J Am Oil Chem Soc (2011) 88:871–875
DOI 10.1007/s11746-010-1739-y

ORIGINAL PAPER

Malaxation of Olive Paste Under Sealed Conditions

Piernicola Masella · Alessandro Parenti · Paolo Spugnoli · Luca Calamai

Received: 21 July 2010 / Revised: 20 October 2010 / Accepted: 28 November 2010 / Published online: 14 December 2010

Abstract Virgin olive oils from pilot-scale malaxation under hermetically sealed conditions were compared with olive oils from industrial conventional open-to-air malaxation (control). Under sealed conditions, large CO_2 emissions coupled with O_2 depletion occur. Oil samples produced under sealed conditions were less oxidized and contained greater concentrations of antioxidant compounds (especially secoiridoids phenols) than the control. These results were attributed to the reduced O_2 concentration in the hermetically sealed pilot-scale malaxer. The amounts and types of volatile compounds present in the oil were only slightly affected by the treatment.

Keywords Malaxation · Carbon dioxide emission · Phenolic compounds · Volatile compounds

Introduction

In recent years, several studies concerning virgin olive oil processing have proven that malaxation, the continuous and slow mixing of olive paste, is a critical step in extracting olive oil [1, 2]. Malaxation strongly affects olive oil quality by reducing oxidation that usually occurs either in the lipid matrix or in the minor compounds, especially in the phenolics fraction. To avoid this critical problem, solutions, such as the covering of the paste with inert gases (mainly with nitrogen), have been tested with the aim of minimizing the contact with atmospheric oxygen [3–5]. Recently, it was observed that when malaxation was carried out in a sealed container, olive pastes produced large amounts of carbon dioxide and rapid oxygen depletion occurred [6, 7]. Furthermore, the feasibility of this malaxation as a solution to improving olive oil quality both in terms of reduction of the oil oxidation and increase in the phenolic concentration has been demonstrated in the laboratory [6, 7]. The present work reports the results of malaxation under sealed conditions in a pilot-scale trial.

Materials and Methods

Pilot-Scale Malaxer

A pilot-scale malaxation apparatus was specifically designed for the present experiment (OMT, Grassina-Florence, Italy). The apparatus consisted of a cylindrical hermetically sealed stainless-steel tank vertically positioned. The malaxation chamber had an internal height of 1,000 mm and an internal diameter of 600 mm, which gives a volume of about 0.28 m^3 . The tank was equipped with a jacketed heating system and temperature control. The sealing cover was equipped with a special sensor for registering the level of olive paste, a double-acting valve to allow loading and removing the paste under sealed conditions, an analog manometer (full-scale = 1 bar), a cone for

P. Masella (✉) · A. Parenti · P. Spugnoli
Dipartimento di Economia, Ingegneria, Scienze e Tecnologie Agrarie e Forestali, Facoltà di Agraria, Università degli Studi di Firenze, Piazzale Cascine 15, 50144 Florence, Italy
e-mail: piernicola.masella@unifi.it

L. Calamai
Centro Interdipartimentale di Spettrometria di Massa, Università degli Studi di Firenze, Polo Scientifico, Via U. Schiff 6, Sesto F.no, 50019 Florence, Italy

L. Calamai
Dipartimento di Scienze delle Produzioni Vegetali, del Suolo e dell'Ambiente Agroforestale, Facoltà di Agraria, Università degli Studi di Firenze, Piazzale delle Cascine 18, 50144 Florence, Italy

a rubber stopper used for gas sampling (CO_2 and O_2 concentrations in the headspace above the paste surface). A gear motor activated a mixing shaft consisting of a central screw conveyor fitted with external helical blades that constantly removed the heated paste from the tank wall thus preventing overheating. The pilot-scale apparatus was introduced into an industrial continuous centrifugation plant (model EUROX15DE, OMT spa, Grassina-Florence, Italy) equipped with a metal crusher, four conventional horizontal open-to-air malaxation tanks (0.8 m^3 internal volume of a single tank), and a horizontal three-phase decanter centrifuge.

Olives

The tests used 4,750 kg of olives consisting of a mixture of Frantoio (80%) and Moraiolo (20%) cultivars. The drupes, in good sanitary conditions and in a medium state of ripeness, were mechanically picked near Florence (Tuscany, Italy) in 1 day and stored for 12 h in aerated fruit baskets until processed. All the extraction tests were performed on the same day.

Experimental Procedures

Malaxation in the pilot-scale apparatus (namely under sealed conditions) was tested in five replicates in comparison to conventional malaxation (i.e., a horizontal open-to-air malaxation tank). The initial set of olive drupes (4,750 kg) was homogenized and divided into five batches of about 950 kg each. Then, each batch was homogenized and divided in two sub-samples, one aliquot of 250 kg and another aliquot of 700 kg. The first olive sub-sample (250 kg) was crushed and the resulting paste was malaxated in the pilot-scale apparatus at 28 °C (sealed conditions). In each trial the pilot-scale malaxer was filled at a paste flow rate of about 1,000 kg h^{-1}, which corresponded to a full filling time of the chamber of about 15 min (250 kg of olive paste and 0.03 m^3 void volume). This moment was formally assumed to be the beginning of malaxation, which was carried out for 45 min. In this way, the kinetics of CO_2 evolution and O_2 consumption in the malaxation chamber were measured (every 5 min) with no variation of the chamber headspace volume until the end of kneading. The second olive sub-sample (700 kg) was crushed and then malaxated in the conventional horizontal open-to-air malaxation tank (control), adopting the same operating conditions (45 min at 28 °C). The filling flow rate was set at 2,800 kg h^{-1} giving the same filling time of the pilot-scale malaxer (about 15 min). In both the cases, the oil was extracted after malaxation by means of a horizontal decanter centrifuge with a 1,000 kg h^{-1} feeding flow rate. The same procedure was repeated for each olive batch (five replicates). As the mill-plant was designed for working without the use of a vertical centrifuge at the end of the processing chain, the virgin olive oil was sampled directly after the decanter centrifuge.

Chemical Analyses

The concentrations of CO_2 and O_2 were measured, as percentages (v/v), by a handheld gas analyzer type CheckPoint O_2/CO_2 (PBI-Dansensor A/S, Ringsted, Denmark). Free acidity, peroxide value (PV), and UV specific extinction coefficients (K232, K270) were carried out according to the European Official Method of Analysis [8]. Total chlorophyll (Chlo) and carotene (Caro) contents were evaluated by measuring their absorbances at 670 and 470 nm, respectively, using absorptivities from the literature [9].

The total hydrophilic phenols were determined colorimetrically as previously reported [10]. The HPLC hydrophilic phenols profile was determined according to the method of the SSOG Technical Commission (2006) as specified in a previous paper [10, 11]. For the sake of simplicity, the identified compounds were grouped into simple phenols (Sph: hydroxytyrosol, tyrosol), secoiridoids (Seco: decarboxymethyl oleuropein aglycone dialdehyde and oxidized forms, decarboxymethyl ligstroside aglycon dialdehyde and oxidized forms, oleuropein aglycon aldehyde and hydroxylic forms and oxidized form, oleuropein, oleuropein aglycon dialdehyde form, ligstroside aglycon dialdehyde form, ligstroside aglycon aldehyde and hydroxylic form and oxidized form), and lignans (Lign: pinoresinol, 1-acetoxy-pinoresinol).

Gas chromatography–mass spectrometry analysis of volatile compounds was performed by using an Agilent 7890 GC apparatus equipped with an Agilent 5975C inert XL MSD quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA), after automated SPME sampling as described in detail in a previous paper [10]. According to Sanchez-Ortiz et al. [5], volatile compounds were clustered into different classes as related to the polyunsaturated fatty acid and the LOX pathway branch origin. Quantitative data reported for every volatile class are the sum of the contents of the following compounds: C6/LnA [(*E*)-hex-2-enal; (*Z*)-hex-3-enol; (*E*)-hex-2-enol]; C6/LA [Hexanal; hexan-1-ol]; C5/LnA [pent-1-en-3-one; pent-1-en-3-ol; (*E*)-pent-2-enal]; Esters [Ethyl acetate, Methyl salicylate]; Amm/Ferm [3-methyl-butanal, 3-Methyl-1-butanol, phenylethyl alcohol]; Others [1-Hepten-3-ol, 1-octen-3-ol (2*E*,4*E*)-Hepta-2,4-dienal, nonanal (*E*)-2-Decenal, Benzaldehyde, Pentanal]. Determinations for all the parameters were repeated in duplicate and the two values averaged. Statistical significance of the investigated treatment was evaluated through paired *t* test, i.e., five pairs

to compare between sealed conditions malaxation and open-to-air malaxation, paired by the initial olive batch processed.

Results and Discussion

Carbon dioxide and oxygen concentrations recorded during pilot-scale malaxation are reported in Fig. 1 as a function of time. Considering that before malaxation the CO_2 and O_2 concentrations in the void chamber correspond to the standard atmospheric composition (0.2 and 21%, respectively), during the filling time a large CO_2 emission and a simultaneous O_2 depletion occur so that at the beginning of malaxation they had a similar mean concentration of about 10% (left part of graph in Fig. 1). The trend of CO_2 emission and O_2 depletion only partially confirm observation in previous laboratory experiments where both CO_2 and O_2 showed variations with time faster than those observed in the present experiment. Along with the variability related to the different characteristics of the olives (i.e., cultivar, ripeness state, etc.), these differences were probably due to differences in exposure of the paste surface to the headspace of the chambers. In fact, the paste surface to the paste amount ratio was 43 $cm^2\ kg^{-1}$ in the laboratory conditions versus 11 $cm^2\ kg^{-1}$ in the present industrial conditions. However, the increase in CO_2 concentration and the O_2 depletion were highly and linearly correlated ($r = 0.85$), supporting the hypothesis that the general phenomenon of CO_2 emission is mainly due to the accelerated cellular respiration [6, 7]. In contrast,

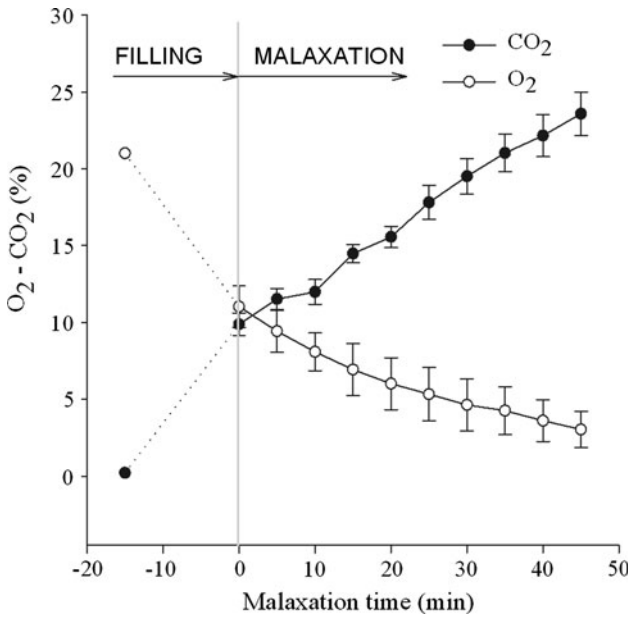

Fig. 1 CO_2 emission and O_2 depletion kinetics during malaxation under sealed conditions

oxygen availability in the malaxer chamber seems to affect the extent of the CO_2 emission from the olive paste very slightly [12]. Therefore, it can be supposed that the respiratory metabolism is not the only phenomenon responsible for carbon dioxide emission. Proietti et al. [13] reported that olive fruits are able to accumulate large amounts of CO_2 in their free space, i.e., between 400 and 800 ml m^{-3}. Thus, we can hypothesize that at least a part of the CO_2 in the head-space of the malaxation chamber is derived from a simple release from cells free space. Moreover, at this stage, the possible contribution of other biochemical processes such as fermentation and/or respiration of a substrate other than sugars cannot be ruled out. According to Servili et al. [12] the oxygen depletion could be partly ascribed to enzymatic consuming activity in the paste. Of course all these occurrences contribute to deviate the linear relationship between oxygen depletion and CO_2 increase and/or to generate deviations from a theoretical respiratory quotient of 1. Further investigations are needed to better clarify the phenomenon of CO_2 emission. Standard quality parameters between conventional malaxation and malaxation under sealed conditions are summarized in Table 1. Oil samples from the pilot-scale apparatus had lower oxidation indexes. However, the differences were significant only for PV, with a reduction of about 10% from the control. These results are probably related to the progressive reduction of oxygen concentration, which leads to a partial inhibition of enzymatic oxidative activity of peroxidase [1]. Similarly, the oxidative activity of other enzymes such as polyphenol oxidase and those involved in the lipoxygenase pathway appear to be affected by the reduced presence of oxygen [1, 2]. In fact, as shown in Table 2, sealed malaxation makes it possible to obtain oils with higher minor components concentrations. In particular, significantly higher concentrations of pigments (chlorophylls and carotenes) and total phenolic compounds (colorimetric method) were observed. Significant variation in the HPLC profile was observed, with nearly 40% higher secoiridoids concentration. In contrast, simple phenols and lignans were not affected by the treatment. The different antioxidant power and susceptibility to oxidation of specific phenols could be the origin of the dissimilar behaviour of individual classes. The volatile compound concentrations are reported in Table 3. A significantly lower concentration of total volatiles (about 15%) was observed for oils produced under sealed conditions; however, the differences were not significant for the individual classes of compounds. Thus, it seems that the reduced oxygen concentration only slightly affects the activity of lipoxygenase enzymes. Virgin [1, 2] olive oil organoleptic characteristics are strictly related both to phenolic and volatile compounds concentrations. On the base of the significant increment of total secoiridoids it is possible to hypothesize that sealed

Table 1 Comparison of virgin olive oil standard quality parameters between open-to-air malaxation and sealed malaxation

Parameter	Open-to-air	Sealed	MD
Free acidity (oleic ac., w/w%)	0.360 (0.017)	0.327 (0.014)	0.032 (0.020)^{ns}
Peroxide value (meq O_2 Kg^{-1})	11.792 (0.594)	10.481 (0.090)	1.311 (0.613)**
K232 (1%, 1 cm)	1.589 (0.070)	1.534 (0.119)	0.055 (0.053)^{ns}
K270 (1%, 1 cm)	0.135 (0.024)	0.125 (0.022)	0.011 (0.021)^{ns}

MD mean difference; *ns* not significant

Data are means of five independent replicated experiments; standard deviations are reported in brackets; significance of mean differences was tested by the paired *t* test, * *p* at 0.05, ** *p* at 0.01

Table 2 Minor components concentrations (mg kg^{-1}) in virgin olive oil from open-to-air malaxation and sealed malaxation

Parameter	Open-to-air	Sealed	MD
Chlo	24.6 (6.7)	35.1 (11.8)	−10.5 (6.0)*
Caro	17.3 (3.2)	21.2 (4.4)	−3.9 (1.4)**
Sph	1.5 (0.7)	2.4 (1.8)	−0.9 (1.2)^{ns}
Lign	38.0 (5.0)	44.4 (4.7)	−6.4 (8.7)^{ns}
Seco	94.9 (21.0)	131.3 (27.7)	−36.4 (25.1)*
Total HPLC	134.4 (23.3)	178.1 (27.1)	−43.7 (26.5)*
Total phenols[#]	142.6 (35.5)	175.8 (39.6)	−33.2 (15.2)**

[#] Colorimetric method

MD mean difference, *ns* not significant

Data are means of five independent replicated experiments; standard deviation are reported in brackets; significance of mean differences was tested by the paired *t* test, * *p* at 0.05, ** *p* at 0.01

Table 3 Volatile compounds concentrations (μg kg^{-1}) in virgin olive oil from open-to-air malaxation and sealed malaxation

Volatile classes	Open-to-air	Sealed	MD
Amm/Ferm	1,428 (136)	1,158 (198)	270 (209)^{ns}
C5/LnA	6,162 (1,753)	5,166 (920)	1,484 (890)^{ns}
C6/LA	20,270 (9,839)	23,422 (19,401)	9,228 (13,006)^{ns}
C6/LnA	145,538 (21,749)	115,054 (30,030)	31,680 (27,779)^{ns}
Esters	2,560 (1,684)	1,666 (178)	1,114 (1,632)^{ns}
Others	17,166 (3,407)	16,516 (3,188)	3,066 (2,322)^{ns}
Total volatiles	193,124 (20,116)	162,982 (15,363)	30,142 (17,559)*

MD mean difference, *ns* not significant

Data are means of five independent replicated experiments; standard deviation are reported in brackets; significance of mean differences was tested by the paired *t* test, * *p* at 0.05, ** *p* at 0.01

malaxation gives oil with a greater perception of bitter and pungency attributes. Such a condition could represent a problem for some consumers, who appreciate virgin olive oils with delicate sensory notes. In these cases, the tested malaxation solution can result in a useful application with an olive variety with a very low amount of phenols, or to process over-ripe fruits assuring a good extraction of phenolic compounds and giving oils with acceptable organoleptic characteristics. Alternatively, the oil produced in sealed malaxation, could be properly used as a base for blending with other oils with different attributes. Further, the plausible improved oil stability during storage caused by the higher phenols concentration probably balances the observed slight decrement in the total volatiles concentration.

Conclusions

The results obtained on a pilot-scale, albeit with some discrepancies compared to observations made in the laboratory, confirmed the phenomenon of CO_2 emission coupled with O_2 depletion during malaxation under sealed conditions. The reduced O_2 concentration, interfering with the activity of some oxidative enzymes, led to oils characterized by less oxidation and a greater antioxidant concentration. In contrast, the enzyme activities involved in the lipoxygenase pathway, seems to be only slightly affected by the low oxygen concentrations during kneading. Clearly we need to obtain a better understanding of the biochemical processes that occur during malaxation under sealed conditions. It is possible to envision real-time measurement of CO_2 and O_2, and controlled supply of O_2 as micro-oxygenation if necessary.

References

1. Servili M, Selvaggini R, Esposto S, Taticchi A, Montedoro GF, Morozzi G (2004) Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. J Chromatogr A 1054:113–127
2. Angerosa F, Servili M, Selvaggini R, Taticchi A, Esposto S, Montedoro GF (2004) Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. J Chromatogr A 1054:17–31

3. Servili M, Selvaggini R, Taticchi A, Esposto S, Montedoro GF (2003) Volatile compounds and phenolic composition of virgin olive oil: optimization of temperature and time of exposure of olive paste to air contact during the mechanical extraction process. J Agric Food Chem 51:7980–7988
4. Servili M, Selvaggini R, Taticchi A, Esposto S, Montedoro GF (2003) Air exposure time of olive pastes during the extraction process and phenolic and volatile composition of virgin olive oil. J Am Oil Chem Soc 80:685–695
5. Sanchez-ortiz A, Romero C, Perez AG, Sanz C (2008) Oxygen concentration affects volatile compound biosynthesis during virgin olive oil production. J Agric Food Chem 56:4681–4685
6. Parenti A, Spugnoli P, Masella P, Calamai L (2006) Carbon dioxide emission from olive oil pastes during the transformation process: technological spin offs. Eur Food Res Technol 222:521–526
7. Parenti A, Spugnoli P, Masella P, Calamai L (2007) Improving olive oil quality using CO_2 evolved from olive pastes during processing. Eur J Lipid Sci Technol 109:1180–1185
8. Off J Eur Communities (2003) European Commission Regulation EEC/1989/2003 of 6 November 2003 amending Regulation (EEC) No. 2568/91 on the characteristics of olive oil and olive pomace oil and on the relevant methods of analysis. L295:57–77
9. Mınguez-Mosquera MI, Rejano-Navarro L, Gandul-Rojas B, Sanchez-Gomez AH, Garrido-Fernandez J (1991) Color pigment correlation in virgin olive oils. J Am Oil Chem Soc 68:332–336
10. Masella P, Parenti A, Spugnoli P, Calamai L (2009) Influence of vertical centrifugation on extra virgin olive oil quality. J Am Oil Chem Soc 86:1137–1140
11. International Olive Oil Council (IOOC) (2006) SSOG Technical Commission—Sub-commission on vegetable oils. Determination of biophenols in olive oils by HPLC. Natural and oxidised oleuropein and ligstroside derivatives, lignans, flavonoids and phenolic acids. Method under public review RESOLUTION NO. RES-4/94-V/06
12. Servili M, Taticchi A, Esposto S, Urbani S, Selvaggini R, Montedoro GF (2008) Influence of the decrease in oxygen during malaxation of olive paste on the composition of volatiles and phenolics compounds in virgin olive oil. J Agric Food Chem 56:10048–10055
13. Proietti P, Famiani F, Tombesi A (1999) Gas exchange in olive fruit. Photosynthetica 36:423–432