Indices of oxidative damage to phenolic compounds in olive fruits for the implementation of olive paste malaxation optimization charts

S. Trapani[a], C. Breschi[a], L. Cecchi[b], L. Guerrini[c], N. Mulinacci[b], A. Parenti[c], V. Canuti[a], M. Picchi[a], G. Caruso[d], R. Gucci[d], B. Zanoni[a, *]

[a]Department of Agricultural, Food and Forestry Systems Management (GESAAF) – Food Science and Technology and Microbiology Section, Università degli Studi di Firenze, Via Donizetti 6, 50144 Florence, ITALY

[b]Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), Università degli Studi di Firenze, Via Schiff 6, 50019 Sesto Fiorentino, ITALY

[c]Department of Agricultural, Food and Forestry Systems Management (GESAAF) – Agricultural, Forest and Biosystem Engineering Section, Università degli Studi di Firenze, Piazzale delle Cascine 15, 50144 Florence, ITALY

[d]Department of Agriculture, Food and Environment, Università degli Studi di Pisa, Via del Borghetto 80, 56124 Pisa, ITALY

*Corresponding author:

Tel.: +39 055 2755507;

Fax.: +39 055 2755500

Email address: bruno.zanoni@unifi.it

Running title:

Malaxation oxidative damage indices

Abstract

An original kinetic study of the transformation phenomena of phenolic compounds in olive paste was carried out at different malaxation time-temperature conditions under exposure to air, using Abencor lab equipment to process olives (Frantoio cv) of a known degree of ripeness.

Empirical kinetic models and the relevant apparent kinetic constants were determined for the following significant indices: total phenolic compound content in vegetation water samples using the Folin-Ciocalteu method; verbascoside and β-OH-verbascoside contents in olive paste samples using HPLC-UV; and 3,4-DHPEA-EDA contents in olive oil samples using HPLC-UV. Two opposite phenolic compound transformation phenomena were proposed to explain the kinetic models: (i) enzymatic oxidative damage of phenolic compounds; (ii) physical and enzymatic release of phenolic compounds from cellular tissues. It was possible to propose a reference optimization chart to predict "selective" time-temperature conditions to maximize the apparent EVOO extraction yield while minimizing the degradation phenomena of phenolic compounds during malaxation.

Keywords:

Kinetics, Malaxation, Modelling, Olive oil, Phenolic compounds, Yield

1. Introduction

Oxidation is the most frequent degradation behaviour of food after microbial spoilage phenomena. Oxidative damage to food consists of oxidation reactions in lipids, proteins and minor compounds, causing a negative effect on food, particularly in terms of sensory and nutritional qualities. Oxidative reactions involve enzymatic or non-enzymatic phenomena and they are proportionally related to food temperature (Diplock et al., 1998).

One of food technology's missions is to minimize oxidative damage in food processing where exposure to oxygen and, in general, operating conditions with high potentials of redox can occur. Therefore, it is necessary to select effective indices to both monitor and optimize operating conditions to control oxidative damage in food.

Extra virgin olive oil (EVOO) extraction processing can be an interesting example of how this approach can be applied in consideration of effects on the phenolic compounds in olive fruits. The phenolic profile has a critical role in the quality of EVOO. The amount of the different phenolic compounds is positively related to the preservation of oil quality from oxidation during shelf life, and it is responsible for EVOO's "bitter" and "pungent" sensory descriptors. Moreover, these compounds prevent ageing phenomena and several chronic diseases in humans (Clodoveo et al., 2014). Biochemical, chemical and physical phenomena that affect EVOO's phenolic profile, including enzymatic oxidative reactions, occur during the ripening of the olive fruits and the oil extraction process (Zanoni, 2014).

An impressive number of phenolic compounds (i.e. particularly oleoside compounds) are present in *Olea europaea* fruits. Secoiridoids, such as oleuropein, demethyloleuropein and ligstroside represent the predominant phenolic oleosides,

whereas verbascoside is the main hydroxycinnamic derivative of olive oil fruits. Simpler phenolic compounds such as hydroxytyrosol and tyrosol are also present. The olive cultivar, geographical area of production, climatic conditions during the crop season, crop load and olive health conditions affect the phenolic profile of olive oil fruits (El Riachy et al., 2011). The concentration of oleuropein declines with the physiological development of the fruit, whilst the concentration of verbascoside and demethyloleuropein increase with ripening (Ryan et al., 2002). Artajo et al. (2007) noted a significant decrease in phenolic compounds in the Arbequina cultivar in relation to the harvest period; in the study by Trapani et al. (2016) oleuropein and oleoeuropein aglycone (3,4-DHPEA-EA) contents showed a linear decrease during olive ripening for the Frantoio and Moraiolo cultivars. Water availability has a considerable effect on phenolic composition and the literature has supported that fruit moisture negatively affects the phenolic content of oil (Talhaoui et al., 2016); some studies have also reported an increase in secoiridoids in water-stressed olive trees (Artajo et al., 2006; Caruso et al., 2014).

However, the phenolic profile of olive oil fruits is not the same as the phenolic profile of extractable EVOO, since numerous transformation phenomena occur during the oil extraction process. Phenolic compounds are distributed greatly between the water and oil phases of olive paste, obtained by crushing the olive fruits. The greater affinity of phenolic compounds towards the water phase means that only 0.3% - 2% of the phenols available in the olive fruits are transferred to the oil (Rodis et al., 2002). Secoiridoids are the compounds with the highest transfer rate from fruits to oil, followed by simple phenols; due to its structure, no verbascoside is found in EVOO (Klen and Vodopivec, 2012; Talhaoui et al., 2016). Moreover, rupturing of the olive cell tissues activates a

series of enzymatic and non-enzymatic phenomena in the phenolic compounds. New phenolic compounds, which are hydrolytic forms of oleoeuropein and ligstroside, appear in the olive paste, whereas some fruit phenols disappear after crushing; therefore, the dialdehydic form of decarboxymethyl oleuropein aglycone (3,4-DHPEA-EDA) is often EVOO's most abundant phenolic compound (Zanoni, 2014; Klen et al., 2015a).

Three main steps in the oil extraction process affect the EVOO's phenolic profile: the crushing of the olive fruits, malaxation of the olive paste, and mechanical separation of the oil. The crushing step causes the initial physical partition of the phenolic compounds into the oil and water phases of the olive paste and activates the enzymatic (i.e. β-glucosidase activity) and non-enzymatic hydrolytic phenomena that transform oleoeuropein and ligstroside into their respective aglycones and decarboxymethylated forms (Clodoveo et al., 2014; Leone et al., 2015). The malaxation step consists of slow and continuous kneading of the olive paste to induce physical phenomena (i.e. oil droplet coalescence, rising of oil to the surface) that improve the oil process yield (Trapani et al., 2017); in general, malaxation is expected to continue the above hydrolytic phenomena without any enzymatic oxidative degradation (i.e. polyphenol oxidase and peroxidase activities) of the phenolic compounds (Clodoveo, 2012). Finally, the processing parameters during separation of the oil by centrifugation (i.e. use of a horizontal centrifuge with screw conveyor, namely "decanter") from the solid and water phases of olive paste have to be planned and controlled to maximize phenolic compound dissolution in the extractable EVOO (Altieri et al., 2013; Caponio et al., 2014).

In view of the various possible combinations of operating conditions, such as time, temperature, oxygen exposure and kneading tools, several studies on the effect of

malaxation on the phenolic profile of EVOO can be reported (Angerosa et al., 2001; Ranalli et al., 2001; Parenti and Spugnoli, 2002; Ranalli et al., 2003; Kalua et al., 2006; Migliorini et al., 2006; Artajo et al., 2007; Parenti et al., 2008; Servili et al., 2008; Boselli et al., 2009; Gomez-Rico et al., 2009; Migliorini et al., 2009; Espinola et al., 2011; Migliorini et al., 2012; Catania et al., 2013; Taticchi et al., 2013; Tamborrino et al., 2014a; Klen et al., 2015a). The literature data shows that the malaxation behaves in a more complex way than the one described above. The secoiridoid profile depends on a combination of the following three kinds of opposite phenomena: (i) enzymatic oxidative degradation catalyzed by polyphenol oxidases (PPOs) and peroxidases (PODs), which cause a decrease in the phenolic compound content; (ii) enzymatic (i.e. β-glucosidase activity) and non-enzymatic hydrolytic phenomena that transform oleoeuropein and ligstroside into their respective aglycones and decarboxymethylated forms, especially the 3,4-DHPEA-EDA compound; (iii) physical and enzymatic (i.e. pectinase and cellulase activities) phenomena which promote the release of phenolic compounds from cellular tissues and then cause an increase in the phenolic compound content. Among the cinnamic acids, verbascoside content decreases, whereas its derivatives, such as the β-OH-verbascoside diastereoisomers, increase during malaxation.

The literature data shows an incomplete and not uniform overview of the overall effect of the above phenomena on the phenolic profile of EVOO (relevant remarkable data are presented as supplementary material in Table S1). However, two common behaviours seemed to be observed: the content of the most representative phenolic compounds tends to decrease with malaxation time at a constant temperature, while it tends to increase with malaxation temperature at a constant time. These effects inversely depend

on the oxygen exposure of the olive paste during malaxation: the higher the partial oxygen pressure, the greater the above decrease in phenolic compound content with time and the smaller the above increase in phenolic compound content with temperature.

No modelling based on pseudo *n*-order kinetics has been carried out on either the phenomena involved or the relationships of relevant rate constants with temperature. Therefore, the lack of quantitative time-temperature relationships makes it more difficult to apply the literature data to control olive paste malaxation. A kinetic approach to phenolic compound transformation phenomena may also link up to our previous time-temperature kinetic study to predict the potential effect of malaxation on extraction yield (Trapani et al., 2017), in order to strike a balance between oil yield and oil quality characteristics.

The aim of this work is to apply a kinetic approach to phenolic compound transformation phenomena in order to select technological indices for the implementation of olive paste malaxation optimization charts.

2. Material and methods

2.1. Malaxation trials

The olive fruits were harvested in a high-density (513 trees ha^{-1}), fully productive olive (*Olea europea* L., cv. Frantoio) orchard located at the experimental farm of University of Pisa (Caruso et al., 2013). A sample of approx. 40 kg of fruits was harvested by hand from two adjacent trees on 21 October 2015 and quickly transported to the laboratory.

~~The olive oil fruits (*Olea europea* L. Frantoio cv.) were supplied by the Pisa University experimental farm located in Venturina (Livorno, Italy) during the 2015 crop season.~~

~~The ripe olive oil fruits were picked by hand at 08:00 a.m. at the end of October. Approximately 40 kg of olive oil fruits, which presented no infection or physical damage, were quickly transported to the laboratory.~~

The kinetic study was performed using Abencor lab equipment (Abencor analyser, MC2 Ingegneria Y Sistemas S.L., Seville, Spain) following Trapani et al. (2017). With respect to its usual use, the equipment was utilized both for the olive crushing and olive paste malaxation, but not for the olive paste centrifugation. The equipment consisted of an "MM-100" hammer mill (with 5.5 mm-diameter crusher holes) and a thermostated water bath (Thermo-mixer TB-100), with eight work sites; the work sites consisted of eight stainless steel mixing jars (speed of mixing blades: 50 rpm) under exposure to air, so that several olive paste malaxation treatments could be simulated in parallel. It was deliberately decided to perform the malaxation in this manner to make the oxidative degradation phenomena more evident.

The malaxation trials were carried out in triplicate at 22, 27, 32 and 37°C for 0, 20, 40, 60, 80 and 100 minutes; the water and paste temperatures were monitored using a type T thermocouple thermometer (Testo 926, Milan, Italy). Approximately 2.1 kg of olive paste, separated into six mixing jars each containing 350 g of olive paste, were used for each malaxation trial.

The olive paste samples were partly used to measure the phenolic compound content and partly to measure the apparent oil extraction yield, as reported below in the description of the analysis methods.

2.2. Analysis methods on olive oil fruits

188 The olive samples were analysed for weight, pulp/stone ratio and Maturity Index
189 (Anonymous, 2011). The Maturity Index was based on the evaluation of the olive skin
190 and pulp colours. The values ranged from 0 (deep green skin colour) to 7 (black skin
191 colour with all the flesh purple to the stone).
192 A homogeneous batch of olives (i.e. approx. 300 g) were crushed in a laboratory crusher
193 (Zeutec, Rendsburg, Germany), and the olive paste was used to make chemical analyses
194 of the water and oil contents. The water content of the olive paste was measured by
195 heating 60 g of the sample in an oven at 105°C until a constant weight was reached. The
196 total oil content was determined on 5 g of dried olive paste (see the above oven
197 method). Samples were extracted using hexane in an automatic extractor (Randall
198 mod.148, VELP Scientifica, Milan, Italy), following the method of Cherubini et al.
199 (2009). The characteristics of the processed olive oil fruits are given as supplementary
200 material in Table S2.
201
202 *2.3. Chemical analysis methods on the olive paste, olive oil and vegetation water*
203 The phenolic compound content was extracted and determined on olive paste,
204 vegetation water and olive oil samples. The olive oil and vegetation water samples were
205 obtained by centrifugation (type 4239R, Alc Int. s.r.l, Milan, Italy); the olive paste
206 samples, in 50 mL screw-cap tubes, were centrifuged at 4000 rpm (1800 G) for 15 min
207 followed by a second centrifugation at 7000 rpm (5400 G) for 10 min. The oil and water
208 phases were collected separately using a Pasteur pipette and then put into 15 mL test
209 tubes for the following chemical analyses.
210 Phenolic compound content by Folin-Ciocalteu (Singleton and Rossi, 1965)

Olive paste. A 4.0 g olive paste sample was weighed in a 100 mL screw-cap tube and 80 mL of MeOH/H_2O solution (60/40, v/v) was added. The tube was shaken for 30 min and then was centrifuged at 4000 rpm for 15 min; the MeOH/H_2O phase was collected. The above extraction method was repeated and the collected MeOH/H_2O phases were brought to volume with MeOH/H_2O solution (60/40, v/v) in a 200 mL flask, which was stored in a freezer at least for 2 hours; then, the above solution was filtered (FN 7 Munktell, Ahlstrom Falun AB, Falun, Sweden). 1.0 mL of the filtered phenolic extract was added to 5 mL of Folin-Ciocalteu reagent and 20 mL of Na_2CO_3 solution (20% w/v); the solution was brought to volume with purified water in a 100 mL flask and was stored for 1 hour at room temperature. The total phenolic compound content was detected at 765 nm (Lambda 35 UV/Vis Spectrometer, Perkin Elmer, Waltham, MA) and quantified using a gallic acid calibration curve (r^2 = 0.997) as mg gallic acid kg^{-1} of olive paste.

Olive oil. A 5.0 g olive oil sample was weighed in a 100 mL screw-cap tube and 10 mL of MeOH/H_2O solution (80/20, v/v) was added. The tube was shaken for 30 min and then was centrifuged at 4000 rpm for 10 min; the MeOH/H_2O phase was collected. The above extraction method was repeated and the collected MeOH/H_2O phases were brought to volume with MeOH/H_2O solution (80/20, v/v) in a 25 mL flask, which was stored in a freezer at least for 5 hours; then, the above solution was filtered. 1.0 mL of the filtered phenolic extract was added to 10 mL of Folin-Ciocalteu reagent (1/10 diluted) and the solution was brought to volume with Na_2CO_3 solution (7.5% w/v) in a 20 mL flask; it was stored for 2 hours at room temperature. The total phenolic compound content was detected at 765 nm and quantified using a gallic acid calibration curve (r^2 = 0.997) as mg gallic acid kg^{-1} of olive oil.

Vegetation water. The vegetation water sample was filtered and 1.0 g of the filtered vegetation water sample was weighed and then it was brought to volume with purified water in a 20 mL flask. 1.0 mL of the phenolic extract was added to 50 ml of purified water, 5 mL of Folin-Ciocalteu reagent and 20 mL of Na_2CO_3 solution (20% w/v); the solution was brought to volume with purified water in a 100 mL flask and was stored for 1 hour at room temperature. The total phenolic compound content was detected at 765 nm and quantified using a gallic acid calibration curve ($r^2 = 0.997$) as mg gallic acid kg^{-1} of vegetation water.

Phenolic compound content by HPLC-UV

Olive paste. The phenolic compounds were extracted from the olive paste using the Cecchi *et al.* (2013) method. An 8.0 g olive paste sample was added to a 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, v/v solution) and 30 mL of EtOH/H_2O solution (80/20, v/v). The mixture was homogenized with ULTRA-TURRAX at 11,000 rpm in an ice bath for 3 min and centrifuged (type PK121R, Alc Int. s.r.l, Milan, Italy) at 4,000 rpm (2000 G) at 0°C for 10 min. Then the supernatant was added to a 100 mL flask and it was stored in a freezer. The extraction procedure was repeated with 30 mL of EtOH/H_2O solution (80/20, v/v), and the obtained supernatant was added to the flask.

The obtained solution was concentrated in a vacuum at approx. 35°C, added to 2.5 mL of Milli-Q-Water (Millipore SA, Molsheim, France), washed twice with 25 mL of hexane in a separating funnel to remove lipid component, centrifuged at 14,000 rpm (24540 G) at 0°C for 5 min, and poured into a 10 mL flask. Five mL of methanol was added to the solution, which was brought to volume with Milli-Q-Water. The

MetOH/H_2O solution of the phenolic extract was immediately used for the chromatographic analysis.

Chromatographic analyses were carried out using an HP1200L Liquid Chromatograph (Agilent Technologies, Palo Alto, CA), equipped with an autosampler, a column heater module, a quaternary pump, and coupled with DAD and MS detectors.

A Poroshell 120 EC-C18 column (3.0 mm, internal diameter; 150 mm, length; 2.7 µm, particle size) (Agilent Technologies, Palo Alto, CA) was used. It was equipped with a pre-column of the same phase. Elution was performed at a flow rate of 0.4 mL min^{-1} with a multistep linear gradient, using H_2O brought to pH 3.2 by formic acid (solvent A) and acetonitrile (solvent B). The three-step linear gradient of both solvents A and B changed as follows: from 95% A/5% B to 60% A/40% B in 40 min, with isocratic elution for 5 min, to 0% A/100% B in 5 min, with isocratic elution for 3 min, then to 95% A/5% B in 2 min. The total time of analysis was 55 min. All the solvents used were of HPLC grade. Syringic acid was chosen as the internal standard. The phenolic compounds were quantified at 280 nm; syringic acid and tyrosol were chosen as external calibration standards to evaluate the relative response factor (i.e. RRF = 4.74) and phenolic compound content values were expressed as mg_{tyr} kg^{-1} of olive paste. Verbascoside and β-OH-verbascoside diastereoisomers were also quantified at 330 nm; syringic acid and verbascoside were chosen as external calibration standards to evaluate the relative response factor (i.e. RRF = 3.04) and verbascoside and β-OH-verbascoside diastereoisomers content values were expressed as mg_{verb} kg^{-1} of olive paste.

Olive oil. The extraction, identification and determination of phenolic compounds were performed on the olive oil samples in agreement with the official IOC method (Anonymous, 2009). The hydrophilic phenolic compound was extracted from the oil

using a MeOH/H_2O (80/20, v/v) solution. The phenolic compounds in the mixture were separated and determined by an HPLC series 200 LC (Perkin Elmer Inc., Waltham, MA) consisting of a Perkin Elmer series 200 autosampler and a quaternary pump, coupled with a 9050 UV-Vis detector (Varian Inc, Palo Alto, CA). The analytical conditions were: pre-column: LiChroCART® 4-4 Purospher® STAR RP-18E, 5 µm (Merck KGaA, Darmstadt, Germany); HPLC column: LiChroCART® 250-4.6 Purospher® STAR RP-18E, 5 µm (Merck KGaA, Darmstadt, Germany); injection volume: 20 µl; solvent: acid H_2O (0.2% H_3PO_4)/acetonitrile/methanol gradient as described in the official method; wavelength: 280 nm.

Syringic acid was used as the internal standard; syringic acid and tyrosol were chosen as the external calibration standards to evaluate the relative response factor (i.e. RRF = 5.40). The phenolic compound content values were expressed as $mg_{tyr}\,kg^{-1}$ of olive oil.

2.4. Physical analysis methods on the olive paste, olive oil and vegetation water

Partition coefficient

Partition coefficients (*P*) were determined in order to compare the difference in solubility of the phenolic compound content in the different phases during malaxation. The ratio between the total phenolic compound content in olive oil and vegetation water ($P_{o/w}$) and the ratio between the total phenolic compound content in olive oil and olive paste ($P_{o/p}$) were determined using analytical data from the Folin-Ciocalteu and HPLC-DAD methods, respectively.

Apparent oil extraction yield

An apparent Extractability Index (EI_{app}) of oil during malaxation was measured following Trapani et al. (2017). This method permitted a quick measurement of the

potential extraction performance by centrifugation of an olive paste malaxation treatment; hence, at increasing values this index would increase the effect of the malaxation on the olive paste, thus making the oil easier to extract industrially by way of centrifugation using a "decanter".

The apparent Extractability Index (EI_{app}) was calculated using the following ratio:

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

where the extraction yields are expressed as percentage ratios of the mass of extracted oil and the mass of centrifuged olive paste; *EY (%)* is the percentage extraction yield and EY_{max} (%) is the percentage maximum oil extraction yield (Table S2).

2.5. Data processing

The analytical data were statistically processed according to a multifactor ANOVA using Statgraphics Centurion software (ver. XV, Statpoint Technologies, Warrenton, VA). Type III sums of squares were chosen and the contribution of each factor (i.e. time, temperature and replication) was measured after removing the effects of all of the other factors. The P-value test measured the statistical significance of each of the factors.

Time-temperature models were set up following the common kinetic approach to express the relationships between data and time as pseudo-chemical kinetics and then to correlate the relevant rate constant of the reactions with temperature. The kinetic data were processed using Table Curve 2D Version 4 software (Systos Software Inc., Richmond, CA).

3. Results and discussion

In our study the choice of which phenolic compounds to measure was based on criteria of both analytical effort and the relevance of the compounds in the literature in order to study the effect of malaxation on EVOO quality (Klen et al., 2015a). Therefore, measurements using the Folin-Ciocalteu method were carried out on olive paste, olive oil and vegetation water samples to determine the total phenolic compound content in a simple way; measurements using HPLC-UV methods were carried out both to determine the total phenolic compound content, as well as the verbascoside and β-OH-verbascoside diastereoisomer contents in the olive paste samples, and to determine the total contents of phenolic compounds and oleuropein and derivatives in the olive oil samples.

In order to determine the kinetic models a prior assessment was performed of the statistic significance of the time-temperature variations of the measured indices (Table 1). Significant chemical indices were highlighted for every type of sample; among these were indices of known importance (i.e. total phenolic compounds by Folin-Ciocalteu and 3,4-DHPEA-EDA) and indices about which less is known (i.e. verbascoside and β-OH-verbascoside diastereoisomers). Of the physical indices, the apparent Extractability Index proved to be significant, confirming what was reported by Trapani et al. (2017). Instead, the partition coefficients did not prove to be significant. These indices assumed values on average between 4 and 5%, similarly to the studies by Artajo et al. (2007). The fact that there were no variations suggests that the transformations of the phenolic compounds during malaxation did not display significant mass transfer phenomena between the water and oil phases of olive paste. The mean values of all the above significant indices are presented as supplementary material in Table S3.

354 *3.1 Kinetic models of phenolic compound transformation phenomena*

355 Table 2 shows the kinetic models of the phenolic compound transformation phenomena,
356 which were produced by normalizing the data in Table S3, that is, by processing the
357 data to determine their relative variation in relation to the data measured at time t = 0
358 ($\triangle_{rel}$). In the case of the β-OH-verbascoside diastereoisomers it was preferred to
359 determine the kinetic model relating to the sum of their contents. As they are complex
360 phenomena all the kinetic models are empirical and the kinetic constants are apparent.

361 In the vegetation water samples the normalized total phenolic compound content by
362 Folin-Ciocalteu decreased linearly with time at the different tested temperatures (Fig.
363 1); a maximum decrease of approx. 40% occurred at 27°C after 100 min of malaxation.
364 The apparent decreasing rates ($K_{f(\vartheta)}$) showed an irregular trend with temperature: they
365 increased from 22 to 27°C, then they decreased, at 37°C reaching a similar value to
366 what was seen at 22°C. A polynomial model with a maximum point was suitable to
367 describe this relationship (Table 2). Figure 1 shows an agreement between the
368 experimental and predicted data.

369 The normalized verbascoside content in the olive paste samples strongly decreased with
370 time; verbascoside disappeared almost completely at 27°C after 100 min of malaxation
371 (Fig. 2). Nevertheless, this decrease assumed a different trend as a function of
372 temperature, with a clear concave curve at 22 and 37°C, but an exponential curve at
373 27°C. The general trend modelled by kinetics combined an apparent lag phase of
374 verbascoside decrease with an apparent decreasing exponential phase (Table 2). The
375 relationships of the apparent kinetic constants of the above phases ($t_{lag\ f(\vartheta)}$, $K_{f(\vartheta)}$) with
376 temperature were described by polynomial models with a minimum point (Table 2).
377 Figure 2 shows an agreement between the experimental and predicted data.

A similar behaviour compared with verbascoside content was evidenced for normalized 3,4 DHPEA-EDA content in the olive oil samples (Fig. 3). As a result, the relevant kinetics were comparably modelled (Table 2). Figure 3 shows an agreement between the experimental and predicted data.

The normalized sum of the β-OH-verbascoside diastereoisomer content increased linearly with time at the different tested temperatures (Fig. 4); an increase of four times occurred at 37°C after 100 min of malaxation. It was possible to significantly describe the experimental data using a pseudo zero-order kinetics with a rate constant ($K_{f(\vartheta)}$) that was temperature dependent through the Arrhenius equation (Table 2). Figure 4 shows an agreement between the experimental and predicted data.

The overall vision of the above kinetics can coherently suggest that the phenolic compound transformation phenomena were caused by two opposing phenomena during olive paste malaxation, in line with the literature data (Boselli et al., 2009; Clodoveo, 2012; Taticchi et al., 2013; Clodoveo et al., 2014; Klen et al., 2015a): (i) a decreasing phenomenon probably due to enzymatic oxidative damage of the phenolic compounds; (ii) an increasing phenomenon probably due to a physical and enzymatic release of phenolic compounds from the cellular tissues. The effects of the above combination of phenomena were time-temperature dependent. In relation to the decreasing phenomenon it can be assumed that, after an activation phase, the speed increases as the temperature increases. In relation to the increasing phenomenon it can be assumed that it was absent or limited to 22 and 27°C, to then become present, at an increasing speed, at 32 and 37°C, so much so that at 37°C it cancelled out the effects of the decreasing phenomenon; this phenomenon tends to die out in time, seeing as at 37°C, even after a long period of malaxation, the effects of the decreasing phenomenon were seen. Hence,

402 the irregular variation of the apparent decreasing rates with the temperature of the total
403 phenolic compounds by Folin-Ciocalteu reflected the combination of the different
404 speeds of the two aforesaid phenomena (Fig. 1). Similarly, the apparent lag phases of
405 verbascoside and 3,4 DHPEA-EDA kinetics reflected either a slow decreasing
406 phenomenon or an increasing phenomenon which disguised the effects of the decreasing
407 phenomenon (Fig.e 2 and 3). The upshot is also that the different kinetics between the
408 verbascoside and its β-OH diastereoisomers must be related to the aforesaid
409 transformation phenomena resulting from the verbascoside (Figs. 2 and 4). The linear
410 and exponentially temperature-dependent increase in β-OH verbascoside
411 diastereoisomers could just be the expression of the decreasing phenomenon; that is,
412 these diastereoisomers could be considered products of the verbascoside degradation
413 due to a hydroxylation reaction, probably of an enzymatic nature. This consideration
414 can be added to what was reported by Klen et al. (2015b).
415
416 *3.2 Apparent oil extraction yield kinetic models*
417 According to Trapani et al. (2017), the modelling of the evolution of the oil extraction
418 yield, expressed as an apparent Extractability Index (EI_{app}), by pseudo first-order
419 kinetics was statistically significant at every malaxation temperature (Table 2). It was
420 reasonably assumed that for $t = 0$, $EI_{app} = 0$ and that EI_{app} tends in time to
421 asymptotically reach a maximum value of 100% ($EI_{app,max}$). The rate constant ($K_{f(\vartheta)}$)
422 was also significantly temperature dependent through the Arrhenius equation (Table 2).
423 Figure 5 shows an agreement between the experimental and predicted data.
424 Compared to the data of Trapani et al. (2017), the kinetic models were characterized by
425 lower values of Arrhenius constants: $K_0 = 3500$ min^{-1} vs. $K_0 = 7.50\ 10^7$ min^{-1} and $Ea =$

28064 J mol^{-1} vs. *Ea* = 54512 J mol^{-1}. As a result, there was a faster increase in the apparent extraction yield during malaxation. It is thought that this was possible thanks to the greater oil content (24%) and the greater Maturation Index (3.2) of the olive oil fruits (Table S2) compared to those referring (i.e. oil content = 20%; Maturation Index = 1.1) to the olives used in the experiment by Trapani et al. (2017). The data of Espinola et al. (2011) tend to confirm this hypothesis.

3.3. A malaxation time-temperature optimization chart

The direct application of the above kinetics enabled the construction of a synoptic chart to predict the potential effect of malaxation on phenolic compound content in isothermal conditions.

The chart was outlined with a logarithmic scale on the y-axis showing the malaxation time, and a linear scale on the x-axis showing the malaxation temperature (Fig. 6). On the chart it was possible to plot different relationships between the times and temperatures of malaxation, corresponding to defined quantitative levels of apparent phenolic compound oxidative damage, represented by the above selected indices. As the objective was to choose just one representative index for the vegetation water, the olive paste and the olive oil samples, it was opted to show the following defined medium-low levels of apparent oxidative damage by way of example in the synoptic chart: 10% and 20% apparent decrease in the total phenolic compound content using the Folin-Ciocalteu method in the vegetation water, 10% and 20% apparent increase in the sum of β-OH verbascoside diastereoisomers in the olive paste and 10%, and 20% apparent decrease in 3,4 DHPEA-EDA in the olive oil.

449 The malaxation time (t) to reach the above set of apparent oxidative damage levels as a
450 function of the olive paste malaxation temperature (ϑ) was calculated according to the
451 relevant kinetics models in Table 2 as follows:

452 • for the total phenolic compound content using the Folin-Ciocalteu method:

453 $$t = \left(1 - \Delta_{rel,ref}\right) \cdot \frac{1}{K_{f(\vartheta)}} \quad [2]$$

454 where $\Delta_{rel,ref}$ is the chosen normalized value of reference (i.e. in our case 0.9 or 0.8
455 corresponding respectively to a 10% and 20% apparent decrease value);

456 • for the sum of β-OH verbascoside diastereoisomer content:

457 $$t = \left(\Delta_{rel,ref} - 1\right) \cdot \frac{1}{K_{f(\vartheta)}} \quad [3]$$

458 where $\Delta_{rel,ref}$ is the chosen normalized value of reference (i.e. in our case 1.1 or 1.2
459 corresponding respectively to a 10% and 20% apparent increase value);

460 • for 3,4 DHPEA-EDA content:

461 $$t = \frac{ln\left(\frac{1+\exp\left(-K_{f(\vartheta)} \cdot t_{lag,f(\vartheta)}\right)-\Delta_{rel,ref}}{\Delta_{rel,ref}}\right)+K_{f(\vartheta)} \cdot t_{lag,f(\vartheta)}}{K_{f(\vartheta)}} \quad [4]$$

462 where $\Delta_{rel,ref}$ is the chosen normalized value of reference (i.e. in our case 0.9 or 0.8
463 corresponding respectively to a 10% and 20% apparent decrease value);

464 The synoptic chart (Fig. 6) shows how together the three chosen indices give an overall
465 vision of the effects of the malaxation time-temperature conditions on the
466 transformation phenomena of phenolic compounds.

467 The sum of β-OH verbascoside diastereoisomer content proved to be the most sensitive
468 index among those chosen at the malaxation time-temperature conditions (i.e. levels of
469 damage reached in lower malaxation times at the same temperature). Forming a straight

line, it can be considered the index that expresses the substantially oxidative damage of the phenolic compounds only.

The total phenolic compound content using the Folin-Ciocalteu method proved to be the least sensitive index among those chosen to the malaxation time-temperature conditions (i.e. levels of damage reached in longer malaxation times at the same temperature). Forming a convex curve, it can be considered the overall and simple measurement index that expresses the combination of phenolic compound damage and release phenomena.

The trends of time as a function of malaxation temperature to reach set levels of apparent degradation of 3,4 DPHEA-EDA took on the appearance of highly convex curves; this is due to the kinetics dealt with in the previous paragraph which, thanks to measuring a specific compound (and not a set of compounds like in the case of total phenolic compound content by Folin-Ciocalteu) made it easier to separate the phenolic compound degradation and release phenomena. As such, the 3,4 DPHEA-EDA content proved to be the index that best represents the effect of the malaxation time-temperature conditions on the phenolic compounds in the extractable oil.

The synoptic chart can also be used for optimization purposes, for example, if it plots, using straight lines, the different relationships between the times and temperatures of malaxation, corresponding to values of 60% and 80% (i.e. expression of insufficient and satisfactory oil process yields, respectively) of the apparent Extractability Index (Fig. 7). Please see Trapani et al. (2017) for the equation that expresses the malaxation time to reach the above set of apparent extraction levels as a function of the olive paste malaxation temperature, according to the relevant kinetic model in Table 2.

It is evident how in the adopted strong oxidative impact experimental conditions an acceptable apparent yield is not compatible with a lower degradation of the sum of β-

OH verbascoside diastereoisomer content; from the synoptic chart it can be deduced that a lower degradation is compatible with an apparent yield of less than 50% or that an 80% apparent yield determines degradation of around 50% of the sum of β-OH verbascoside diastereoisomer content.

However, should the combination between phenolic compound degradation and release phenomena be considered, an apparent acceptable yield appears compatible with lower apparent degradation of the total phenolic compound content by Folin-Ciocalteu. Instead, an acceptable apparent yield only seems compatible with a lower apparent degradation of the 3,4-DHPEA-EDA content for some time-temperature combinations. For example, by moving along the straight line corresponding to 80% of the apparent yield, three zones can be seen with reference to the adopted experimental conditions: (i) a zone with an approximate temperature of < 23°C for time = 40 min compatible with a reduced apparent degradation of 3,4 DHPEA-EDA; (ii) a zone with an approximate temperature of > 23°C for times between 40 and 30 min responsible for a high apparent degradation of 3,4 DHPEA-EDA; (iii) a zone with an approximate temperature of > 33°C for time < 30 min compatible with a lower apparent degradation of 3,4 DHPEA-EDA.

4. Conclusions

This research is based on an original kinetic approach which enabled the prediction of the effects of time-temperature conditions of malaxation treatment under exposure to air on the transformation phenomena of phenolic compounds in olive paste.

It was possible to identify and quantify two contrasting phenolic compound transformation phenomena, which were measured on samples both of olive paste and its

518 vegetation water and oil components: (i) a decreasing phenomenon probably due to
519 enzymatic oxidative damage of phenolic compounds; (ii) an increasing phenomenon
520 probably due to a physical and enzymatic release of phenolic compounds from the
521 cellular tissues. These phenomena could be significantly monitored by three different
522 but complementary technological indices. The sum of β-OH verbascoside
523 diastereoisomer content in the olive paste samples proved to be a very sensitive index in
524 expressing the degradation phenomena. The total phenolic compound content by Folin-
525 Ciocalteu and the 3,4 DHPEA-EDA proved to express the combination of the two
526 aforesaid transformation phenomena in the vegetation water and oil samples,
527 respectively; of the two, the second appeared of particular interest as it specifically
528 refers to the most important phenolic component present in EVOO.
529 With regard to the experimental conditions adopted in this work, it was possible to
530 propose a reference optimization chart in order to predict "selective" time-temperature
531 conditions to maximize the apparent EVOO extraction yield while minimizing the
532 degradation phenomena of phenolic compounds during malaxation treatment when the
533 olive paste is exposed to oxygen. The chart shows how an acceptable apparent yield is
534 not compatible with a lower degradation of the phenolic compounds. Nevertheless, in
535 consideration of the presence of phenolic compound release phenomena too, time-
536 temperature combinations can be seen that are compatible for example with a
537 minimization of the apparent degradation of the 3,4 DHPEA-EDA content.
538 Our kinetic approach could be also a useful reference to understand and quantify the
539 potential efficacy on the optimization of malaxation treatment of several production
540 elements. The effects of cultivar and the degree of ripeness of the olive oil fruits
541 (Espinola et al., 2011, Caruso et al., 2013, Caruso et al., 2014[GC1]), of technological

innovations in the pre-treatment of olive paste prior to malaxation based on ultrasound or microwave techniques (Clodoveo et al., 2013; Tamborrino et al., 2014) and of malaxation treatment under no or controlled exposure of the olive paste to oxygen (Servili et al., 2008; Leone et al., 2014; Catania et al., 2016) could be compared with the kinetic model devised in this work.

Lastly, the proposed approach to quickly heat the olive paste to a particular temperature using a tubular heat exchanger, leave the paste in the malaxer for the desired time and then send it for extraction in a “decanter” seems a good idea in order to best exploit what is shown in this work (Veneziani et al., 2015; Leone et al., 2016). Such an approach would pave the way towards real control of malaxation treatments in order to achieve the desired phenolic profiles in EVOO.

Acknowledgements

This research was supported by the Tuscan regional government "NUTRIFOROIL Project DD 6107/2013 - Progetti integrati di ricerca in agricoltura a favore di Università ed Enti di ricerca operanti in Toscana", which was published in Tuscan regional government Official Gazette (BURT) no. 18 of 05/02/2013.

559 **References**

560 Altieri, G., Di Rienzo, G.C., & Genovese, F. (2013). Horizontal centrifuge with screw
561 conveyor (decanter): Optimization of oil/water levels and differential speed during
562 olive oil extraction. *Journal of Food Engineering*, 119, 561-572.

563 Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2001). Influence of malaxation
564 temperature and time on the quality of virgin olive oils. *Food Chemistry*, 72, 19-28.

565 Anonymous, (2009). *IOC/T.20/Doc No 29. Official methods of analysis. Determination*
566 *of biophenols in olive oils by HPLC.* International Olive Council, Madrid, Spain.

567 Anonymous, (2011). *COI/OH/Doc. No 1. Guide for the determination of the*
568 *characteristics of oil-olives* (pp. 1-41). International Olive Council, Madrid, Spain.

569 Artajo, L.S., Romero, M.P., Suarez, M., & Motilva, M.J. (2007). Partition of phenolic
570 compounds during the virgin oil industrial extraction process. *European Food*
571 *Research Technology*, 225, 617-625.

572 Artajo, L.S., Romero, M.P., Tovar, M.J., & Motilva, M.J. (2006). Effect of irrigation
573 applied to olive trees (*Olea europaea* L.) on phenolic compound transfer during olive
574 oil extraction. *European Journal of Lipid Science and Technology*, 108, 19-27.

575 Boselli, E., Di Lecce, G., Strabbioli, R., Pieralisi, G., & Frega, N.G. (2009). Are virgin
576 olive oils obtained below 27°C better than those produced at higher temperatures?
577 *LWT - Food Science and Technology*, 42, 748-757.

578 Caponio, F., Summo, C., Paradiso, V.M., & Pasqualone, A. (2014). Influence of
579 decanter working parameters on the extra virgin olive oil quality. . *European Journal*
580 *of Lipid Science and Technology*, 116, 1626-1633.

581 Caruso, G., Gucci, R., Urbani, S., Esposto, S., Taticchi, A., Di Maio, I., Selvaggini, R.,
582 & Servili, M. (2014). Effect of different irrigation volumes during fruit development

583 on quality of virgin olive oil of cv. Frantoio. *Agricultural Water Management*, 134,
584 94-103.
585 Caruso, G., Rapoport, H.F., & Gucci, R. (2013). Long-term evaluation of yield
586 components of young olive trees during the onset of fruit production under different
587 irrigation regimes. *Irrigation Science*, 31, 37-47.
588 Catania, P., Vallone, M., Farid, A., & De Pasquale, C. (2016). Effect of O_2 control and
589 monitoring on the nutraceutical properties of extra virgin olive oils. *Journal of Food*
590 *Engineering*, 169, 179-188.
591 Catania, P., Vallone, M., Pipitone, F., Inglese, P., Aiello, G., & La Scalia, G. (2013). An
592 oxygen monitoring and control system inside a malaxation machine to improve extra
593 virgin olive oil quality. *Biosystems Engineering*, 114, 1-8.
594 Cecchi, L., Migliorini, M., Cherubini, C., Giusti, M., Zanoni, B., Innocenti, M., &
595 Mulinacci, N. (2013). Phenolic profiles, oil amount and sugar content during olive
596 ripening of three typical Tuscan cultivars to detect the best harvesting time for oil
597 production. *Food Research International*, 54, 1876-1884.
598 Cherubini, C., Migliorini, M., Mugelli, M., Viti, P., Berti, A., Cini, E., & Zanoni, B.
599 (2009). Towards a technological ripening index for olive oil fruits. *Journal of Food*
600 *Science and Agriculture*, 89, 671-682.
601 Clodoveo, M.L. (2012). Malaxation: Influence on virgin olive oil quality. Past, present
602 and future - An overview. *Trends in Food Science & Technology*, 25, 13-23.
603 Clodoveo, M.L. Durante, V., La Notte, D., Punzi, R., & Gambacorta, G. (2013).
604 Ultrasound-assisted extraction of virgin olive oil to improve the process efficiency.
605 *European Journal of Lipid Science and Technology*, 115, 1062-1069.
606 Clodoveo, M.L., Hbaieb, R.,H., Kotti, F., Scarascia Mugnozza, G., & Gargouri, M.

(2014). Mechanical strategies to increase nutritional and sensory quality of virgin olive oil by modulating the endogenous enzyme activities. *Comprehensive Reviews of Food Science and Food Safety*, 13, 135-154.

Diplock, A.T., Charleux, J.L., Crozier-Will, G., Kok, F.J., Rice-Evans, C., Roberfroid, M., Stahl, W., & Vina-Ribes J. (1998). Functional food science and defence against reactive oxidative species. *British Journal of Nutrition*, 80, Suppl. 1:S77-S112.

El Riachy, M., Priego-Capote, F., Leon, L., Rallo, L., & Luque de Castro, M.D. (2011). Hydrophilic antioxidants of virgin olive oil. Part 2: Biosynthesis and biotransformation of phenolic compounds in virgin olive oil as affected by agronomic and processing factors. *European Journal of Lipid Science and Technology*, 113, 692-707.

Espinola, F., Moya, M., Fernandez, D.G., & Castro, E. (2011). Modelling of virgin olive oil extraction using response surface methodology. *International Journal of Food Science and Technol*ogy, 46, 2576-2583.

Gomez-Rico, A., Inarejos-Garcia, A.M., Salvador, M.D., & Fregapane, G. (2009). Effect of malaxation conditions on phenol and volatile profiles in olive paste and the corresponding virgin olive oils (*Olea europaea* L. Cv. Cornicabra). *Journal of Agriculture and Food Chemistry*, 57, 3587-3595.

Kalua, C.M., Bedgood, D.R., Bishop, A.G., & Prenzler, P.D. (2006). Changes in volatile and phenolic compounds with malaxation time and temperature during virgin olive oil production. *Journal of Agriculture and Food Chemistry*, 54, 7641-7651.

Klen, T.J., & Vodopivec, B.M. (2012). The fate of olive fruit phenols during commercial olive oil processing: Traditional press versus continuous two- and three-phase centrifuge. *LWT - Food Science and Technology*, 49, 267-274.

631 Klen, T.J., Wondra, A.G., Vrhovsek, U., & Vodopivec, B.M. (2015b). Phenolic
632 profiling of olives and olive oil process-derived matrices using UPLC-DAD-ESI-
633 QTOF-HRMS analysis. *Journal of Agricultural and Food Chemistry*, 63, 3859-3872.
634 Klen, T.J., Wondra, A.G., Vrhovsek, U., Sivilotti, P., & Vodopivec, B.M. (2015a).
635 Olive fruit phenols transfer, transformation, and partition trail during laboratory-scale
636 olive oil processing. *Journal of Agricultural and Food Chemistry*, 63, 4570-4579.
637 Leone, A., Esposto, S., Tamborrino, A., Romaniello, R., Taticchi, A., Urbani, S., &
638 Servili, M. (2016). Using a tubular heat exchanger to improve the condition process
639 of olive paste: evaluation of yield and olive oil quality. *European Journal of Lipid*
640 *Science and Technology*, 118, 308-317.
641 Leone, A., Romaniello, R., Zagaria, R., & Tamborrino, A. (2014). Development of a
642 prototype malaxer to investigate the influence of oxygen on extra-virgin olive oil
643 quality and yield, to define a new design of machine. *Biosystems Engineering*, 118,
644 95-104.
645 Leone, A., Romaniello, R., Zagaria, R., Sabella, E., De Bellis, L., & Tamborrino, A.
646 (2015). Machining effects of different mechanical crushers on pit particle size and oil
647 drop distribution in olive paste. *European Journal of Lipid Science and Technology*,
648 117, 1271-1279.
649 Migliorini, M., Cecchi, L., Cherubini, C., Trapani, S., Cini, E., & Zanoni, B. (2012).
650 Understanding degradation of phenolic compounds duirng olive oil processing by
651 inhibitor addition. *European Journal of Lipid Science and Technology*, 114, 942-950.
652 Migliorini, M., Cherubini, C., Zanoni, B., Mugelli, M., Cini, E., & Berti, A. (2009).
653 Influenza delle condizioni operative di gramolatura sulla qualità dell'olio extra
654 vergine di oliva. *La Rivista Italiana delle Sostanze Grasse*, LXXXVI, 92-102.

655 Migliorini, M., Mugelli, M., Cherubini, C., Viti, P. & Zanoni, B. (2006). Influence of
656 O_2 on the quality of virgin olive oil during malaxation. *Journal of the Science of*
657 *Food and Agriculture*, 86, 2140-2146.
658 Parenti, A., & Spugnoli, P. (2002). Influenza della temperatura delle paste di oliva sul
659 contenuto polifenolico degli oli estratti. *La Rivista Italiana delle Sostanze Grasse*,
660 LXXIX, 97-100.
661 Parenti, A., Spugnoli, P., Masella, P., & Calamai, L. (2008). The effect of malaxation
662 temperature on the virgin olive oil phenolic profile under laboratory-scale conditions.
663 *European Journal of Lipid Science and Technology*, 110, 735-741.
664 Ranalli, A., Contento, S., Schiavone, C., & Simone, N. (2001). Malaxing temperature
665 affects volatile and phenol composition as well as other analytical features of virgin
666 olive oil. *European Journal of Lipid Science and Technology*, 103, 228-238.
667 Ranalli, A., Pollastri, L., Contento, S., Iannucci, E., & Lucera, L. (2003). Effect of olive
668 paste kneading process time on the overall quality of virgin olive oil. *European*
669 *Journal of Lipid Science and Technology*, 105, 57-67.
670 Rodis, P.S., Karathanos, V.T., & Mantzavinou, A. (2002). Partitioning of olive oil
671 antioxidants between oil and water phases. *Journal of Agricultural and Food*
672 *Chemistry*, 50, 596-601.
673 Ryan, D., Antolovich, M., Prenzler, P., Robards, K., & Lavee, S. (2002).
674 Biotransformation of phenolic compounds in *Olea europea* L.. *Scientia*
675 *Horticulturae*, 92, 142-176.
676 Servili, M., Taticchi, A., Esposto, S., Urbani, S., Selvaggini, R., & Montedoro G.F.
677 (2008). Influence of the decrease in oxygen during malaxation of olive paste on the

composition of volatiles and phenolic compounds in virgin olive oil. *Journal of Agriculture and Food Chemistry*, 56, 10048-10055.

Singleton, V.L., & Rossi JR., J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144-158.

Talhaoui, N., Gomez-Caravaca, A.M., Leon, L., De la Rosa, R., Fernandez-Gutierrez, A., & Segura-Carretero, A. (2016). From olive fruits to olive oil: phenolic compound transfer in six different olive cultivars grown under the same agronomical conditions. *International Journal of Molecular Sciences*, 17, 337, 1-14.

Tamborrino, A., Pati, S., Romaniello, R., Quinto, M., Zagaria, R., & Leone, A. (2014a). Design and implementation of an automatically controlled malaxer pilot plant equipped with an in-line oxygen injection system into the olive paste. *Journal of Food Engineering*, 141, 1-12.

Tamborrino, A., Romaniello, R., Zagaria, R., & Leone, A. (2014b). Microwave-assisted treatment for continuous olive paste conditioning: Impact on olive oil quality and yield. *Biosystems Engineering*, 127, 92-102.

Taticchi, A., Esposto, S., Veneziani, G., Urbani, S., Selvaggini, R., & Servili, M. (2013). The influence of the malaxation temperature on the activity of poliphenoloxidase and peroxidase and on the phenolic composition of virgin olive oil. *Food Chemistry*, 136, 975-983.

Trapani, S., Guerrini, L., Masella, P., Parenti, A., Canuti, V., Picchi, M., Caruso, G., Gucci, R., & Zanoni, B. (2017). A kinetic approach to predict the potential effect of malaxation time-temperature conditions on extra virgin olive oil extraction yield. *Journal of Food Engineering*, 195, 182-190.

Trapani, S., Migliorini, M., Cherubini, C., Cecchi, L., Canuti, V., Fia, G., & Zanoni, B. (2016). Direct quantitative indices for ripening of olive oil fruits to predict harvest time. *European Journal of Lipid Science and Technology*, 118, 1202-1212.

Veneziani, G., Esposto, S., Taticchi, A., Selvaggini, R., Urbani, S., Di Maio, I., Sordini, B., & Servili, M. (2015). Flash thermal conditioning of olive pastes during the oil mechanical extraction process: cultivar impact on the phenolic and volatile composition of virgin olive oil. *Journal of Agriculture and Food Chemistry*, 63, 6066-6074.

Zanoni, B. (2014). Which processing markers are recommended for measuring and monitoring the transformation pathways of main components of olive oil? *Italian Journal of Food Science,* 26, 3-11.

Nomenclature

a, b, c	regression coefficients (min^{-1} or min)
Ea	activation energy ($J\ mol^{-1}$)
EI_{app}	apparent Extractability Index (%)
$EI_{app,\ max}$	maximum apparent Extractability Index (%)
EY	extraction yield (%)
EY_{max}	maximum extraction yield (%)
$K_{f(\vartheta)}$	apparent kinetic constants as a function of malaxation temperature (min^{-1})
k_0	frequency factor (min^{-1})
R	gas constant ($J\ mol^{-1}\ K^{-1}$)
T	malaxation absolute temperature (K)
t	malaxation time (min)
t_{lag}	apparent lag phase (min)
$\triangle_{rel}$	relative variation with time at different temperatures
$\triangle_{rel,ref}$	relative variation chosen as reference
ϑ	malaxation temperature (°C)

731 FIGURE CAPTIONS

732

733 FIGURE 1. Kinetics of normalized total phenolic compound content using the Folin-
734 Ciocalteu method in vegetation water samples at 22°C (**a**), 27°C (**b**), 32°C (**c**) and 37°C
735 (**d**). The symbols ■ and ——— are for experimental and predicted data, respectively.

736

737 FIGURE 2. Kinetics of normalized verbascoside content in olive paste samples at 22°C
738 (**a**), 27°C (**b**), 32°C (**c**) and 37°C (**d**). The symbols ■ and ——— are for experimental
739 and predicted data, respectively.

740

741 FIGURE 3. Kinetics of normalized 3,4 DHPEA-EDA content in olive oil samples at
742 22°C (**a**), 27°C (**b**), 32°C (**c**) and 37°C (**d**). The symbols ■ and ——— are for
743 experimental and predicted data, respectively.

744

745 FIGURE 4. Kinetics of the normalized sum of β-OH-verbascoside diastereoisomer
746 content in olive paste samples at 22°C (**a**), 27°C (**b**), 32°C (**c**) and 37°C (**d**). The
747 symbols ■ and ——— are for experimental and predicted data, respectively.

748

749 FIGURE 5. Kinetics of the apparent Extractability Index (EI_{app}) at 22°C (**a**), 27°C (**b**),
750 32°C (**c**) and 37°C (**d**). The symbols ■ and ——— are for experimental and predicted
751 data, respectively.

752

753 FIGURE 6. Time-temperature synoptic chart of olive past malaxation in relation to
754 phenolic compounds: in green the curves referring to the total phenolic compounds, in

755 red the curves referring to 3,4 DHPEA-EDA, in blue the curves referring to the sum of
756 β-OH verbascoside diasteroisomers. The unbroken curves show a variation of 20%, and
757 the dashed curves 10%.
758
759 FIGURE 7. Olive paste malaxation time-temperature optimization chart obtained by
760 overlapping the synoptic chart shown in Figure 6 with the black straight lines of
761 apparent yield, unbroken to indicate an 80% yield and dashed for a 60% yield.