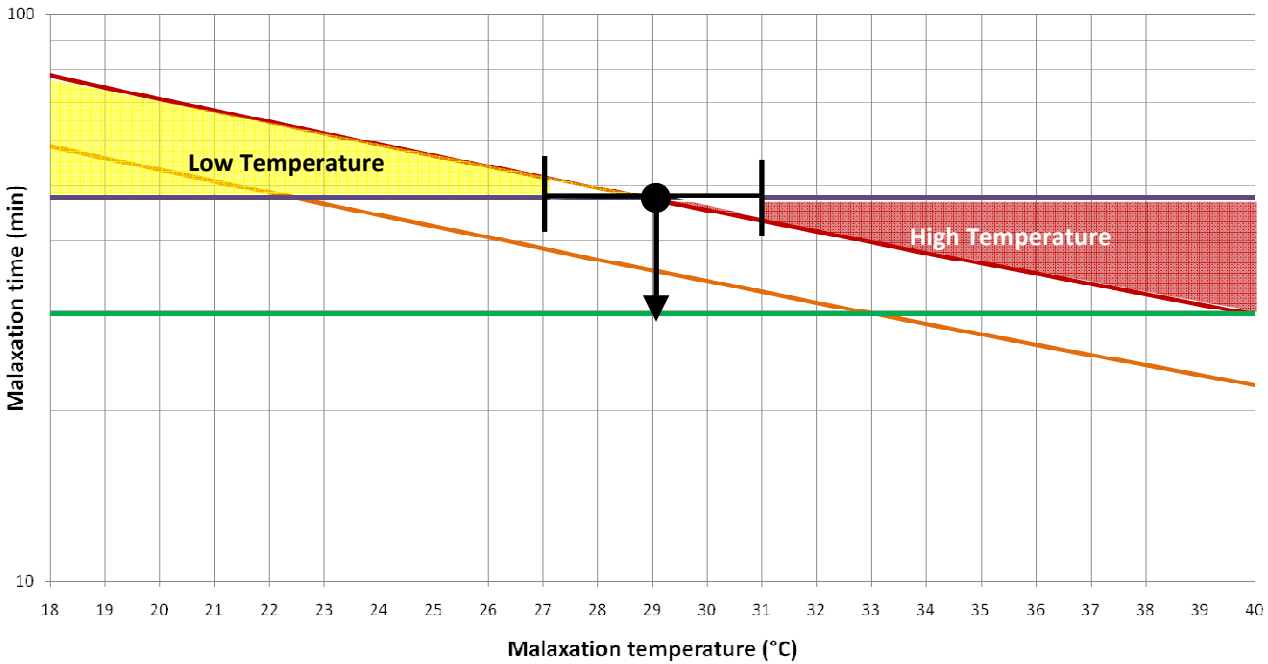

Figure 4.1.6. Chart of optimization of malaxation time-temperature conditions: *EI* = 80% ( ), *EI* = 60% ( ), $OD/OD_{t=0}$ = 2.6 ( ), $OD/OD_{t=0}$ = 2.0 ( ).

4.2.4 References

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4.2 Time-temperature kinetics of phenolic compounds during malaxation: a preliminary study

4.2.1 Introduction

Quality characteristics of virgin olive oil depend on several transformation phenomena of oil composition during processing. Consumers require to obtain high extra virgin olive oil quality, with specific attention to nutritional, sensorial and GMP (Good Manufacturing Practice) requests. With the aim to satisfy their requests there is need to optimize productive process. 1.2 section shows taht phenolic content is a crucial parameter because define nutritional and sensorial consumers requests. The malaxation is the critical operation to control the evolution of olive oil phenolic content but actually little is known about the phenomena that occurring. The role of time-temperature conditions for malaxation was investigated but contradictory results were often obtained. The compromise of the operating condition of time and temperature isn't simple to identify, as on one hand the heating will tend to increase the effectiveness of the mixing, with an increase in oil yield, on the other hand affects the quality of the oil with a deterioration of the sensory olfactory and sensorial notes (which the fault reheating, the loss of volatile substances, with decrease of the notes relating to fruity) and lower nutritional properties due to the degradation of phenolic compounds (Zanoni, 2014).

The use of low temperatures and short times (<30°C for 30-45 min) is the general criterion of optimization in order not to inactivate the enzymes of "lipoxygenase pathway", to reduce the activity of the polyphenol oxidase and the lipase, as well have a good extraction yield in oil (Ranalli et al., 2001). A further compromise is related to the exposure of oil to oxygen pastes for the final quality of the oil and for its shelf-life, which enzymatic oxidation, which occurs in load of phenolic compounds.

To prevent oxidation is increasingly using systems that prevent the contact of the olives pasta during malaxation with atmospheric oxygen. Moreover, during malaxation the pastes

will release carbon dioxide, which can be used as protection against oxidation, provided that the malaxator is closed (Parenti *et al.,* 2005).

On the other hand the lack of oxygen can promote the risk of not developing enzymatic oxidation of "lipoxygenase pathway". From this, through a series of reactions, where they are involved unsaturated fatty acids free, it generates a wide variety of volatile compounds, which show a marked positive influence on the sensory characteristics of aroma and flavor, such as fruity and grass. The influence of the temperature and time during malaxation on the overall quality of the olive oil has been studied by various authors in literature. In table 4.2.1 there is a summary of literature works about evaluation of phenolic compounds during malaxation.

Table 4.2.1 shows that the samples (monocultivar or mixed), type of extraction system (industrial or laboratory scale), time (from 0 to 120 minutes) and temperature (15 to 60 °C) condition during malaxation were heterogeneous.

Gomez-Rico *et al.,* 2009 and Taticchi *et al.,* 2013 carried out the analysis of phenolic compounds in olive paste while other authors evaluated analytical results on the content of phenolic compounds in olive oil extracted.

Table 4.2.1. Literature data about phenolic content at different time-temperature condition during malaxation.

	Amirante *et al.*, 2001	**Ranalli *et al.*,2001**	**Ranalli *et al.*,2003**	**Kalua *et al.*, 2006**	**Parenti *et al.*, 2008**	**Boselli *et al.*,2009**	**Gomez-Rico *et al.*, 2009**	**Taticchi *et al.*,2013**
Cultivar and quantity	Mix Moraiolo, Leccino, Frantoio 450-500 Kg	Caroleo, Leccino, Dritta 600 Kg/cv	Caroleo, Leccino, Dritta 600 Kg/ cv	Frantoio 1 Kg batch	Frantoio 3,6 Kg/ batch	Frantoio, Leccino Coratina 465 Kg	Cornicabra 200 Kg	Coratina, Ogliarola, Moraiolo, Peranzana 150 Kg/cv
Extraction system	Industrial *Alfa Laval* + 19-42% water in decanter	Industrial	Industrial *Rapanelli*	Laboratory *Abencor* + 100 ml water /Kg olives	Laboratory *Paar-Physica GmbH*	Industrial *Pieralisi*	Industrial *Pieralisi* Laboratory *Abencor*	Industrial *Rapanelli*
Time and temperature during malaxation	t: 30, 45, 60min T: 27, 32, 35°C	T: 20, 25, 30, 35°C	t:0,15,30,456 0, 75 min T: 30°C	t: 30, 60, 90, 120 min T: 15, 30, 45, 60°C	t: 45 min T: 21, 24, 27, 30, 33, 36°C	t: 45 min T: 25, 35, 45°C	t:30,60,90m in T:20, 28,40°C	t: 0, 10, 20, 30, 40 min T: 20, 25, 35°C
Chemical method for olive paste	–	–	–	–	–	–	HPLC	HPLC
Chemical method for oil	HPLC, Colorimetric	C-NMR, Colorimetric	Colorimetric	HPLC, LC-ESI-MS	HPLC, Colorimetric	HPLC, Colorimetric	HPLC	HPLC
Results - Optimal malaxation conditions	27°C 60 min 32% dilution	25°C and 30°C	30°C shortest time	45 and 60°C 30 min	27°C - 30°C 45 min	35°C – 45 min cv Frantio and Leccino 45°C – 45 min cv Coratina	24 – 28°C time doesn't affect	35°C 40 min

According to the literature there isn't a univoc optimal malaxation condition: Ranalli identify 30°C for shortest time but Gomez-Rico results show that the time doesn't affect, Boselli found difference among cultivars. There is little uniformity in the literature and especially none expresses the data in kinetic. The aim of this work was to carry out a preliminary time-temperature kinetic study of phenolic content in the olive paste and olive oil during malaxation.

4.2.2 Materials and Methotds

4.2.2.1 Olives

During 2014 crop season, olive oil fruits (*Olea europea* L. Frantoio cv.) were supplied by a farm located in Venturina (Livorno, Italy). Ripe olive oil fruits were picked by hand at 08:00 a.m. at the end of October along plant circumference at approx. 1.7 m from the soil. Approximately 30 kg of olive oil fruits were quickly transported to the laboratory. Olive samples were analysed for average weight, pulp/stone ratio and Maturity Index (Anonymous, 2011). A homogeneous batch of olives (i.e. approx. 300 g) was crushed in a laboratory crusher (Zeutec, Rendsburg, Germany), and the olive paste was used for chemical analyses of water and oil contents. The water content was measured on olive paste by heating 60 g of sample in an oven at 105°C until reaching constant weight. The total oil content was determined on 5 g of dried olive paste (see the above oven method). Samples were extracted with hexane in an automatic extractor (Randall mod.148, VELP Scientifica, Milan, Italy), following the method of Cherubini *et al.* 2009 (data not shown).

4.2.2.2 Process

An Abencor lab equipment (Abencor analyser, MC2 Ingegneria Y Sistemas S.L., Seville, Spain) was used both for olive crushing and for olive paste malaxation. The equipment consisted of a hammer mill MM-100 (crusher holes 5.5 mm in diameter) and a thermostated water bath (Thermo-mixer TB-100) with eight working sites; the working sites consisted of eight mixing jars (velocity of mixing blades: 50 rpm) in stainless steel. Oil and vegetation

water was extracted from malaxed olive paste by a table centrifuge (type 4235 CWS,Alc Int. s.r.l, Milan, Italy) at 2000 rpm for 2 min or at 5000 rpm for 40 min. The malaxation trials were carried out at 23, 28, 33 and 37°C for 0, 20, 40, 60, and 80 minutes in triplicate; water and paste temperatures were monitored by a type T thermocouple thermometer (Testo 926, Milan, Italy). Approximately 1.75 kg of olive paste, divided in five mixing jars with 350 g of olive paste, was used for each malaxation trial. Malaxed olive paste samples were partly used to measure phenolic content and partly to extract oil and vegetation water, which were used to measure phenolic content too.

4.2.2.3 Chemical Analysis on olive pastes

Colorimetric method

According to internal methodology, developed by PromoFirenze (Special Agency of the Chamber of Commerce of Florence) Division Chemical Laboratory, have been weighed 4 g of olive paste in test tubes to plastic centrifuge. To each test tube were added 80 ml of solution MeOH: H_2O = 60: 40 (v: v), the mixture was stirred for 30 min, centrifuged at 4000 rpm for 10 min and the supernatant was recovered (procedure repeated for two times) in a 200 ml flask and frozen for 2 h. The extracts were filtrate with No. 1 Whatman paper and get at least 10 mL of clear solution for UV test. 1 mL of the filtrate was transferred to 100 mL volumetric flask, were added 5mL of Folin-Ciocalteu's phenol reagent, added 20 mL of sodium carbonate solution (20 g in 100ml), made volume up to 100 mL exactly with HPLC grade water. Was stopped the flask and was mixed thoroughly by inverting it several times. After 1 hour was recorded the UV absorption at 760 nm: the same solution without the extraction solution but with 1 mL Met OH: H20 (60:40) as blank solution. To prepare a calibration curve, were added 0, 1, 2, 3, 5, and 10 mL of the acid gallic solution (0.500 g of dry gallic acid in10 mL with MetOH: H_20) into 100 mL volumetric flasks, and then dilute to volume with water. These solutions will have phenol concentrations of 0, 50, 100, 150, 250, and 500 mg/L gallic acid, the effective range of the assay. With the above colorimetric reaction, the absorbance was measured at 765 nm and calibration curve was carried out. The data were expressed as mg of gallic acid/kg of olive paste.

HPLC method

The total phenolic content was evaluated as previously reported in 3.1.2.2 section and in Cecchi *et al.*, 2013.

4.2.2.3 Chemical Analysis on vegetation water

The vegetation water has been extracted from the pasta by centrifugation (type 4235 CWS,Alc Int. s.r.l, Milan, Italy) at 4000 rpm for 20 min. It has been diluted 1:20, filtered and the colorimetric reaction was carried out according to (Anonymus, 2009).

4.2.2.3 Chemical Analysis on oil

The oil has been extracted from the pasta by centrifugation (type 4235 CWS,Alc Int. s.r.l, Milan, Italy) at 4000 rpm for 20 min. The phenolic compounds by colorimetric method and HPLC method were quantified according to the COI/T.20/Doc. no. 29 analytical method (Anonymus, 2009).

The free acidity, peroxide number, and UV spectrophotometric indices (K_{232}, K_{270}, ΔK) of the oil samples were determined according to the analytical methods (Anonymus, 1991). All oil samples were classified as extra virgin (data not show).

4.2.2.4 Data processing

Analytical data were processed according to one-way ANOVA followed by Tukey's test (significance level: $p = 0.01$). Time-temperature models were set up following the common kinetical approach to express the relationships between data and time as pseudo-chemical kinetics and then, to correlate relevant rate constant of reactions with temperature using an Arrhenius equation. Kinetics data were processed using Table Curve 2D Version 4 software (Systos Software Inc., Richmond, CA).

4.2.3 Results and Discussion

Table 4.2.2 shows the temperatures in each test calculated by the average of the replicas, for the paste and for the water of the heating tank.

Table 4.2.2. Pasta and water temperatures.

Paste (°C)		Water *tank* (°C)	
Mean	ds	Mean	ds
23.5 °C	± 0.2	24.3 °C	± 0.05
27.6 °C	± 0,7	29.4 °C	± 1.1
32.6 °C	± 0,4	34.0 °C	± 0.5
36.7 °C	± 0,4	39.2 °C	± 0.5

4.2.3.1 Olive paste

Colorimetric method

Table 4.2.3 shows the evolution of total phenolic content measured by colorimetric method during the time at different temperature conditions**.**

Table 4.2.3. Total phenol content in olive paste.

Time (min)	23°C		28°C		33°C		37°C	
	Phenolic content (mg/kg)	ds	Phenolic content (mg/kg)	ds	Phenolic content (mg/kg)	ds	Phenolic content (mg/kg)	ds
0	4670	42	4598	93	3971	957	4463	151
20	4548	228	4203	84	3734	625	3914	556
40	4408	251	4099	67	3336	588	3555	442
60	4274	513	3869	303	3169	348	3330	264
80	4219	434	3747	310	3107	98	3241	260

The total phenolic contents show a common trend to the decrease during malaxation time. It seems also to note an higtest decreases with increasing temperature. In order to

minimize matrix effects (i.e. different phenolic contents values between samples at time t = 0), a relative variation of phenolic contents ($PC/PC_{t=0}$) was also determined (fig. 4.2.1).

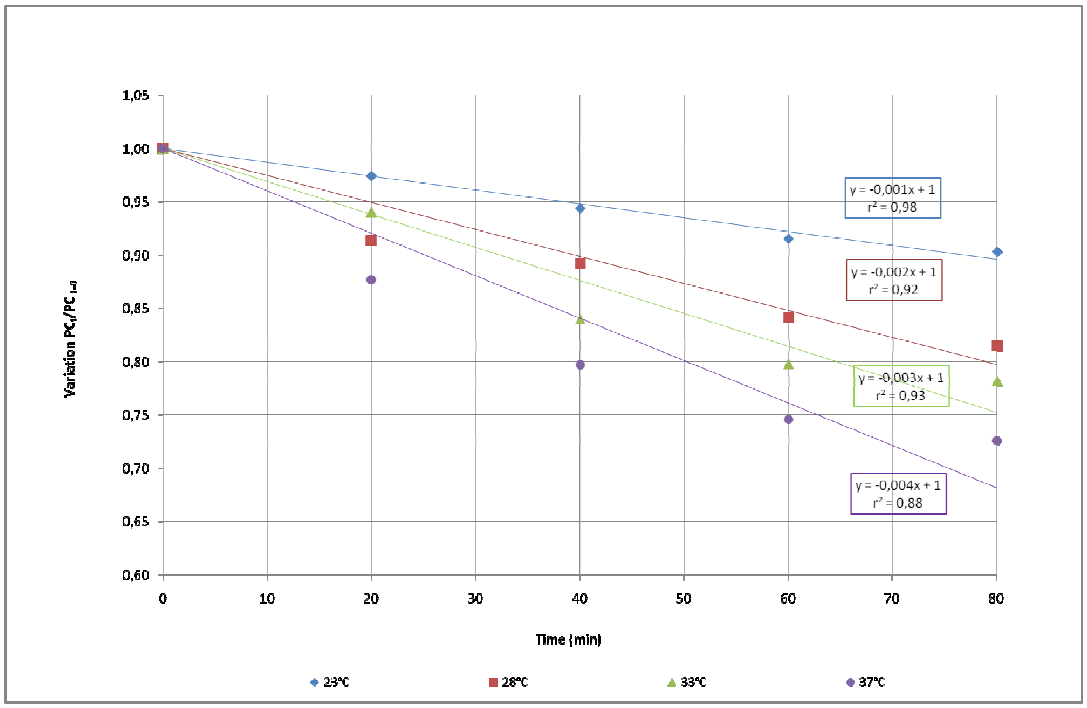

Figure 4.2.1. Variation of phenolic contents ($PC/PC_{t=0}$) at different time and temperature conditions.

The values obtained demonstrate that the decrease of phenolic compounds is time dependent according to a linear trend. The slope found for the 4 different lines, relative to the different experimental temperatures, and expressed by the slope of each equation, indicates that the kinetics of variation is also affected by the temperature. The angular coefficient, in fact, expresses the rate constant (0 order). The rate constant k refers to the constant reported the Arrhenius equation, which shows, experimentally, as the speed of a chemical reaction is influenced by temperature:

$$k_{PC} = k_0 \cdot e^{-E_a/RT} \qquad r^2 = 0.98 \qquad [3]$$

where:

k_0= frequency factor - 1.61 x 10^{10} min^{-1};

E_a = activation energy 74651 J/mol;

R = gas constant 8.314 J/mol K;

T = temperature (K).

The relationship applied for the calculation of the kinetics of variation as a function of time and temperature of phenolic compounds in the paste is as follows:

$$PC_t / PC_{t=0} = 1 - kt \qquad [4]$$

where:

PC_t = concentration of phenolic compounds at time t = 0 min; 20 min; 40 min; 60 min; 80 min;

$PC_{t=0}$ = concentration of phenolic compounds at time t = 0 min;

k = constant speed - slope of the line;

t = time.

Thanks to this relation it was possible estimate the time required to obtain a 10% decrease of phenolic compounds in the paste as a function of temperature during malaxation.

HPLC method

The evolution of total phenolic compounds measured by HPLC method showed a clear decrease but sometimes irregular during time and temperature conditions. Probably these results could be attributed to the large number of individual compounds and their different trends during time-temperature conditions taken during malaxation. Thus, it seems more

useful to study the evolution of individual compounds: verbascoside content (sugar ester of caffeic acid) was selected since the study of its variation, for each test compared to the initial time, has outlined a significant decrease in function of time. Table 4.2.4 shows the evolution of verbascoside content and relative variation ($V/V_{t=0}$) during the time at different temperature conditions.

Table 4.2.4. Verbascoside content evolution and variation during malaxation.

Time (min)	23°C		28°C		33°C		37°C	
	Verbascoside (mg/kg)	V_t / $V_{t=0}$	Verbascoside (mg/kg)	V_t / $V_{t=0}$	Verbascoside (mg/kg)	V_t / $V_{t=0}$	Verbascoside (mg/kg)	V_t / $V_{t=0}$
0	223	1.00	123	1.00	53	1,00	64	1.00
20	184	0.82	96	0.78	–	–	–	–
40	122	0.54	–	–	21	0,39	8	0.12
60	123	0.55	42	0.34	7	0,13	–	–
80	17	0.08	41	0.33	8	0.16	11	0.18

With the same above approach were calculated the k (3.23×10^2 min^{-1}), Ea (26060 J/mol) with r^2 =0.85 and the time required to obtain a decrease 20% of verbascoside as a function of temperature during malaxation [4].

4.2.3.2 Vegetation water

Table 4.2.5 shows the evolution of total phenolic content measured by colorimetric method during the time at different temperature conditions.

Table 4.2.5. Total phenol content in olive paste.

Time (min)	23°C		28°C		33°C		37°C	
	Phenolic content (mg/kg)	Ds	**Phenolic content (mg/kg)**	ds	**Phenolic content (mg/kg)**	ds	**Phenolic content (mg/kg)**	ds
0	3729	13	3613	768	3847	325	4204	467
20	3861	170	3853	51	3751	202	4326	442
40	3249	300	3759	245	3845	134	5323	615
60	3773	116	3897	89	3809	81	4033	270
80	3571	236	3935	429	3594	139	4752	219

In vegetation water there wasn't a significant decreasing trend between phenolic content at different times and temperatures: therefore, the considerations made before can not be repeated for the vegetation water.

4.2.3.3 Oil

Colorimetric method

Table 4.2.6 shows the evolution of total phenolic content measured by colorimetric method during the time at different temperature conditions.

Table 4.2.6. Total phenol content in oil.

tempo (min)	23℃		28℃		33℃		37℃	
	Phenolic content (mg/kg)	ds	Phenolic content (mg/kg)	ds	Phenolic content (mg/kg)	ds	Phenolic content (mg/kg)	ds
0	128	6	77	15	117	6	155	16
20	109	4	58	13	98	12	110	8
40	105	3	47	5	90	5	94	9
60	105	5	56	9	75	7	97	3
80	95	3	50	5	80	7	91	10

The total phenolic contents show a common trend to the decrease during malaxation time. It seems also to note an higtest decreases with increasing temperature. In order to minimize matrix effects (i.e. different phenolic contents values between samples at time t = 0), a relative variation of phenolic contents ($PC/PC_{t=0}$) was also determined (fig. 4.2.2).

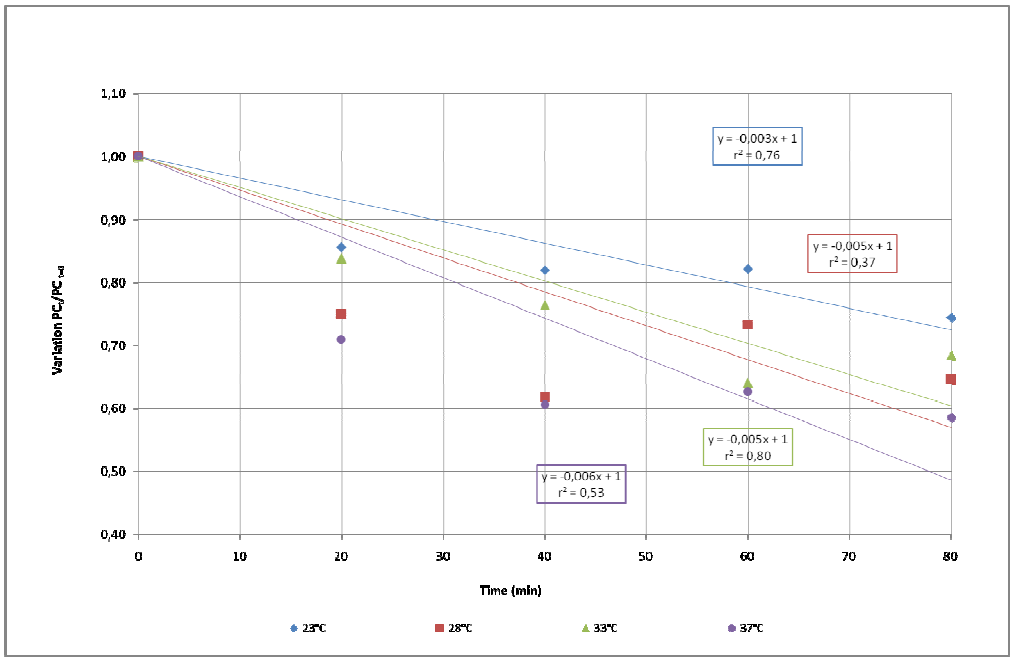

Figure 4.2.2. Variation of phenolic contents ($PC/PC_{t=0}$) at different time and temperature conditions.

The values obtained demonstrate that the decrease of phenolic compounds is time dependent according to a linear trend. According to the same approach (see 4.2.3.1) it was possible estimate the time required to obtain a decrease 20% of phenolic compounds in the oil as a function of temperature during malaxation (k 1.47 x 10^4 min^{-1}, Ea 37911 J/mol, r^2 0.99).

HPLC method

According to 1.2 section where the secoiridoides are considered the phenolic class crucial for their nutritional and sensorial value, it seems more useful to study the evolution of secoiridoides content. Table 4.2.7 shows the evolution of secoirioid content measured by HPLC method during the time at different temperature conditions and relative variation ($S/S_{t=0}$) In order to minimize matrix effects (i.e. different phenolic contents values between samples at time t = 0).

Table 4.2.7. Secoiridoides (S) content evolution and variation during malaxation.

Time (min)	23 ℃		28 ℃		33 ℃		37 ℃	
	S (mg/Kg)	$S_t/S_{t=0}$	S (mg/Kg)	$S_t/S_{t=0}$	S (mg/Kg)	$S_t/S_{t=0}$	S (mg/Kg)	$S_t/S_{t=0}$
0	94	1.00	75	1.00	93	1.00	80	1.00
20	–	–	–	–	–	–	59	0.74
40	88	0.94	72	0.96	–	–	52	0.65
60	88	0.94	73	0.97	63	0.68	–	–
80	67	0.71	69	0.92	68	0.73	–	–

The secoiridoides content show a common trend to the decrease during malaxation time. It seems also to note an higtest decreases with increasing temperature. According to the same approach (see 4.2.3.1) it was possible estimate the time required to obtain a decrease 20% of secoiridoides in the oil as a function of temperature during malaxation (k 1.86 x 10^9 min^{-1}, Ea 67440 J/mol, r^2 0.89).

4.2.3.4 Material balance

The breakdown of the olives in macro-components, calculated chemical characterization, has been expressed in percentage terms as:

- Moisture 49.4%;
- Oil on dry matter 47.6%.

The mass balance allowed the calculation of the relative composition of 1 kg of olive paste, (knowing that the dry pulp is 50.6%) expressed as a percentage:

- Oil 24.1%;
- Water 49.4%;
- Solids 26.5%.

Hypothetically, it was considered a breakdown of the phenolic compounds proportional to the components present in 1 kg of olive paste.

Therefore, the breakdown hypothetical, in mg of phenolic compounds/ kg of paste, was calculated on the basis of the experimental results, obtained at time 0 with the colorimetric method (Folin-Ciocalteu reagent), in relation to oil percentage, water and solid substances present in 1 kg of olive paste.

The results showed a significant difference between real and hypotetic values (tab. 4.2.8).

Table 4.2.8. Mass balance of phenolic compounds: comparison between real and hypothetical data.

	23°C		**28°C**		**33°C**		**37°C**	
	Real mg/kg	**Hypotetic mg/kg**	**Real mg/kg**	**Hypotetic mg/kg**	**Real mg/kg**	**Hypotetic mg/kg**	**Real mg/kg**	**Hypotetic mg/kg**
Oil	128	1126	77	1109	117	957	155	1076
Water	3729	2307	3613	2272	3848	1962	4204	2205
Solids	813	1237	908	1217	6	1052	104	1182
Total	4670	4670	4598	4598	3971	3971	4463	4463

From the table it can be seen as the real distribution of phenolic compounds (for solids was calculated from the difference), deviates from the hypothetical distribution of phenolic compounds proportional to the components in 1kg of paste. It was also calculated the percentage breakdown (tab. 4.2.9).

Table 4.2.9. Percentage breakdown hypothetical and real components of the phenolic compounds.

	Oil	**Water**	**Solids**
Hypotetic	**24.1%**	**49.4%**	**26.5%**
23°C	2.7%	79.8%	17.4%
28°C	1.7%	78.6%	19.7%
33°C	2.9%	96.9%	0.1%
37°C	3.5%	94.2%	2.3%

The real percentage breakdown of phenolic compounds obtained in oil, the vegetation water and solids, revealed, in fact, as in the aqueous phase were the highest concentration of

phenolic compounds; in the oil phase, however, it was transferred just a small percentage; in solid phase the percentage appears to be variable. This distribution can be explained by considering how the molecules of the phenolic compounds are divided into different phases, according to their affinity for water or oil. The phenolic compounds are substances for the most hydrophilic (often in the form with sugars, which favors solubilisation), this results in a reduced transfer them in the oil with respect to the total quantity contained in the olives departure. The operation of crushing due to breaking of the cells of the drupes, freeing the water and the lipid substance contained inside them; during the malaxation the phenolic compounds, which are located at the interface between oil and water, are transferred favorably in the aqueous phase, during the slow and continuous mixing (Zanoni, 2014).

4.2.3.5 Optimization chart

In order to discuss on optimal time-temperature conditions of olive paste to improve the malaxation, it was useful to compare the kinetics of the above phenomena by the same optimization chart (see 4.1.3.4). In this chart (Fig. 4.2.5) it is possible to distinguish three reference areas in which they can be identified different time-temperature combinations to defined percentages of degradation of the phenolic content in the pastes and oil. The verbascoside is very sensible to degradation; in fact, its decrease of 20% is obtained for combinations of time and temperature smaller than the optimum operating conditions to obtain a good extraction.

1) an intermediate zone around 30° C for 45 min in which would be a decrease of 10% of the total phenolic compounds in paste and a decrease of 20% of the total phenolic compounds and secoiridoid in olive oil, in combination with an effective coalescence of the drops of oil (100%> 30 micron) and to an EI of 80%.
2) a zone at low temperatures, below 30°C, in which a good extraction yield would be obtained for long malaxation time. For the combinations detectable between the kinetics relative to the index of extractability of 80% and the kinetics of degradation of 20% of secoiridoid you would get a fair compromise for an oil extractability of 80% and a limited degradation of total phenolic compounds in the pastes (the area is highlighted in the figure 4.2.5). In addition these conditions

would be obtained definitely a maximum extractability, but with a thrust degradation of the phenolic content. For a short time, however, it would not get a good extractability, but could be taken of the combinations of time, temperature optimum for preserving fully the phenolic content. For example at 25°C for 20 min malaxation there isn't the destruction of 20% of verbascoside.

3) A zone at high temperatures, > 30°C, in which, for the combinations of time-temperature detectable below the degradation of the 10% of phenolic compounds in the paste, the degradation of the phenolic content and the extractability of oil would be minimal. For combinations detectable between the kinetics relative to the index of extractability of 80% and the kinetics of decrease of 20% of secoiridoid would be the loss of 10% of the total phenolic compounds in pasta and 20% of secoiridoid in oil, without get a good extractability, therefore, would be operating conditions to be avoided (the area is highlighted in the figure 4.2.5). In addition these conditions, however, would be reached EI 80%, but with a degradation of 20% of the total phenolic compounds in the oil. However, because the kinetics of the decrease of 20% of phenolic compounds in the oil and the index of extractability 80% are between them almost overlapping, the right compromise would be to keep roughly between the two straight lines.

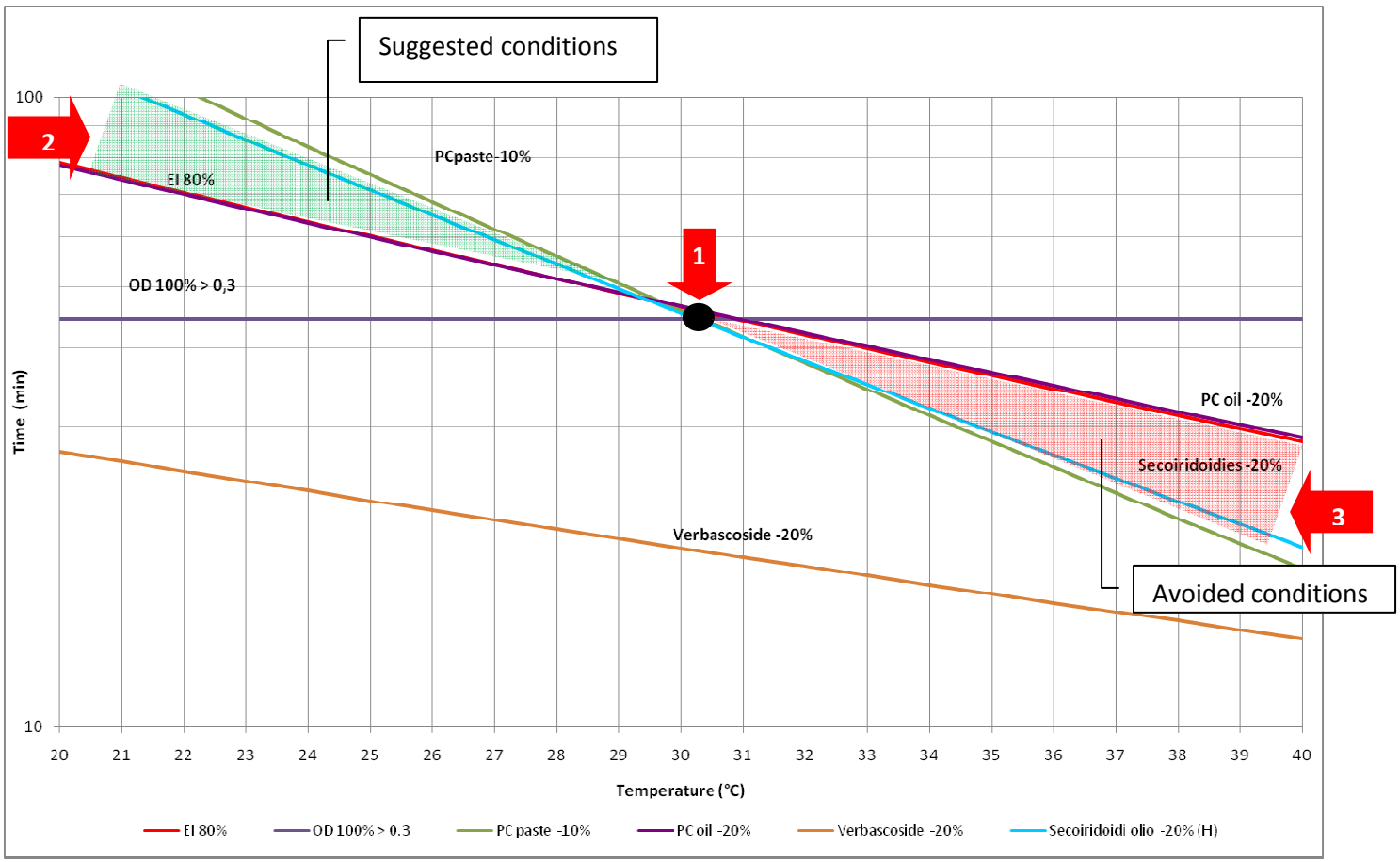

Figure 4.2.5. Chart of optimization of malaxation time-temperature conditions.

Wanting to protect with special care component of secoiridoids the operating conditions to be taken should be identified in the area at low temperatures for long times or between the kinetics of the extractability index and the kinetics of decrease of secoiridoid. In conclusion presumably the optimization map obtained can be used as a hypothetical support tool for operators in managing and directing the malaxation to obtain a limited and controlled degradation of phenolic compounds associated with a good extractability of oil extra virgin olive oil.

4.2.4 References

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5. CONCLUSIONS

The operations of harvesting generally occur over a period of time quite wide, due to labour issues, but mainly dictated by traditions rooted in the territory associated with the postponement of the olives harvest in order to obtain higher yields in oil mill. Following this line, there is no enhancement of the raw material characteristics and no improvement of extracted oils quality occurs. This PhD research project introduce a methodology and an innovative tool that could allows to highlight the optimal time of harvest, in which the fruit is able to simultaneously satisfy two requirements: high oil yield and suitable quality characteristics. Moreover, easy and rapid analytical methods are now available to monitor the olives ripening and decide the optimum date for harvest, using predetermined chemical parameters as reference. It was also evaluated the effect of the fly attack on the oil quality.

On the other hand the time-temperature conditions to be taken during malaxation have not been defined yet in a precise way: some producers gave much attention to preserve the phenolic compounds in the oil, therefore the malaxation is at low temperatures for short times, others, however, to obtain a maximum extraction in oil the malaxation is for very long times, sometimes even at high temperatures causing a significant loss of phenolic content in the oil. This PhD research project was based on an original experimental approach, characterized by (i) the use dell'Abencor lab equipment to simulate parallel several olive paste malaxation treatments; (ii) a simple "on-line" direct measurement of potential extraction performance by centrifugation of an olive paste malaxation treatment; (iii) the direct measurement of the oil droplets coalescence phenomena during malaxation; (iv) the direct measurement of the phenolic compounds during malaxation on olive paste. This allowed to determine for the first time and orders of reaction kinetic constants of oil process yield, oil droplets coalescence and phenolic contents kinetics. It was possible to propose a chart of optimization of malaxation time-temperature conditions to improve process yield oil. This chart can be considered as a useful reference for the operational aspects of improving the process of extraction of extra virgin olive oil, which are connected to the olive paste malaxation. For example, the paper optimization can allow a more informed choice of malaxation operating conditions, showing how to vary malaxation of temperature change in a substantial way the phenomena that affect the process yield oil with consequent variation of malaxation time to apply. Considering concurrently the

phenolic content, also if preliminary, the chart can be used as a hypothetical support tool for operators in managing and directing the malaxation to obtain a limited and controlled degradation of phenolic compounds associated with a good extractability of oil extra virgin olive oil. It may also be the reference for assessing the effect of cultivar, degree of ripeness and water content of the olives on the effectiveness of malaxation. The development of our research will be introduced in the paper optimization other kinetic related to significant phenomena for oil during malaxation, first of all the change in the aromatic profile as well as the confirmation of the phenolic kinetics. A technical modification of Abencor lab equipment will required to assess also the kinetics even in conditions of reduced oxidative impact. A schematic conclusion with principal results and deriverables is given in figure 5.1.

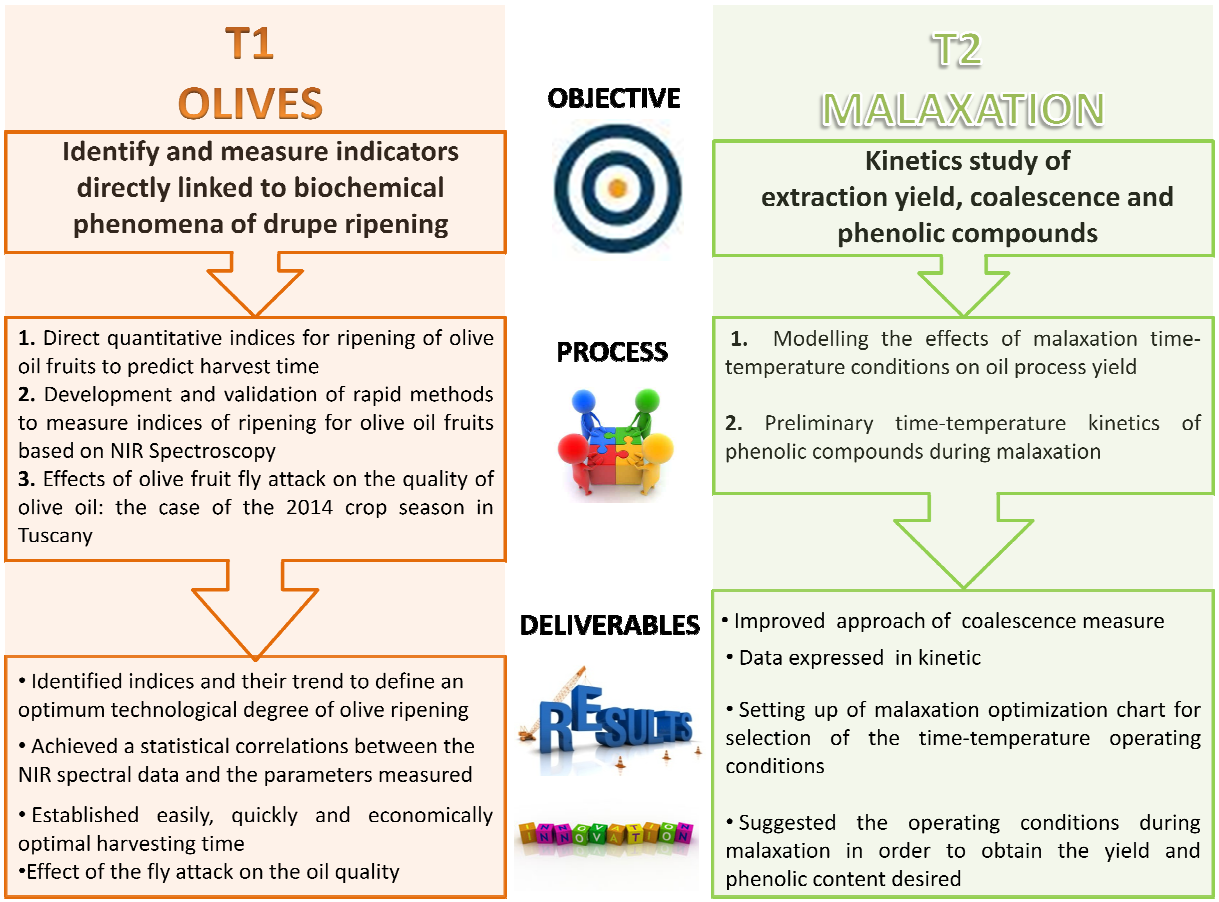

Figure 5.1. Schematic conclusions and deliverables of PhD thesis.

Publications

Scientific Articles

Guerrini S., Mari E., Migliorini M., Cherubini C., Trapani S., Zanoni B., Vincenzini M. 2015. Investigation on microbiology of olive oil extraction process. *Ital. J. Food Sci.* 27:108-114.

Trapani S., Migliorini M., Cherubini C., Cecchi L., Canuti V., Fia G., Zanoni B. 2015 Direct quantitative indices for ripening of olive oil fruits to predict harvest time. *Eur. J. Lipid Sci. Technol.* In press.

Cecchi L., Migliorini M., Cherubini C., Trapani S., Zanoni B. 2015. Effect of olive fruit fly attack on the quality of olive oils: the case of 2014 crop season in Tuscan. *Ital. J. Food Sci.* In press.

Scientific Congress

Oral comunication

Trapani S., Migliorini M., Cherubini C., Cecchi L., Zanoni B. "Harvest time prediction of olive oil fruits by evolution of ripening biochemical Indices", 12th Euro Fed Lipid. Oils, Fats and Lipids: from Lipodomics to Industial Innovation, Montpellier, France, September 2014.

Trapani S. Methods and Indices for Monitoring and Optimization of Extra Virgin Olive Oil Production. 20th Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology. Perugia, Italy, September 2015.

Trapani S., Migliorini M., Bernocchi A., Cherubini C., Cecchi L., Zanoni B. Development and Validation of Rapid Methods to Measure Indices of Ripening for Olive Oil Fruits based on NIR Spectroscopy. 13th Euro Fed Lipid. Oils, Fats and Lipids: New Challenges in Technology, Quality Control and Health, Florence, Italy, September 2015.

Poster

Trapani S. "Methods and Indices for Monitoring and Optimization of Extra Virgin Olive Oil Production" 19th Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology. Bari, September 2014.

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