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Demonstration of the Effectiveness of a Pilot, Variable Speed Crusher Featuring an In-Line Oxygen Dosing System

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At the industrial scale, improvements to extra virgin olive oil (EVOO) processing are an important opportunity to increase both added-value, and the product's organoleptic and chemical parameters. Concerning all the operations used during EVOO extraction, crushing and malaxation have an effect on the extraction efficiency and minor components composition extremely significant. In this context, a pilot industrial-scale crusher is designed and developed that can control the amount of oxygen that is dosed directly into the produced olive paste. In this study, the effectiveness of this new technique, combined with different crushing speeds on EVOO quality, is evaluated. It is demonstrated that supplying oxygen to the paste enriches the volatile fraction, the mean increase is about 30% for the compounds associated to positive sensory notes, while the concentrations of the compounds associated to sensory defects are stationary on 5.2 mg kg^{-1}. ***Practical Applications*****: This result is confirmed by a sensory analysis, notably an increase in fruity intensity. It is also shown that crushing speed affects the extraction of compounds such as biophenols, and increases the bitter taste. Increased bitterness is detected for samples crushed at 3500 rpm compared to those crushed at lower speed (mean 5.7±0.3 compared to 4.5±0.4).**

1. Introduction

Extra virgin olive oil (EVOO) consumption is increasing worldwide due to its nutritional value and characteristic aroma, and the presence of a large number of chemical compounds.[1,2] These sensory and nutritional properties are attributed to its phenolic[3] and volatile[4,5] compounds.

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EVOO is extracted from healthy olive fruit by mechanical action. Of all of the operations that are used, crushing and malaxation have the most significant effect on efficiency and the composition of minor compounds[6,7] and can impair product quality. The pleasant, fruity attribute of EVOO is mainly due to its volatile organic compounds (VOCs). It is well-known that VOCs are affected by several operating factors. The first relate to "in-field" factors, such as the environment, agronomy, genetics, timing, and the type of harvesting; then there are "out-of-field" factors, such as the transport and storage of fruit,[8] operating conditions during extraction,[6,9,10] and oil storage, packaging and transport conditions.[11]

VOC concentrations are low in unbroken olives. However, they increase significantly when the cell structure ruptures during mechanical extraction. The lipoxygenase (LOX) pathway is mainly activated during crushing and malaxation,[12] with the release of molecules that are considered to be responsible for the oil's positive aroma—C6 and C5 compounds of the primary and secondary LOX pathways, respectively. Volatile compounds are a complex mix of aldehydes, alcohols, ketones, acids, hydrocarbons, and esters and are closely associated with both oil flavor and its positive and negative sensory attributes.[13,14]

Drupe crushing and the temperature of the paste are therefore processing steps where great care must be taken as technology can, on the one hand, prevent oxidative or detrimental phenomena or, on the other hand, enhance olive properties. Producers and academics have been working together to better-understand the key elements that modulate the complex series of physical, physicochemical, chemical, and biochemical transformations that occur during EVOO production, in order to develop advanced and sustainable solutions that both increase yield and improve the quality of products.[15]

During crushing, oil is released from the intracellular vacuoles and the enzymatic reactions that affect the volatile profile and the phenolic compound content in the final product are activated.[16] In a laboratory study, Inarejos-García and co-authors[17] examined the effect of grid hole diameter and rotational speed in hammer crushers. They found a significant effect of grid hole diameter on the extraction of secoiridoids (mainly hydroxytyrosol

derivatives). The authors concluded that crushing parameters may be a very useful way to modulate minor compounds and olive oil quality. These results were confirmed in Guerrini and co-authors study[18] who demonstrated that crushing speed affects the extraction of compounds such as chlorophylls, biophenols, 3,4-HPEA-EDA, and p-HPEA-EDA.

Given the various possible combinations of operating conditions, such as the time-temperature relationship, oxygen exposure, and kneading tools, several studies have investigated kneading effects on the phenolic profile of EVOO, and its influence on headspace composition.[19,20,21,22,23,24] These studies confirm the important role of oxygen during malaxation on phenolic compound content and other substances responsible for the attractive aroma of EVOO. Oxygen is another crucial element in LOX reactions; in moderation it is associated with positive sensory notes, but its excess can cause defects.[25] Thus, oxygen concentration is an important parameter that needs to be controlled.[24]

All of the experiments cited above evaluated oxygen concentrations in the headspace of the malaxer, and in the olive paste inside the malaxer tank. One study[24] went further, and investigated the direct injection of oxygen into olive paste during malaxation. Oxygen concentrations in the headspace were controlled with a dedicated system that was implemented in an industrial pilot plant. Drawing on this earlier work, we present an entirely innovative approach. Specifically, we propose a new solution for dosing oxygen concentrations in olive paste that is very different to earlier techniques. A pilot industrial crusher was developed that incorporated a system to directly control the oxygen dosage in the olive paste. The system included a dedicated oxygen injector consisting of a food compressor, a gas flowrate regulator, and a sparger. The injector produced a constant air flow, thanks to a regulation valve controlled by a flow meter, and was designed for installation in an industrial prototype.

This study evaluates the potential of the new system. It investigates the level of dissolved oxygen in olive oil in order to verify consumption, and studies the relationship between the oxygen concentration and crusher speed on EVOO quality.

2. Experimental Section

2.1. Crusher Design

A prototype blade cutter crusher was designed and built to simulate a real industrial process. The key elements of the prototype crusher are:

- A management system to feed olive fruit. The system uses a feedback controller to maintain the instantaneous electrical power adsorbed by the crusher by adjusting the speed of the conveyor belt that feeds fruit to the hop above the crushing chamber.
- A two-step heat exchange system, consisting of an initial heat exchanger fitted around the crushing chamber, and a second exchanger located immediately downstream of the crusher.
- A variable drive to regulate crusher speed.
- An oxygen dosing system, consisting of a coaxial sintered steel sparger plugged between the crusher and the pipe that delivers olive paste to the malaxer. The flowmeter used (Key Instruments FR2000 series, Worldwide Plastics, Inc.) measures the gas flow rate from 0.4 to 5 L min^{-1}.
- The device is a food-grade compressor, suitably sized and interfaced with a gas flow rate regulator to ensure accurate oxygen dosing. The specifics properties are reported: dry oil - less compressor (30/7 PRIME S), flow rate 85 L min^{-1} at 5 bar, max pressure 7 bar, capacity 24 L, Motor power: 0.75 kW – 230 V. A pressure reducer (RIEGLER, RIEGLER & Co. KGSchützenstraße Bad Urach) with setting range 0.5–10 bar, max. 16 bar.

The crushing elements (eight, radially mounted knives) are surrounded by a fixed cylindrical grid with internal diameter 350 and 6.5 mm holes. The crusher was made by a specialized company (MORI-TEM srl, Via Leonardo da Vinci 59, 50 028 Barberino Tavarnelle (FI), Italy).

In **Figure 1**, the details about the solutions tested are reported.

2.2. Olives

Olive (Olea europaea) cultivar *Frantoio* were manually picked in Bucine (Arezzo Italy- ≈43°28' N/11°36' E), in early November 2020. Fruits were in good sanitary/physical conditions, (assessed by visual inspection by company technicians) with no signs of insect or pest infestation, or mechanical damage. The ripening index was assessed as 4,[26] consistently the exterior color of the skin was almost entirely from purple to black, while the flesh was white.

2.3. Experimental Conditions

The pilot crusher was tested in experiments carried out at an industrial-scale olive oil extraction plant. In this experiment, the oxygen dosing system and different crushing speeds was only tested. These two elements were selected as they have most influence on the final product's characteristics (see the Introduction). This choice was also based on the limited literature on the direct injection of oxygen into paste.

A homogeneous batch of 2700 kg of *Frantoio* cultivar was split into sub-batches and crushed at two speeds (2500 and 3500 rpm), corresponding to the peripheral speed of the crushing elements at the outermost part of the crushing chamber. It should be noted that this interval represents the full range of speeds used by the host company during production. Crusher speed was tested in combination with three levels of oxygen injection: a control level (no oxygen injection); a low level (2.2 mg O_2/kg of paste); and a maximum level (5.5 mg O_2/kg). All trials were performed in triplicate, for a total of 18 samples.

After crushing, the olive paste was mixed in a malaxation apparatus (MORI-TEM Srl, Italy). The apparatus consist in five malaxation chambers conditioned, each containing up to 70 kg of paste, inclined and elongated (cylindrical and narrow). In the pilot plant there were two lines of independent electric motors, two motors for each of the tubular elements (5 malaxer chambers). The upper line corresponds to the motors responsible for mixing the paste, the second line of motors is instead responsible for moving the olive paste between the malaxers (filling and emptying) and toward the centrifugal decanter in the case of the last malaxer. The

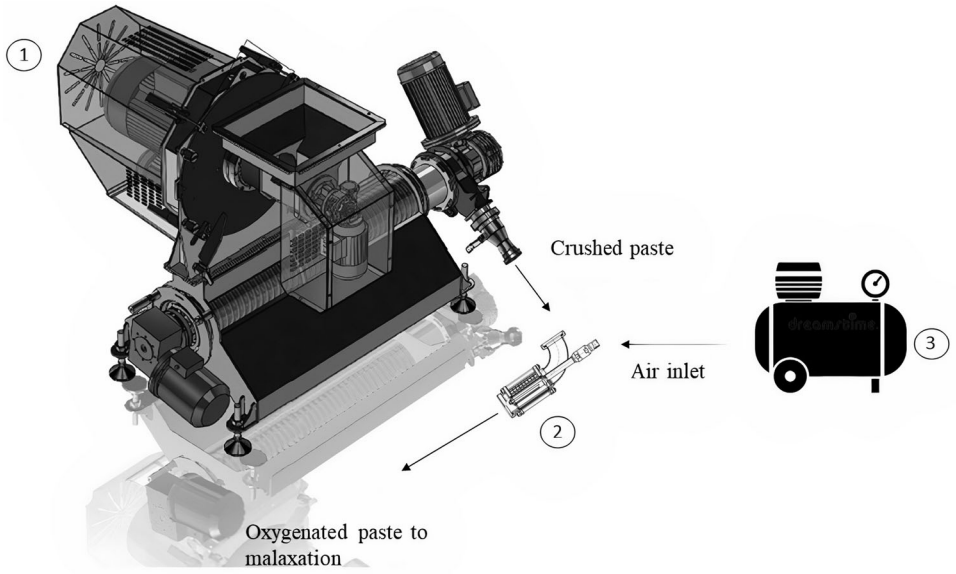

Figure 1. Blade cutter crusher (1), and the key elements of the prototype evidenced (2 steel sparger, 3 air compressor).

malaxations chambers are sealed at the top and all have an electric valve at the paste inlet. Temperature is controlled by thermostatic valves and was set to 25 °C during the whole test. Inside the malaxers, a vertical reel fitted with helical blades moves and mixes the olive paste.

Malaxation was carried out in continuous in two of the five malaxation chamabers for 25 min.

A two-phase decanter (MORI–TEM srl Via Leonardo da Vinci 59, 50 028 Barberino Tavarnelle (FI), Italy) separated the oil from water and pomace at a rate of 840 kg h^{-1}. Finally, the oil that was produced was immediately filtered using a stainless steel prefilter and a filter press.

2.4. Monitoring of the Oxygen Level to Validate the Micro-oxygenator Sparger

Oxygen concentration was measured after filtration to determine whether the injected oxygen was entirely consumed during malaxation. Measurements were carried out with an oxygen analyser (InPro 6850i, Mettler Toledo, Italy).

2.5. Chemical Analysis

Oil samples obtained from trials were analysed for free fatty acids (% oleic acid), peroxide value (meq O_2 per kg of oil) and UV spectroscopic indices (K232, K268. and ΔK) according to official methods.[27]

Biophenolic fractions were extracted and identified following the International Olive Council (IOC) official method.[28] Phenolic compounds were extracted using a methanol:water 80:20 v/v solution. HPLC analysis was performed using a HP 1100 coupled with both a DAD and MS detector, the latter equipped with an HP1100 MSD API-electrospray interface (Agilent Technologies, Palo Alto, CA). A Poroshell 120, EC-C18 column (150 × 3.0 mm^2 id, 2.7 μm particle size; Agilent Technologies, Palo Alto, CA) was used for separation. According to the official method, acetonitrile, H_2O and methanol were adopted as elution solvents following the elution gradient described by the IOC. The chromatogram was recorded at 280 nm, using syringic acid as internal standard, while the phenolic concentration was expressed as mg kg^{-1} of tyrosol.

2.6. Volatile Organic Compounds Analysis

Identification and quantification of VOCs (volatile organic compounds) was performed by headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) using the multiple internal standard method, as described by Fortini.[29]

Briefly, analyses involved weighing, into 20 mL screw cap vials fitted with a PTFE/silicone septa, 4.3 g of an oil sample and 0.1 g of an internal standard (ISTD mix). After 5 min equilibrium at 60 °C, a SPME fibre (50/30 μm DVB/CAR/PDMS, Supelco, St. Louis, USA) was exposed for 20 min in the vial headspace under orbital shaking (500 rpm). Then, the fiber was immediately desorbed for 2 min in a gas chromatograph injection port operating in splitless mode at 260 °C.

Compounds were identified and quantified (mg/kg) by comparison of their mass spectra and retention times with those of the ISTD mix, consisting of the following 11 compounds: 3,4-dimethylphenol, 4-methyl-2-pentanol, hexanoic acid-d11, 1-butanol-d10, ethyl acetate-d8, toluene-d8, ethyl hexanoate-d11, acetic acid 2,2,2-d3, 6-chloro-2-hexanone, 3-octanone, and trimethyl acetaldehyde. The same amount of ISTD mix was added to calibration scales to normalize each analyte concentration of the calibration curve to that of the respective ISTD mix.

Table 1. Mean and standard deviation of dissolved oxygen in EVOO samples. Letters indicate statistical differences $p \leq 0.05$. All trials were performed in triplicate, for a total of 18 samples ($n = 3$).

Oxygen level	Control	Low level	High level	Control	Low level	High level	*p*		
Crusher speed [rpm]	2500	2500	2500	3500	3500	3500	Oxygen level	Crusher speed	Interaction
Oxygen monitoring [mg L^{-1}]	8.20 ± 0.26^a	8.30 ± 0.26^a	8.50 ± 0.17^a	8.30 ± 0.17^a	8.20 ± 0.30^a	8.23 ± 0.45^a	ns	ns	ns

The GM-MS identification of VOCs was performed using a Trace CG-MS Thermo Fisher Scientific, equipped with a ZB-FFAP capillary column (Zebron) 30 m × 0.25 mm ID, 0.25 µm df. The temperature of the column was controlled as follows: 36 °C for 10 min, increase to 156 °C at 4 °C min^{-1}, increase to 260 °C at 10 °C min^{-1}, decrease to 250 °C at 10 °C min^{-1}, with hold time of 2 min. Helium was used as the carrier gas at constant flow of 0.8 mL min^{-1}. The temperature of both the ion source and transfer line was 250 °C. The mass detector was operated in scan mode within a 30–330 Th mass range at 1500 Th s^{-1}, with an ionization energy of 70 eV.

VOC quantification was carried out by comparing each mass spectra and retention time with those of injected authentic standards. The stock external standard mix contained 71 analytes in refined oil, which was previously verified to be free of any interferent. The analytes and their concentration ranges were chosen based on previous works on Italian virgin olive oils.

2.7. Sensory Analysis

Sensory evaluation of EVOO samples was performed by a panel of eight assessors, who had been trained according to the IOC's method for organoleptic assessment, which is described in EEC Department of Early Education and Care regulations.[27] Sensory evaluation was performed in three separate sessions and samples were randomized between assessors.

2.8. Statistical Analysis

A two-way ANOVA tested for significant differences between crusher speed and the injected oxygen level. The significance level was set to $p < 0.05$. The post-hoc Tukey honestly significant difference (HSD) test was applied to assess differences among mean values of variables where appropriate. All trials were performed in triplicate, for a total of 18 samples.

The values are represented with mean and standard deviation as in function of interaction between crusher speed and oxygen level. In tables are reported the significatively of each factor, while in the figures are reported the main effect of the ANOVA.

All statistical analyses were performed using the R software package (version 3.6.2).

3. Results

The crusher machine was tested in an industrial line in order to demonstrate its applicability and establish the effects of our innovative solution on EVOO quality. No inherent problems related to the operation of the pilot plant were observed.

As reported in **Table 1**, the dissolved oxygen level was constant in the produced EVOO and no significant difference was found between treatments.

3.1. Quality Parameters and VOCs in EVOO

The ANOVA did find significant differences related to crushing speed. However, no significant interaction was found between crushing speed and oxygen level for any parameter. Crusher speed did not affect free fatty acid concentrations, peroxide number, or UV coefficients (**Table 2**). On the other hand, biophenol concentrations were affected.

Simple phenols (tyrosol, hydroxytyrosol, and hydroxytyrosol acetate) were not affected by changes in crusher speed.[30] Tyrosol concentrations were found to be within the range 1.5–1.9 mg kg^{-1}; hydroxytyrosol concentrations minimally fluctuated from 1.7 to 2.2 mg kg^{-1}; and levels of hydroxytyrosol acetate were determined to be within an interval ranging from 0.75 to 1.36 mg kg^{-1}.

Decarboxymethyl oleuropein aglycone dialdehyde (3,4-DHPEA-EDA), was the most abundant biophenol (roughly 35% of total biophenols). This compound, together with oleuropein aglycone dialdehyde (3,4-DHPEA-EA) is the major contributor to olive oil oxidative stability.[31]

It is known that an increase in malaxation time and also different malaxation temperatures significantly decreased the concentrations of aglycone isomers of oleuropein and ligstroside but, conversely, increased the oleocanthal and oleacein contents.[32,33]

Our results showed that its concentration is very sensitive to crushing speed. The 3,4- DHPEA-EDA content of oil from olives crushed at 2500 rpm is lower, by about 128 mg kg^{-1}, compared to olives crushed at 3500 rpm (160 mg kg^{-1}). The ligstroside derivative p-HPEA-EDA follows the same trend. Concentrations increase as a function of crushing speed and range from a minimum of 47 mg kg^{-1} (at 2500 rpm) to a maximum of 55 mg kg^{-1} (at 3500 rpm). Aglycone secoiridoids such as 3,4-DHPEA-EDA, p-HPEA-EDA, p-HPEA-EA, and 3,4-DHPEA-EA appear during crushing due to the hydrolysis of oleuropein, dimethyl-oleuropein, and ligstroside. Furthermore, the content of oleuropein, ligstroside, and their derivatives was proportional to the intensity of bitterness and pungency.

We found no effect of the oxygen level with respect to the main chemical parameters, but we did find a significant increase in VOCs. Our experimental data identified several statistically significant differences (p-value < 0.05) in the LOX pathway.

In particular, **Table 3** shows that significant differences were found for five compounds, namely Heptanal, Z3-hexenal, E2-hexenal, 1-Hexanol, and Z3-Hexen-1-ol due to

Table 2. Mean and standard deviation for oils obtained in different crushing conditions and oxygen dosages. Letters indicate statistical differences, $p \leq 0.05$. ns = not significant. All trials were performed in triplicate, for a total of 18 samples ($n = 3$).

Oxygen level	Control	Low level	High level	Control	Low level	High level	*p*		
Crusher speed [rpm]	2500	2500	2500	3500	3500	3500	Oxygen level	Crusher speed	Interaction
Free fatty acids [%]	0.16 ± 0.03^a	0.16 ± 0.02^a	0.16 ± 0.02^a	0.18 ± 0.01^a	0.15 ± 0.00^a	0.15 ± 0.01^a	ns	ns	ns
Peroxide number [meqO2 kg^{-1}]	2.83 ± 0.59^a	2.93 ± 0.38^a	2.67 ± 0.38^a	2.73 ± 0.15^a	2.43 ± 0.15^a	2.67 ± 0.61^a	ns	ns	ns
K232	1.79 ± 0.09^a	1.82 ± 0.10^a	1.84 ± 0.04^a	1.83 ± 0.03^a	1.86 ± 0.05^a	1.85 ± 0.05^a	ns	ns	ns
K268	0.14 ± 0.02^a	0.15 ± 0.02^a	0.15 ± 0.02^a	0.15 ± 0.01^a	0.16 ± 0.01^a	0.15 ± 0.02^a	ns	ns	ns
ΔK	0.00 ± 0.01^a	0.00 ± 0.00^a	0.01 ± 0.01^a	0.01 ± 0.00^a	0.01 ± 0.01^a	0.01 ± 0.01^a	ns	ns	ns
Hydroxytyrosol	1.77 ± 0.47^a	2.08 ± 0.61^a	2.33 ± 0.75^a	1.81 ± 0.49^a	2.00 ± 0.54^a	2.07 ± 0.45^a	ns	ns	ns
Tyrosol	1.56 ± 0.48^a	1.71 ± 0.35^a	1.73 ± 0.32^a	1.85 ± 0.12^a	1.87 ± 0.11^a	1.81 ± 0.29^a	ns	ns	ns
Vanillic acid + Caffeic acid	1.00 ± 0.42^a	1.06 ± 0.47^a	1.03 ± 0.31^a	1.16 ± 0.34^a	1.10 ± 0.09^a	1.17 ± 0.03^a	ns	ns	ns
Vanillin	1.63 ± 0.03^a	1.58 ± 0.38^a	1.71 ± 0.20^a	1.67 ± 0.12^a	1.80 ± 0.18^a	1.80 ± 0.30^a	ns	ns	ns
Para-coumaric acid	1.43 ± 0.98^a	0.86 ± 0.34^a	0.96 ± 0.37^a	0.97 ± 0.19^a	1.39 ± 0.77^a	1.19 ± 0.52^a	ns	ns	ns
Hydroxytyrosyl acetate	0.91 ± 0.02^a	0.92 ± 0.13^a	1.03 ± 0.52^a	0.77 ± 0.19^a	1.35 ± 1.11^a	0.75 ± 0.34^a	ns	ns	ns
Ferulic acid	2.07 ± 0.89^a	2.08 ± 0.85^a	2.07 ± 0.24^a	2.35 ± 0.16^a	2.14 ± 0.54^a	2.27 ± 0.34^a	ns	ns	ns
Ortho-coumaric acid	2.03 ± 0.61^a	1.80 ± 0.77^a	2.13 ± 1.05^a	1.94 ± 0.67^a	1.98 ± 1.20^a	2.87 ± 3.01^a	ns	ns	ns
Decarboxymethyl oleuropein aglycone, oxidised dialdehyde	3.19 ± 0.62^a	3.87 ± 1.74^a	3.93 ± 2.93^a	3.76 ± 0.72^a	3.89 ± 2.63^a	3.14 ± 1.54^a	ns	ns	ns
(3,4-DHPEA-EDA)	125.05 ± 26.08^b	126.73 ± 37.23^b	133.83 ± 20.66^b	154.00 ± 22.14^a	159.13 ± 31.40^a	163.34 ± 34.88^a	ns	0.04	ns
Oleuropein	16.34 ± 3.76^a	15.00 ± 0.63^a	13.91 ± 6.04^a	18.18 ± 4.95^a	16.17 ± 6.11^a	20.64 ± 0.92^a	ns	ns	ns
(3,4-DHPEA-EA)	65.46 ± 15.28^a	83.02 ± 14.65^a	80.03 ± 13.23^a	78.52 ± 12.12^a	84.93 ± 5.77^a	80.49 ± 10.65^a	ns	ns	ns
Tyrosilacetate	16.31 ± 6.11^a	20.20 ± 2.90^a	17.42 ± 7.42^a	17.31 ± 3.20^a	18.18 ± 6.24^a	18.63 ± 2.35^a	ns	ns	ns
Decarboxymethyl ligstroside aglycone, oxidized dialdehyde	16.52 ± 3.22^a	19.91 ± 0.66^a	18.86 ± 4.32^a	16.92 ± 1.22^a	18.55 ± 0.62^a	18.44 ± 3.23^a	ns	ns	ns
(p-HPEA-EDA)	46.23 ± 8.87^b	47.39 ± 9.41^b	49.14 ± 4.34^b	53.70 ± 8.04^a	53.24 ± 9.25^a	56.18 ± 9.98^a	ns	0.05	ns
Pinoresinol, 1 acetoxy-pinoresinol	54.55 ± 10.48^a	62.65 ± 9.51^a	57.47 ± 5.27^a	56.43 ± 3.06^a	58.40 ± 2.94^a	59.31 ± 6.58^a	ns	ns	ns
Cinnamic acid	8.88 ± 0.86^a	8.70 ± 3.01^a	8.96 ± 2.26^a	9.64 ± 1.08^a	9.91 ± 0.90^a	7.77 ± 1.27^a	ns	ns	ns
(p-HPEA-EA)	9.49 ± 2.42^a	13.72 ± 4.17^a	11.16 ± 2.52^a	9.76 ± 1.47^a	10.23 ± 0.37^a	11.40 ± 0.75^a	ns	ns	ns
Oleuropein aglycone, oxidized aldehyde and hydroxylic	11.61 ± 1.50^b	9.76 ± 3.54^b	11.00 ± 2.54^b	13.37 ± 3.42^a	13.13 ± 0.97^a	12.86 ± 1.94^a	ns	0.05	ns
Luteolin	23.05 ± 5.08^b	24.50 ± 3.48^b	22.29 ± 5.71^b	25.06 ± 5.02^a	26.19 ± 4.96^a	26.60 ± 4.48^a	ns	ns	ns
Oleuropein aglycone, aldehyde and hydroxylic	44.14 ± 9.88^b	48.78 ± 4.12^b	49.67 ± 6.61^b	53.50 ± 4.16^a	56.02 ± 6.93^a	53.22 ± 7.39^a	ns	0.05	ns
Ligstroside aglycone, oxidized aldehyde and hydroxylic	18.90 ± 0.90^a	19.11 ± 2.30^a	19.49 ± 3.39^a	21.05 ± 2.53^a	19.55 ± 2.10^a	18.96 ± 2.86^a	ns	ns	ns
Apigenin	3.06 ± 1.42^a	1.78 ± 0.30^a	1.24 ± 0.18^a	1.90 ± 0.48^a	2.38 ± 0.52^a	2.32 ± 1.94^a	ns	ns	ns
Methyl-luteolin	8.13 ± 2.03^a	8.39 ± 0.56^a	8.09 ± 0.98^a	8.74 ± 0.84^a	9.61 ± 1.82^a	9.79 ± 1.17^a	ns	ns	ns
Ligstroside aglycone, aldehyde and hydroxylic	15.24 ± 3.29^a	14.92 ± 2.33^a	15.14 ± 3.20^a	15.11 ± 1.62^a	15.74 ± 4.14^a	15.04 ± 3.52^a	ns	ns	ns
Total phenolic compounds	503.50 ± 92.36^a	545.16 ± 80.97^a	534.63 ± 75.39^a	570.97 ± 43.70^a	588.89 ± 65.70^a	592.05 ± 82.39^a	ns	ns	ns

the addition of oxygen. Concentrations increased in treated samples. Furthermore, our results show that even a low level of oxygen injection was enough to significantly increase concentrations. The greatest difference was obtained for E-2-hexenal. In our experiment, its concentration increased in samples where oxygen was added to the paste. Concentrations for control samples were about 12.5 mg kg^{-1}, while at a minimum level of oxygen injection this increased to 15.7 mg kg^{-1}. As the odour threshold is 0.25 mg kg^{-1} it was clearly perceivable in all samples.

The same trend was observed for Z3-hexenal, 1-Hexanol, and Z3-Hexen-1-ol. Namely, samples treated with the minimum dosage of oxygen were statistically different to control samples. Z-3-hexenal and E-2-hexenal are described as having "green leaves" and "green and sweet" sensory notes.[34] The low odour threshold means that they are the most important VOCs in the LOX

Table 3. Volatile compounds and total VOC content (mg kg^{-1}) for oils obtained at different crushing speeds and dosages of oxygen. Letters indicate statistical differences, $p \leq 0.05$. ns = not significant. All trials were performed in triplicate, for a total of 18 samples (n = 3).

Oxygen Level	Control	Low Level	High Level	Control	Low Level	High Level	*P*		
Crusher speed (rpm)	2500	2500	2500	3500	3500	3500	Oxygen Level	Crusher speed	Interaction
Ethyl propionate	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	ns	0.020	ns
Heptanal	0.69 ± 0.01^{a}	0.68 ± 0.00^{b}	0.69 ± 0.01^{b}	0.70 ± 0.00^{a}	0.68 ± 0.01^{b}	0.68 ± 0.00^{b}	0.02	ns	ns
*Z*3-Hexenal	0.51 ± 0.04^{b}	0.53 ± 0.05^{ab}	0.63 ± 0.07^{a}	0.46 ± 0.03^{b}	0.51 ± 0.01^{ab}	0.56 ± 0.11^{a}	0.03	ns	ns
*E*2-Hexenal	12.29 ± 1.28^{b}	14.76 ± 0.16^{a}	17.10 ± 0.92^{a}	13.04 ± 2.78^{b}	16.43 ± 0.87^{ab}	16.6 ± 0.77^{a}	0.01	ns	ns
1-Pentanol	0.01 ± 0.01^{b}	0.01 ± 0.01^{a}	0.00 ± 0.01^{a}	0.01 ± 0.02^{a}	0.02 ± 0.00^{ab}	0.023 ± 0.00^{a}	ns	ns	ns
*Z*2-Penten-1-ol	0.23 ± 0.01^{b}	0.24 ± 0.01^{b}	0.24 ± 0.01^{b}	0.25 ± 0.02^{a}	0.26 ± 0.01^{ab}	0.254 ± 0.01^{a}	ns	0.013	ns
*E*2- Hexenyl acetate	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	ns	ns	ns
2-Heptanol	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.011 ± 0.00^{a}	ns	ns	ns
*Z*3- Hexenyl acetate	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.036 ± 0.00^{a}	ns	ns	ns
1-Hexanol	0.08 ± 0.05^{b}	0.20 ± 0.02^{a}	0.22 ± 0.11^{a}	0.08 ± 0.04^{b}	0.18 ± 0.03^{a}	0.249 ± 0.08^{a}	0.01	ns	ns
*E*3-Hexen-1-ol	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.02 ± 0.02^{a}	0.03 ± 0.01^{a}	0.026 ± 0.00^{a}	ns	ns	ns
*Z*3-Hexen-1-ol	0.37 ± 0.01^{b}	0.45 ± 0.03^{a}	0.46 ± 0.02^{a}	0.41 ± 0.03^{b}	0.46 ± 0.06^{a}	0.477 ± 0.05^{a}	0.01	ns	ns
2-Nonanone	0.08 ± 0.01^{a}	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	0.07 ± 0.02^{a}	0.07 ± 0.01^{a}	0.074 ± 0.01^{a}	ns	ns	ns
*Z*2 + *E*2-Hexen-1-ol	0.27 ± 0.21^{a}	0.19 ± 0.26^{a}	0.11 ± 0.28^{a}	0.18 ± 0.41^{a}	0.19 ± 0.23^{a}	0.162 ± 0.12^{a}	ns	ns	ns
Nonanal	0.43 ± 0.05^{a}	0.41 ± 0.04^{a}	0.38 ± 0.03^{a}	0.39 ± 0.24^{a}	0.35 ± 0.16^{a}	0.378 ± 0.06^{a}	ns	ns	ns
2,4-Hexadienal	0.95 ± 0.04^{a}	0.97 ± 0.1^{a}	0.94 ± 0.07^{a}	0.93 ± 0.09^{a}	1.02 ± 0.05^{a}	0.954 ± 0.05^{a}	ns	ns	ns
2-Octanol	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.014 ± 0.00^{a}	ns	ns	ns
1-Heptanol	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.019 ± 0.00^{a}	ns	ns	ns
(*E*,*E*)-2,4-Heptadienal	0.06 ± 0.01^{a}	0.06 ± 0.02^{a}	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	0.06 ± 0.02^{a}	0.073 ± 0.00^{a}	ns	ns	ns
Propanoic acid	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	0.026 ± 0.01^{a}	ns	ns	ns
1-Octanol	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.02 ± 0.00^{a}	0.03 ± 0.01^{a}	0.02 ± 0.00^{a}	0.025 ± 0.00^{a}	ns	ns	ns
2-Heptanone	0.05 ± 0.00^{b}	0.05 ± 0.00^{ab}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{ab}	0.049 ± 0.00^{a}	0.02	ns	ns
Butanoic acid	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	ns	ns	ns
Nonanol	0.18 ± 0.01^{a}	0.17 ± 0.00^{a}	0.16 ± 0.00^{a}	0.16 ± 0.04^{a}	0.15 ± 0.02^{a}	0.156 ± 0.01^{a}	ns	ns	ns
*E*2-Decenal	1.74 ± 0.37^{a}	2.53 ± 0.53^{a}	2.51 ± 1.4^{a}	3.04 ± 1.89^{a}	2.49 ± 1.88^{a}	3.843 ± 1.56^{a}	ns	ns	ns
(*E*,*E*)-2,4-Nonadienal	0.05 ± 0.01^{a}	0.05 ± 0.00^{a}	0.05 ± 0.01^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.042 ± 0.01^{a}	ns	ns	ns
(*E*,*E*)-2,4-Decadienal	1.03 ± 0.04^{a}	0.99 ± 0.02^{a}	0.99 ± 0.06^{a}	1.03 ± 0.09^{a}	1.10 ± 0.04^{a}	1.046 ± 0.01^{a}	ns	ns	ns
Phenol	0.07 ± 0.00^{a}	0.07 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.02^{a}	0.07 ± 0.01^{a}	0.063 ± 0.00^{a}	ns	ns	ns
4-Ethyl-2-methoxyphenol	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.004 ± 0.00^{a}	ns	0.001	ns
4-Ethyl phenol	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.02^{a}	0.04 ± 0.00^{a}	0.037 ± 0.00^{a}	ns	ns	ns

pathway,[4,13,30] along with several others that contribute to the fruity attribute.

Figure 2 shows total concentrations of LOX VOCs from the C6 and C5 branches. There is a statistically significant lower concentration in LOX VOC content for the control sample without oxygen addition. The ANOVA highlighted a significant (*p*-value < 0.05) main effect of oxygen dosage. In fact, even the minimum level of oxygen was enough to trigger a significant change in concentrations of these important compounds that are mainly responsible for the fruity attribute in EVOO. The mean increase was about 20%.

3.2. Sensory Evaluation

To understand if the observed increase in concentrations (especially of compounds associated with positive notes) was perceptible to the consumer, we conducted a sensory test. Here, the aim was to understand if our proposed solution could increase (desirable) perceptions of green notes. **Figure 3** reports the intensity of the main descriptors. Overall, judges were unable to detect any sensory defects in all olive oil samples. The sensory evaluation found a value of 0 for all defect attributes, and values greater than 0 for fruity attributes. Hence, all samples could be considered as EVOO under European regulations. However, some differences between samples were detected.

Control samples (without oxygen addition) were perceived as less fruity ($p < 0.05$) than the other two that were treated with oxygen. Average fruity intensity registered for samples with added oxygen was 5.2 ± 0.2, compared to 4.1 ± 0.2 for the other samples.

Crusher speed did have a significant effect on the bitter attribute ($p < 0.05$). Increased bitterness was detected for samples crushed at 3500 rpm compared to those crushed at lower speed

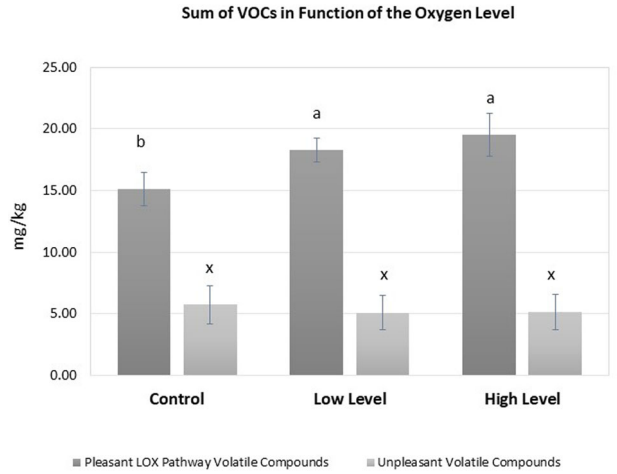

Figure 2. Means and standard deviations of total LOX pathway VOCs (letters a, b) and total of unpleasant compounds (*x*) as a function of the oxygen level ($n = 6$). Letters indicate a statistical difference ($p < 0.05$).

(mean 5.7 ± 0.3 compared to 4.5 ± 0.4). Judges detected no difference for the pungent attribute

4. Discussion

The first aim of the research was to find to validate the oxygen dosing system. This goal was achieved through the monitoring effectuated during the extraction trials, measuring the content of dissolved oxygen in the EVOO. The results obtained confirmed the satisfactory performance of the oxygen sparger system, and that the selected range did not increase the dissolved oxygen level in EVOO. The mean value was about 8.30 mg L^{-1} at $T = 15$ °C.

For the qualitative parameters, the increase in phenols with crusher speed it was observed. This trend was also observed by Inarejos-García[17] at laboratory scale, and by Guerrini[18] in industrial trials. In general, a comparison of crushing technologies shows that devices that lead to the more violent rupture of fruit tissues produce a higher concentration of phenols.[35] A higher crushing speed may decrease the diameter of oil droplets, increasing the oil/water emulsion interphase area, and facilitating the mass transfer of phenols to the lipid phase following the action of β-glucosidase.[30]

No significant results were found for lignans and flavonoids as a function of crushing speed. Our finding confirms other research carried out in recent years.[17,18] Finally, high, but not statistically significant values of total biophenols (p-value = 0.08) were registered in samples produced at higher crusher speed.

Concerning the concentration of aromatic volatile compounds, the results showed an increment of their concentrations related to the addition of oxygen. In particular, samples treated with the minimum dosage of oxygen were statistically different to control samples. Our experimental data identified several statistically significant differences (p-value<0.05) in the LOX pathway. The highest difference was observed for E-2-hexenal. This compound provides the characteristic "green" note of olive oil, and is the most abundant C6 aldehyde, representing about 90% of C6 compounds. These compounds are synthetized from free polyunsaturated fatty acids during the extraction process, through a cascade of enzymatic reactions known as the lipoxygenase (LOX) pathway. Consequently, their abundance depends on the concentration and activity of the enzymes involved in the LOX pathway.[36,4]

On the light of these results, to suggest that probably the amount of oxygen taken up by the olive paste during crushing is enough to trigger the LOX cascade, and therefore of the concentration increase of these volatile compounds.

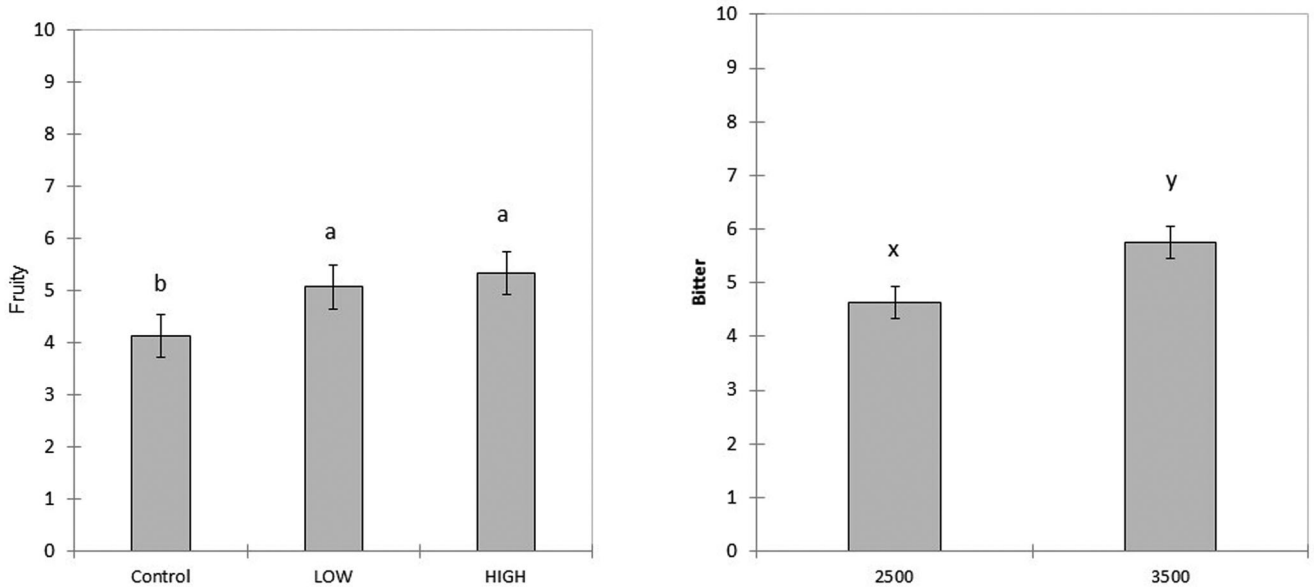

Figure 3. On the left means and standard deviations of the fruity intensity as a function of the oxygen level ($n = 6$. Letters a, b), on the right means and standard deviations of the bitter intensity as a function of the crusher speed ($n = 9$. Letters x, y). Different letters indicate a statistical difference ($p < 0.05$).

Our results are in good agreement with Tamborrino[24] who saturated the malaxer with nitrogen and injected air continuously into the olive paste. The latter study also observed an increase in E-2-hexenal and Z-3-hexenal compounds. Consistent with results reported for other crushers, in our experiment VOC synthesis was minimally affected by changes in rotor speed.[16,18]

Another interesting result relates to compounds that are perceived as unpleasant. Here, no significant differences were found either as a function of oxygen treatment or crusher speed. Overall concentrations were very low with respect to positive attributes. Mean totals were 5.2 mg kg^{-1}, and concentrations were stable independent of the operative condition.

The sensory analysis confirms the trend observed in the VOC analysis. Namely, the oxygen addition did increase fruity notes, while the crusher speed had no influence.

Moreover, samples crushed at higher speed had higher concentrations of several phenolic compounds, result that was confirmed by chemical analysis. Bitterness and pungency are mainly related to the quali-quantitative presence of phenolic compounds in EVOO.[37]

Regarding pungency notes, the sensory analysis not found difference. Andrewes and co-authors[38] found a significant relationship between the concentration of p-HPEA-EDA and pungency. Although we found significant differences in p-HPEA-EDA concentration as a function of crushing speed, our analysis did not identify a relationship between this compound and pungency. However, a difference in concentration of about 10 mg/kg cannot be detected by the panel, as reported in another study.[18]

5. Conclusions

Our study evaluated an innovative pilot industrial crusher. Notably, the system directly controls the oxygen dosage in the olive paste after crushing. It can easily be adapted to the industrial scale and is technically feasible. The system was tested with different crusher configurations and oxygen dosage.

Our results show that the supply of oxygen, even at a minimum level enriches the volatile fraction. This result was confirmed by a sensory analysis, with an increase in fruity intensity. Crushing speed affects the extraction of compounds such as biophenols, in particular 3,4-HPEA-EDA, and p-HPEA-EDA (as reported in previous studies) but does not affect VOCs. Furthermore, there is no interaction between crusher speed and oxygen level, and this result is considered positive as it is possible to produce different oils by setting these two variables independently. Moreover, these two variables were selected in order to improve the qualitative performance of the crusher, and they can easily be set to increase or modulate the chemical composition of olive oil.

The higher crusher speed produced oils with a higher concentration of biophenols and a more bitter and pungent taste. On the other hand, dosing oxygen directly into the olive paste results in oils with higher VOC concentrations and higher fruity intensity. VOCs are unaffected by a change in crusher speed, and were not formed compounds that involves sensory defects with the addition of oxygen.

VOCs are unaffected by a change in crusher speed and were not formed compounds that involves sensory defects with the addition of oxygen. These crusher innovations can be easily configured in order to produce EVOO with specific characteristics that are a function of operational conditions, and with better, and more diverse chemical and organoleptic characteristics.

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[Correction added on 12 May 2022, after first online publication: CRUI-CARE funding statement has been added.]

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

G.A.: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft. L.G.: Conceptualization; Data curation. Ferdinando Corti: Data curation; Investigation. A.S.: Data curation; Methodology. L.C.: Data curation; Methodology; Resources. A.P.: Funding acquisition; Project administration; Resources; Supervision. P.M.: Conceptualization; Data curation; Formal analysis; Methodology; Supervision; Validation; Visualization.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

biophenols, olive oil extraction plants, oxygen dosage, volatile compounds

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