Food Bioscience 53 (2023) 102706

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Effect of enzymatic and talc treatment on olive oil extraction process at the industrial scale

Antonia Tamborrino [b], Roberto Selvaggini [a], Gianluca Veneziani [a,*], Antonio Berardi [b], Alessandro Leone [b], Maurizio Servili [a]

[a] Department of Agricultural, Food and Environmental Sciences, University of Perugia, via S. Costanzo, 06126, Perugia, Italy
[b] Department of Soil, Plant and Food Science (DiSSPA), University of Bari Aldo Moro, via Amendola 165/a, Bari, I-70126, Italy

ARTICLE INFO

Keywords:
Technological coadjuvants
Enzymes
Talc
Oil yield
Olive oil quality

ABSTRACT

Technological coadjuvants were applied at the beginning of the malaxation phase of the olive oil mechanical extraction process at the industrial scale. An enzymatic formulation consisting of 35% pectinase, 28% pectinmethylesterase and 7% polygalacturonase % (v/v/v) and talc was added during the kneading of olive paste to evaluate its impact on oil extractability and on olive oil quality characteristics. Quantitative and qualitative evaluation of hydrophilic phenols and volatile compounds involved in the main health and sensory properties of high-quality olive oil was carried out. The addition of a combination of enzymatic complex and talc, for the first time at industrial scale, increased the oil extractability by 5.6% (absolute value), improving the degradation of the cell wall structure of olive paste and breaking down the oil-in-water emulsions with a more efficient separation of the oil during the extraction process. The use of the enzymatic complex and talc leads to a percentage increase in the phenolic content in the range of 12%–16% without altering the legal quality parameters and volatile profile of the final product.

1. Introduction

The olive oil sector focuses on improving the mechanical extraction process with the dual aim of increasing the performance of the extraction plant and enhancing the quality characteristics of the final product. The increase in performance mainly concerns the increase in working efficiency and oil extractability of olive mills, whereas enhancing quality involves preservation and/or improvement of the main olive oil quality characteristics linked to health and sensory properties and potentially influenced by oil extraction plants and by the management of technological processes. Careful management of technological parameters, such as time, temperature, oxygen and coadjuvants, during the most important phase of the process, namely, the crushing and malaxation steps, has a significant impact on the improvement of processing methods to obtain high-quality standard EVOO (Angerosa et al., 2001; Caponio et al., 2016; Kalua et al., 2006; Squeo et al., 2020; Veneziani et al., 2018). In recent years, many technological research studies have been carried out, introducing new innovations in the mechanical extraction process in an attempt to control the main enzymatic activity of endogenous enzymes (Leone et al., 2015; Kalogianni et al., 2019; Nucciarelli et al., 2022; Pérez et al., 2021; Taticchi et al., 2019; Tamborrino et al., 2021; Tamborrino et al., 2022; Leone et al., 2022; Veneziani et al., 2022). All this was developed to improve the coalescence of oil droplets to increase oil yield, to prevent the oxidation of phenolic fractions mainly due to poliphenoloxydase (PPO) and peroxidase (POD), and to achieve the neoformation of a high level of volatile compounds induced by lipoxygenase, which is responsible of the main characteristic sensory notes of a high-quality olive oil. In the continuous evolution of the olive oil industry, the addition of technological coadjuvants brought further developments and was the object of several studies (Table 1). Some studies focused on the use of talc (Caponio et al., 2016; Espínola et al., 2015; Moya et al., 2010; Sadkaoui, Jimenez, Aguilera, et al., 2017; Vidal et al., 2020), a physical aid that is also permitted in the European Union (EU) since no talc-induced chemical and biochemical alterations to the mechanical extraction process have been discovered, thereby preserving the definition of EVOO as a natural oil extracted only by physical and mechanical technology (Council of the European Union, Council Regulation (EC) No 1513/2001). However, other authors have different opinions on the impact of this coadjuvant on the physicochemical composition of VOO, showing an improvement in quality

* Corresponding author.
E-mail address: gianluca.veneziani@unipg.it (G. Veneziani).

https://doi.org/10.1016/j.fbio.2023.102706
Received 8 March 2023; Received in revised form 26 April 2023; Accepted 26 April 2023
Available online 4 May 2023

parameters (Caponio et al., 2016; Koprivnjak et al., 2016; Sánchez et al., 2022; Squeo et al., 2020). The role of talc is linked to the increase in oil yield, most of all when the extraction process is carried out with "difficult fruits" such as overwatered and/or overripe olives. Micronized natural talc is able to interfere with the water content of olive paste during the malaxation phase and to promote the breakdown of oil-in-water emulsions (Caponio et al., 2016; Sadkaoui et al., 2016). The performance of this physical activity, which improves the final separation step of the oily phase, is highly related to the concentration of coadjuvant, ripening stage and physico-chemical characteristics of raw materials and malaxation parameters such as time and temperature (Moya et al., 2010; Sadkaoui, Jimenez, Pacheco, & Beltran, 2017; Peres et al., 2016; Squeo et al., 2020; Vidal et al., 2020). The activity of other coadjuvants was also examined, such as the addition of enzymatic complexes during the malaxation phase (De Faveri et al., 2008; Hadj--Taieb et al., 2012; Polari & Wang, 2020) or the use of sodium chloride, calcium carbonate and silica (Espínola et al., 2015; Koprivnjak et al., 2016; Moya et al., 2010; Squeo et al., 2020; Tamborrino et al., 2017). The use of alternative coadjuvants to talc are not allowed in the EU in the olive oil mechanical extraction process, also included the addition of enzymes during the kneading of olive paste that is in contrast with the definition of extra or virgin olive oil category (OJEC, 2001). The addition of enzymatic mixtures, mainly consisting of pectinase, cellulase, hemicellulose and xylanase, improve the activity of endogenous enzymes of olive fruits, thereby promoting the breakdown of cellular structures of pulp during the kneading of olive paste, both increasing the oil extractability and the solubilization of phenolic compounds into the oily phase (De Faveri et al., 2008; Hadj-Taieb et al., 2012; Polari & Wang, 2020). During a laboratory-scale optimization of olive oil extraction, both the addition of talc and enzymes were found to have a positive impact on oil yield (Peres et al., 2016). Informed by this previous research, this study investigated the use of both talc and enzymatic complexes and their combination as technological coadjuvants during the olive oil mechanical extraction process for the first time at the industrial scale with the dual purpose of evaluating the effects on oil extractability and quality (Table 1).

2. Materials and methods

2.1. Plant material

Olive fruits (*Olea europaea* L.) cv. Coratina, were harvested in January 2022 in Puglia (Italy). The olives were collected from irrigated land. A homogeneous lot of approximately 18000 kg was used for the experimental trials, including the cleaning runs performed when switching into the next test condition. The olive fruits were processed within 24 h of harvest at Evo Campania s.c.a.r.l. mill Campagna – SA (Italy). The olive maturity index was 3.7, measured as reported by Squeo et al. (2017).

2.2. Industrial olive oil extraction plant

The oil extraction plant used for the experimental tests was built by Pieralisi (Pieralisi MAIP SpA, Jesi, Ancona, Italy) and involves a hummer crusher model cooling system, a group of 6 malaxers (the Panorama model), a two-phase horizontal centrifugal model (Scopion 5.7) and a separator model (Bravo).

During the experimental test, the operating parameters used were as follows.

- mass flow rate equal to 3000 kg h^{-1}
- grid hole diameter of the crusher: 5.7 mm
- malaxation temperature equal to 27 °C
- malaxation time: 30 min
- no water added to the horizontal centrifuge.

2.3. Experimental design

To analyse the activity of the technological aid, the control trials (CONTROL) were alternated and compared with trials that included only the use of the talc (TALC), only the use of the enzymes (ENZYMES) and the combination of the use of the talc and enzymes (ENZ + TALC). The talc used was a hydrated magnesium silicate, added at a

Table 1
Comparison among different additions of coadjuvants during mechanical extraction process and their impact on olive oil yield and quality.

Coadjuvants	Application	Technological effects[a]			References
		Oil yield	Phenolic compounds	Volatile compounds	
Talc *(1–2% w/w)* Enzymes *pectolytic enzymes*	Review article	I	NC	NC	Di Giovacchino et al., 2002 https://doi.org/10.1002/1438-9312(200210)104:9/10<587::AID-EJLT587>3.0.CO;2-M
Enzymes *pectinase, hemicellulase and cellulase; pectinase and hemicellulase; pectinase*	Research article *Lab-scale*	NC	I	NC	De Faveri et al., 2008 https://doi.org/10.1016/j.bej.2008.04.007
Enzymes *pectinases, xylanases and cellulases*	Research article *Lab-scale*	I	I	NC	Hadj-Taieb et al., 2012 https://doi.org/10.1016/j.bej.2011.04.003
Talc and calcium carbonate *(0.3- 1% w/w)*	Research article *Industrial-scale*	I	I	NC	Moya et al., 2010 https://doi.org/10.1016/j.jfoodeng.2009.09.015
Talc *(0.04–0.46 w-%)* Enzymes *(0.003–0.117 w-%)*	Research article *Lab-scale*	I	ND	NC	Peres et al., 2016 https://doi.org/10.1016/j.foodchem.2016.05.022
Sodium cloride and talc *(1–3% w/w)*	Research article *Lab-scale*	I	ND/I	I	Koprivnjak et al., 2016 https://doi.org/10.1002/ejlt.201500014
Talc, calcium carbonate and silica (0–*2% w/w)*	Research article *Lab-scale*	I/R	I	NC	Espínola et al., 2015 https://doi.org/10.1007/s00217-015-2501-3
Talc *(0–1% w/w)*	Research article *Lab-scale*	I	NC	NC	Sadkaoui et al., 2016 https://doi.org/10.1002/ejlt.201600039
Calcium carbonate *(0–4% w/w)*	Research article *Industrial-scale*	ND	R	ND	Tamborrino et al., 2017 https://doi.org/10.1016/j.jfoodeng.2017.02.019
Talc *(0.6 - 2.9 w/w)*	Research article *Industrial-scale*	I/R	I/R	I/R	Vidal et al., 2020 https://doi.org/10.1016/j.lwt.2018.08.001
Talc and calcium carbonate *(0–2% w/w)*	Research article *Lab-scale*	NC	I/R	I/R	Squeo et al., 2020 https://doi.org/10.1016/j.lwt.2020.109887
Talc *(0.7% w-w)* **Enzymes** *pectinase, pectinmethylesterase and polygalacturonase (0.015% v/w)*	**Research article** ***Industrial-scale***	**I**	**I**	**ND**	**Tamborrino et al., 2023**

[a] NC = not calculated; ND = not detected; I = increase; R = reduction.

concentration of 0.7% w/w. Regarding the enzymes, a complex of pectolytic enzymes with depolymerising action composed of 35% pectinase – 28% pectinmethylesterase – 7% polygalacturonase % (v/v/v), was used at a concentration of 0.015% v/w. Talc, enzymes and their combination were added at the beginning of malaxation phase. For each test condition, a homogeneous 700 kg lot of olives was used. Each test condition was repeated 5 times. To analyse the quantitative performance of the mill and olive oil quality, five samples of olives, paste, pomace and olive oil were collected for each trial.

2.4. Quantitative performance of the plant

The quantitative performance of the plant was evaluated by determining (i) the amount of oil lost in the pomace and (ii) the extractability (E), according to Leone et al., 2015. E is the ratio between the percentage of oil extracted during the process and the percentage of oil contained in the olives.

2.5. Analysis of olive oil quality

2.5.1. Reference compounds

Tyrosol (*p*-HPEA) and hydroxytyrosol (3,4-DHPEA) were supplied by Cabru s.a.s. (Arcore, Milan, Italy) and Fluka (Milan, Italy). The other phenolic compounds belonging to secoiridoids were obtained from VOO following the method described by Selvaggini et al. (2014): aglyconic derivatives of oleuropein, [the dialdehydic forms of decarboxymethyl elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA or oleacein) and 3,4- (dihydroxyphenyl)ethanol elenolic acid (3,4-DHPEA-EA or an isomer of the oleuropein aglycon)], aglyconic derivatives of ligstroside [the dialdehydic forms of decarboxymethyl elenolic acid linked to tyrosol (*p*-HPEA-EDA or oleocanthal) and *p*-(hydroxyphenyl) ethanol elenolic acid (*p*-HPEA-EA or ligstroside aglycon)] and lignans [(+)-pinoresinol and (+)-1-acetoxypinoresinol]. All the other solvents and chemical compounds were supplied by Merck (Merck KGaA, Darmstadt, Germany).

2.5.2. Legal quality parameters

Free acidity, peroxide value and spectrophotometric constants (K_{232}, K_{270} and ΔK) of oils extracted with different technological aids used during the malaxation phase were evaluated according to Regulation (EU) 2015/1830 (OJEC, 2015).

2.5.3. Phenolic compounds

The oleuropein and ligstroside derivatives and lignans were separated and purified from VOO with semipreparative HPLC following the method described by Selvaggini et al. (2014). The extraction of the phenolic fraction from samples of olive oil and the next HPLC analyses of the main phenols were carried out according to Antonini et al. (2015) using a Spherisorb ODS1 250 mmx 4.6 mm column with a particle size of 5 μm (Waters, Milford, MA, USA). The HPLC equipment was composed of an Agilent Technologies 1100 series LC system (Agilent Technologies, Palo Alto, CA, USA). The management of all the parts of the equipment and the processing of the chromatographic data were carried out with ChemStation Rev. A. 10.02 (Agilent Technologies, Palo Alto, CA, USA). The amount of each phenolic molecule, expressed as the concentration of mg kg^{-1} of oil, was evaluated using the data obtained by the calibration curve as the response factor.

2.5.4. Volatile compounds

Quantity and quality evaluation of volatile compounds in VOOs were carried out by headspace-solid phase microextraction (HS-SPME) followed by gas chromatography−mass spectrometry (HS-SPME-GC/MS). The sampling of the headspace of each volatile compound and the relative gas chromatography analysis were performed according to Taticchi et al. (2021). The GC/MS analysis of the volatile compounds was conducted with an Agilent Technologies GC 7890B equipped with a "Multimode Injector" (MMI) 7693A (Agilent Technologies, Santa Clara, CA, USA) and a thermostated PAL3 RSI 120 autosampler equipped with a fibre conditioning module and an agitator (CTC Analytics AG, Zwingen, Switzerland). The detection system was an Agilent 5977B single quadrupole GC/MSD with an EI Extractor (XTR) source (Agilent Technologies, Santa Clara, CA. USA). Saturated and unsaturated aldehydes, alcohols, esters at C_5 and C_6 and ketones at C_5 and C_8 were quantitatively and qualitatively identified by comparison of their mass spectra and retention times with reference compounds and with the spectra in the NIST 2014 mass spectral library. The concentration of volatile molecules was evaluated using calibration curves for each compound by internal standard calculation, and the data were expressed as μg kg^{-1} of oil.

2.5.5. Data processing

The quantitative and qualitative results of the different theses compared were evaluated statistically with one-way analysis of variance (ANOVA) carried out with SigmaPlot Software 12.3 (Systat Software Inc., San Jose, CA, USA).

3. Results and discussion

3.1. Olive oil extractability

The quantitative results (Table 2) demonstrated that when enzymes or talc were used, there was a significant increase in extractability from 87.9% to values of 89.7% and 89.2%, respectively. The use of enzymatic formulation, selected as exogenous enzymes with pectolytic action able to depolymerise pectins and cause the maceration of olive tissues, showed a significant improvement in oil yield with an increase of 2.9% (absolute value) in olive oil extractability (Table 2). The data were confirmed by the residual oil in pomace. In fact, when enzymes or talc were used, the percentage of oil lost in pomace was significantly and equally lower than that in the control. Other studies concerning the addition of technological coadjuvants during the kneading phase also confirmed these results due to the hydrolytic processes induced by the complex consisting of pectinase, pectinmethylesterase and polygalacturonase performed on the cell wall of olive fruit mesocarp cells containing oil droplets in the vacuole (Hadj-Taieb et al., 2012; Peres et al., 2016; Vierhuis et al., 2001). A similar increasing trend in oil extractability was observed for the TALC samples, with an improvement of 2.3% (absolute value) compared to the control test (Table 2). In contrast, during these trials, the significant impact on oil yield was due to the physical action of talc coadjuvant and its effect on the breakdown of oil/water emulsions that encourages oil separation and extractability without interfering with chemical and biochemical processes (Caponio et al., 2016; Espínola et al., 2015; Peres et al., 2016; Sadkaoui et al., 2016; Vidal et al., 2020). When the combination of enzymes and talc was used for the first time at industrial scale, a significant increase in extractability exceeding 92% was found. The combined coadjuvant formulation based on enzymatic complex and physical aid showed a higher effect than the use of enzymes or talc added alone to the malaxed olive paste with a further enhancement of oil extractability that reached

Table 2
Moisture, oil content of olive pomaces and olive oil extractability.

Test conditions	Pomace		Extractability (%)
	Moisture (%)	**Oil (%. db)**	
CONTROL[a]	61.5 ± 1.4 a	7.3 ± 1.0 a	86.9 ± 1.9 a
ENZYMES	61.6 ± 0.5 a	5.6 ± 0.8 b	89.7 ± 1.8 b
TALC	61.5 ± 0.7 a	5.7 ± 0.8 b	89.2 ± 1.7 b
ENZ + TALC	61.2 ± 0.4 a	4.4 ± 0.1 c	92.5 ± 0.7 c

[a] Data are expressed as the mean value of three different trials ± standard deviation. Different letters in each rows denotes significant statistical differences according to Tukey test ($p < 0.05$).

increasing values of 5.6% (absolute value) compared to the control test (Table 2). These results are also confirmed by the analysis of the oil lost in the pomace. Indeed, when the combination of enzymes and talc was used, the oil loss in the pomace was significantly lower than that in the other three conditions. Even though the trials conducted with the addition of enzymes showed significant performance in extractability and quality of the final product compared to the control test we need to underline that there is not possibility of an industrial application in the virgin olive oil mechanical extraction process of the EU as regulated by European legislation (OJEC, 2001).

3.2. Olive oil quality

The experimental trials carried out by using enzymatic preparation, talc or the combination of both, used for the first time at industrial scale during the malaxation phase of the mechanical extraction process, did not determine any significant alteration to the legal quality parameters of the olive oils when compared to the control samples (Table 3). The data confirmed the results of other studies on the use of technological coadjuvants (Espínola et al., 2015; Vidal et al., 2020). All the values of acidity, peroxide index and spectrophotometric constants were also abundantly above the legal limit of the category of EVOO, showing high-quality characteristics of the final product (OJEC, 2015), even if the olive oil extracted with the use of enzymatic formulation cannot be classified in merchandise categories by European Regulation (OJEC, 2001) that excludes oils obtained using adjuvants having a chemical or biochemical action.

Relative to the phenolic fraction, the bioactive compounds showed a significant increase when the oils were extracted with the addition of enzyme formulation. The concentration of total phenols increased by 16.3%, with the amounts of oleacein, oleocanthal and oleuropein aglycon that were rinsed being 93.1, 20.8 and 42.2 mg kg^{-1}, respectively (Table 4). The addition of enzymatic aid based on pectinase, pectin methylesterase and polygalacturonase into the olive paste improved the activity of endogenous enzymes, increasing the breakdown process of the cellular olive tissues during the mechanical extraction process of the oil (Vierhuis et al., 2001). This phenomenon determines a higher release of intracellular liquid in the olive paste, improving the physico-chemical interaction between water and the oily phase during the malaxation step with a consequent increase in the solubilization process of the phenolic fraction into the olive oil (De Faveri et al., 2008; Hadj-Taieb et al., 2012; Polari & Wang, 2020). In contrast, when only talc coadjuvant was added to the olive paste in the malaxation phase, no significant effects were determined on the concentration of the main secoiridoid aglycons (Table 4), as reported by different studies (Carrapiso et al., 2013; Koprivnjak et al., 2016; Moya et al., 2010; Vidal et al., 2020). For that reason, the only use of talc that

Table 3
Legal quality parameters of olive oils extracted with the addition of different technological coadjuvants, according to Regulation (EU) 2015/1830.

Test conditions	Acidity (%)	Peroxide value (meq O_2/Kg oil)	K_{232}	K_{270}	ΔK
Legal limits for EVOO	*≤0.8*	*≤ 20.0*	*≤ 2.50*	*≤ 0.22*	*≤ 0.01*
CONTROL[a]	0.22 ± 0.01 a	6.0 ± 0.5 a	1.64 ± 0.12 a	0.13 ± 0.01 a	−0.003 ± 0.001 a
ENZYMES	0.22 ± 0.01 a	5.6 ± 0.7 a	1.77 ± 0.06 a	0.15 ± 0.01 a	−0.005 ± 0.001 a
TALC	0.22 ± 0.01 a	5.8 ± 0.5 a	1.75 ± 0.06 a	0.14 ± 0.01 a	−0.004 ± 0.001 a
ENZ + TALC	0.22 ± 0.01 a	6.9 ± 0.8 a	1.78 ± 0.02 a	0.15 ± 0.00 a	−0.005 ± 0.001 a

[a] Data are expressed as the mean value of three different trials ± standard deviation. Different letters in each rows denotes significant statistical differences according to Tukey test ($p < 0.05$).

Table 4
Phenolic composition of olive oils extracted with the addition of different technological coadjuvants. Data expressed as mg kg^{-1}.

	CONTROL	ENZYMES	TALC	ENZ + TALC
3,4-DHPEA (hydroxytyrosol)[a]	12.0 ± 3.3 a	10.4 ± 4.0 a	6.8 ± 2.5 a	7.8 ± 2.3 a
p-HPEA (tyrosol)	10.4 ± 4.0 a	8.7 ± 4.5 a	5.6 ± 2.5 a	6.1 ± 1.9 a
Vanillic acid	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a
3,4-DHPEA-EDA (oleacein)	586.6 ± 25.2 b	679.7 ± 33.7 a	598.1 ± 22.5 b	670.8 ± 12.2 a
p-HPEA-EDA (oleocanthal)	154.4 ± 1.8 b	175.2 ± 6.2 a	150.9 ± 4.1 b	163.9 ± 5.5 a
(+)-1-Acetoxypinoresinol	19.6 ± 0.3 ab	21.3 ± 0.4 a	19.0 ± 0.5 b	20.5 ± 1.1 ab
(+)-Pinoresinol	12.2 ± 0.6 a	12.3 ± 0.2 a	11.0 ± 2.5 a	13.3 ± 0.6 a
3,4-DHPEA-EA (oleuropein aglycon)	165.1 ± 8.5 a	207.3 ± 10.4 b	182.7 ± 20.5 ab	191.6 ± 7.2 ab
p-HPEA-EA (ligstroside aglycone)	18.5 ± 1.4 a	23.1 ± 2.1 a	19.3 ± 2.4 a	21.9 ± 0.9 a
Total phenols	978.9 ± 28.5 b	1138.2 ± 37.8 a	993.6 ± 37.3 b	1096.1 ± 17.2 a
Sum of oleuropein derivatives	763.7 ± 26.8 b	897.4 ± 35.5 a	787.6 ± 30.6 b	870.2 ± 14.4 a
Sum of ligustrside derivatives	183.2 ± 4.6 a	207.0 ± 7.9 b	175.8 ± 5.4 a	191.9 ± 5.9 a
Sum of lignans	31.8 ± 0.7 a	33.6 ± 0.5 a	30.0 ± 2.6 a	33.8 ± 1.3 a

[a] Data are expressed as the mean value of three different trials ± standard deviation. Different letters in each rows denotes significant statistical differences according to Tukey test ($p < 0.05$). Oleuropein derivatives (sum of 3,4-DHPEA, 3,4-DHPEA-EDA, and 3,4-DHPEA-EA); ligstroside derivatives (sum of *p*-HPEA, *p*-HPEA-EDA and ligstroside aglycone); lignans (sum of (+)-1-acetoxypinoresinol and (+)-pinoresinol).

is allowed in the olive oil mechanical extraction process is as a technological aid, provided that the physical impact on the improvement of oil extractability does not directly determine any chemical and/or biochemical reaction. Squeo et al. (2020) showed that micronized natural talc (MNT) had a weak influence on the activity of polyphenol oxidase (PPO) and peroxidase (POD) but did not modify the phenolic content of olive oils, as shown by other oil coadjuvants. For example, calcium carbonate enhanced the activity of oxidase enzymes, reducing VOO quality (Tamborrino et al., 2017), and sodium chloride was able to increase the concentration of ortho-diphenols in oil (Koprivnjak et al., 2016). However, we need to note that the results regarding talc are not confirmed by other authors, who highlight a positive impact on the phenolic fraction of the final product with the addition of talc during the extraction process, even if the mechanism of why this happens is currently unclear (Caponio et al., 2015; Cert et al., 1996; Espínola et al., 2015; Sadkaoui, Jimenez, Aguilera, et al., 2017; Sánchez et al., 2022). The data obtained are, however, also influenced by other variables such as the time and temperature of malaxation and the dosage and type of MNT (Caponio et al., 2016; Sánchez et al., 2022; Vidal et al., 2020). The combined use of enzymatic formulation and talc showed the same effect on the phenolic compounds of olive oils, improving their concentration compared with both CONTROL and TALC trials. The increase in total phenols was 12.0% and 10.3%, respectively, whereas no differences were shown between the ENZYMES test and ENZ + TALC test (Table 4). The simultaneous addition of enzymatic complex and physical coadjuvant showed a positive effect on the oil mechanical extraction process at the industrial scale, confirming the preliminary data obtained at the laboratory scale by Peres et al. (2016), who highlighted a quantitative impact on the increase in oil yield and phenolic content due to the different cultivars processed. The increase of secoiridoid derivatives of oleuropein and ligstroside improved the bitter and pungent sensory notes of the olive oils. Even if the experimental plan was carried out with olive oils characterized by a high phenolic content, so further investigation should also be done with different cultivars characterized by low and/or medium phenolic concentrations to better understand the quantitative and qualitative impact of enzymatic complexes used alone or with talc coadjuvant during the extraction of oils from olive fruits belonging to different genetic origins at different maturity stages.

Even if the use of enzymatic formulation did not modify the concentration of volatile compounds from a statistical point of view, the data showed a slight increasing trend of aldehydes, alcohols and esters in the olive oils obtained by using the addition of enzymes in the malaxation phase (Table 5). These results could provide advice to examine the impact of the enzymatic coadjuvant with other cultivars and perhaps at different maturation indices with the aim of evaluating the reaction of the lipoxygenase (LOX) pathway, characterized by a different genetic origin and by a different level of activity, in the formation of volatile molecules responsible for olive oil flavour (Sanchez-Ortiz et al., 2012; Veneziani et al., 2018).

The use of talc, as shown for the phenolic concentration, confirmed the absence of effects on the volatile fraction of olive oils (Table 5) and its role as a technological aid that is chemically and biochemically inert (Moya et al., 2010; Squeo et al., 2020) and that is able to exert physical action on malaxed olive paste, reducing the oil-in-water emulsion and increasing the industrial yield without altering the olive oil quality characteristics (Sadkaoui et al., 2016). The data were also confirmed by Squeo et al. (2020), who showed that even though there was minor LOX activity when micronized natural talc was used, no effect of the coadjuvant on the concentration of aldehydes was found in VOOs. The data on the aldehyde concentration were also confirmed by Koprivnjak et al. (2016) in oils extracted from Buža olive fruits that, however, showed an increase in alcohol content when talc was added at a higher concentration of 3%. As explained for the studies of the impact on the phenolic fraction, the correlation between the degree of talc addition and the levels of volatile compounds is unclear, and the effect cannot be due to a direct action of talc on the residual activity of LOX during the malaxation phase (Caponio et al., 2015; Koprivnjak et al. 2016; Vidal et al., 2020). The ENZ + TALC test showed the same trend as the other trials, and no significant variations were observed relative to the concentration of volatile compounds (Table 5).

Table 5
Volatile compounds of olive oils extracted with different coadiuvants. Data expressed as μg kg^{-1}.

	CONTROL	ENZYMES	TALC	ENZ + TALC
Aldehydes				
Pentanal[a]	42 ± 6 a	38 ± 3.5 a	39 ± 3 a	45 ± 4 a
(*E*)-2-Pentenal	12 ± 1 a	11 ± 2 a	13 ± 2 a	10 ± 1 a
Hexanal	529 ± 45 a	564 ± 42 a	521 ± 48 a	527 ± 43 a
(*E*)-2-Hexenal	8032 ± 632 a	9304 ± 546 a	8039 ± 835 a	8529 ± 732 a
(*E,E*)-2,4-Hexadienal	48 ± 4 a	50 ± 4 a	43 ± 5 a	42 ± 5 a
Summ of the aldehydes at C_5 and at C_6	8662 ± 633 a	9967 ± 548 a	8655 ± 837 a	9153 ± 734 a
Alcohols				
1-Pentanol	51 ± 4 a	53 ± 3 a	49 ± 3 a	37 ± 5 b
1-Penten-3-ol	197 ± 8 a	194 ± 18 a	200 ± 13 a	179 ± 18 a
(*E*)-2-Penten-1-ol	29 ± 2 a	30 ± 0.3 a	30 ± 3 a	28 ± 2 a
(*Z*)-2-Penten-1-ol	160 ± 15 a	168 ± 7 a	153 ± 2 a	147 ± 5 a
1-Hexanol	2778 ± 239 a	2804 ± 231 a	2765 ± 201 a	2722 ± 260 a
(*E*)-2-Hexen-1-ol	5440 ± 547 a	5562 ± 372 a	5422 ± 414 a	5436 ± 472 a
(*Z*)-3-Hexen-1-ol	717 ± 70 a	715 ± 28 a	716 ± 39 a	711 ± 49 a
Sum of alcohols at C_5 and at C_6	9371 ± 601 a	9526 ± 439 a	9334 ± 462 a	9259 ± 542 a
Esters				
Hexyl acetate	54 ± 5 a	60 ± 4 a	46 ± 8 a	48 ± 3 a
(*Z*)-3-Hexenyl acetate	116 ± 5 a	134 ± 10 a	114 ± 9 a	121 ± 9 a
Sum of esters at C_6	171 ± 7 ab	194 ± 11 a	160 ± 12 b	168 ± 10 ab
Ketones				
3-Pentanone	34 ± 3 a	37 ± 3 a	34 ± 2 a	33 ± 3 a
1-Penten-3-one	124 ± 10 a	131 ± 12 a	135 ± 7 a	117 ± 5 a
6-Methyl-5-hepten-2-one	14±	15 ± 1 a	15 ± 0.4 a	14 ± 1 a
Sum of ketones at C_5 and at C_8	173 ± 10 a	183 ± 13 a	184 ± 8 a	164 ± 6 a

[a] Data are expressed as the mean of three different trials ± standard deviation. Different letters in each rows denotes significant statistical differences according to Tukey test ($p < 0.05$).

4. Conclusion

The evaluation of olive oil coadjuvants added during the beginning of the malaxation phase of an industrial olive oil extraction process showed a significant effect on increasing both the oil yield and the hydrophilic phenol concentration of the final product. The combined use of an enzymatic complex, based on the activity of pectinase, pectinmethylesterase and polygalacturonase, and talc obtained the best performance in oil extractability, maintaining a higher content of secoiridoid derivatives characterized by high antioxidant activity compared to the other trials. This effect was due to the simultaneous action of hydrolytic processes that improved the degradation of the olive cell wall structure, promoting the solubilization of phenols into the oily phase, and of the breakdown of oil-in-water emulsions induced by the physical coadjuvant that resulted in a better separation of olive oils during the mechanical extraction process. Further investigation should be done on the impact of the addition of combined technological formulation on different cultivars at different maturation stages to evaluate the quantitative and qualitative variation in oil extractability and on the minor compounds responsible for the main health and sensory properties of high-quality olive oil.

Funding

This work has been funded by the European Union - NextGenerationEU under the Italian Ministry of University and Research (MUR) National Innovation Ecosystem grant ECS00000041 - VITALITY. and by the European Union - Next Generation EU – funding from Ministry for University and Research Decree 737/2021 –research project "ECOnomia circolare, Sostenibilità e profili di evoluzione Normativa nella produzione dell'Olio extravergine Di Oliva - (ECOSNODO)", Unique Project Code H91I21001700006.

Author contributions

The authors declare that they have contributed to the same extent to the present study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

Authors grateful acknowledge to Evo Campania s.c.a.r.l. mill Campagna – SA (Italy) that kindly provided olives and industrial extraction plant necessary to accomplish the present study. Authors also thank

Domenico Tarantino and Leonardo Feola for technical support and University of Perugia, MUR and University of Bari Aldo Moro for support with the projectsVitality and Ecosnodo.

References

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2001). Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chemistry, 72*, 19–28. https://doi.org/10.1016/S0308-8146(00)00194-1

Antonini, E., Farina, A., Leone, A., Mazzara, E., Urbani, S., Selvaggini, R., Servili, M., & Ninfali, P. (2015). Phenolic compounds and quality parameters of family farming versus protected designation of origin (PDO) extra-virgin olive oils. *Journal of Food Composition and Analysis, 43*, 75–81. https://doi.org/10.1016/j.jfca.2015.04.015

Caponio, F., Squeo, G., Difonzo, G., Pasqualone, A., Summo, C., & Paradiso, V. M. (2016). Has the use of talc an effect on yield and extra virgin olive oil quality? *Journal of the Science of Food and Agriculture, 96*, 3292–3299. https://doi.org/10.1002/jsfa.7658

Caponio, F., Squeo, G., Monteleone, J. I., Paradiso, V. M., Pasqualone, A., & Summo, C. (2015). First and second centrifugation of olive paste: Influence of talc addition on yield, chemical composition and volatile compounds of the oils. *LWT–Food Science and Technology, 64*, 439–445. https://doi.org/10.1016/j.lwt.2015.05.007

Carrapiso, A. I., García, A., Petrón, M. J., & Martín, L. (2013). Effect of talc and water addition on olive oil quality and antioxidants. *European Journal of Lipid Science and Technology, 115*, 583–588. https://doi.org/10.1002/ejlt.201200252

Cert, A., Alba, J., León-Camacho, M., Moreda, W., & Pérez-Camino, M. C. (1996). Effects of talc addition and operating mode on the quality and oxidative stability of virgin oils obtained by centrifugation. *Journal of Agricultural and Food Chemistry, 44*, 3930–3934. https://doi.org/10.1021/jf9603386

De Faveri, D., Aliakbarian, B., Avogadro, M., Perego, P., & Converti, A. (2008). Improvement of olive oil phenolics content by means of enzyme formulations: Effect of different enzyme activities and levels. *Biochemical Engineering Journal, 41*, 149–156. https://doi.org/10.1016/j.bej.2008.04.007

Di Giovacchino, L., Simona Sestili, S., & Di Vincenzo, D. (2002). Influence of olive processing on virgin olive oil quality. *European Journal of Lipid Science and Technology, 104*, 587–601.

Espínola, F., Moya, M., de Torres, A., & Castro, E. (2015). Comparative study of coadjuvants for extraction of olive oil. *European Food Research and Technology, 241*, 759–768. https://doi.org/10.1007/s00217-015-2501-3

Hadj-Taieb, N., Grati, N., Ayadi, M., Attia, I., Bensalem, H., & Gargouri, A. (2012). Optimisation of olive oil extraction and minor compounds content of Tunisian olive oil using enzymatic formulations during malaxation. *Biochemical Engineering Journal, 62*, 79–85. https://doi.org/10.1016/j.bej.2011.04.003

Kalogianni, E. P., Georgiou, D., & Hasanov, J. H. (2019). Olive oil processing: Current knowledge, literature gaps, and future perspectives. *Journal of the American Oil Chemists' Society, 96*, 481–507. https://doi.org/10.1002/aocs.12207

Kalua, C. M., Bedgood, D. R., Jr., 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 Agricultural and Food Chemistry, 54*(20), 7641–7651. https://doi.org/10.1021/jf061122z

Koprivnjak, O., Brkić Bubola, K., & Kosić, U. (2016). Sodium chloride compared to talc as processing aid has similar impact on volatile compounds but more favorable on ortho-diphenols in virgin olive oil. *European Journal of Lipid Science and Technology, 118*, 318–324. https://doi.org/10.1002/ejlt.201500014

Leone, A., Romaniello, R., Zagaria, R., & Tamborrino, A. (2015). Mathematical modelling of the performance parameters of a new decanter centrifuge generation. *Journal of Food Engineering, 166*, 10–20. https://doi.org/10.1016/j.jfoodeng.2015.05.011

Leone, A., Tamborrino, A., Esposto, S., Berardi, A., & Servili, M. (2022). Investigation on the effects of a pulsed electric field (PEF) continuous system implemented in an industrial olive oil plant. *Foods, 11*(18). https://doi.org/10.3390/foods11182758

Moya, M., Espínola, F., Fernández, D. G., de Torres, A., Marcos, J., Vilar, J., Josue, J., Sánchez, T., & Castro, E. (2010). Industrial trials on coadjuvants for olive oil extraction. *Journal of Food Engineering, 97*, 57–63. https://doi.org/10.1016/j.jfoodeng.2009.09.015

Nucciarelli, D., Esposto, S., Veneziani, G., Daidone, L., Urbani, S., Taticchi, A., Selvaggini, R., & Servili, M. (2022). The use of a cooling crusher to reduce the temperature of olive paste and improve EVOO quality of Coratina, Peranzana, and Moresca cultivars: Impact on phenolic and volatile compounds. *Food Bioprocess Technology, 15*, 1988–1996. https://doi.org/10.1007/s11947-022-02862-9

OJEC. (2001). Council of the European Union, Council Regulation (EC) No 1513/2001 of 23 July 2001 amending Regulations No 136/66/EEC and (EC) No 1638/98 as regards the extension of the period of validity of the aid scheme and the quality strategy for olive oil. *Official Journal of the European Communities, L201*, 4–7. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32001R1513&from=en.

OJEC. (2015). *Official journal of the European community commission delegated regulation (EU) 2015/1830 of 8 july 2015 amending regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis*. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32015R1830.

Peres, F., Martins, L. L., Mourato, M., Vitorino, C., Antunes, P., & Ferreira-Dias, S. (2016). Phenolic compounds of 'Galera Vulgar' and 'Cobrancosa' olive oils along early ripening stages. *Food Chemistry, 211*, 51–58. https://doi.org/10.1016/j.foodchem.2016.05.022

Pérez, M., López-Yerena, A., Lozano-Castellón, J., Olmo-Cunillera, A., Lamuela-Raventós, R. M., Martin-Belloso, O., & Vallverdú-Queralt, A. (2021). Impact of novel technologies on virgin olive oil processing, consumer acceptance, and the valorization of olive mill wastes. *Antioxidants, 10*, 417. https://doi.org/10.3390/antiox10030417

Polari, J. J., & Wang, S. C. (2020). Comparative Effect of hammer mill screen size and cell wall-degrading enzymes during olive oil extraction. *ACS Omega, 5*(11), 6074–6081. https://doi.org/10.1021/acsomega.0c00036

Sadkaoui, A., Jimenez, A., Aguilera, M. P., Pacheco, R., & Beltran, G. (2017). Virgin olive oil yield as affected by physicochemical talc properties and dosage. *European Journal of Lipid Science and Technology, 119*, Article 1600112. https://doi.org/10.1002/ejlt.201600112

Sadkaoui, A., Jiménez, A., Pacheco, & R., & Beltrán, G. (2016). Effect of micronized natural talc physicochemical characteristics and dose on the breakdown of oil-in-water emulsions. *European Journal of Lipid Science and Technology, 118*, 545–552. https://doi.org/10.1002/ejlt.201500112

Sadkaoui, A., Jimenez, A., Pacheco, R., & Beltran, G. (2017). Micronized natural talc affects the proteins and pectic cell wall polysaccharides during "Hojiblanca" virgin olive oil extraction. *European Journal of Lipid Science and Technology, 119*, Article 1600039. https://doi.org/10.1002/ejlt.201600039

Sanchez-Ortiz, A., Romero-Segura, C., Sanz, C., & Perez, A. G. (2012). Synthesis of volatile compounds of virgin olive oil is limited by the lipoxygenase activity load during the oil extraction process. *Journal of Agricultural and Food Chemistry, 60*, 812–822. https://doi.org/10.1021/jf204241e

Sánchez, S., Olivares, I., Puentes, J. G., Órpez, R., La Rubia, M. D., Pacheco, R., & García Martín, J. F. (2022). Use of natural microtalcs during the virgin olive oil production process to increase its content in antioxidant compounds. *Processes, 10*, 950. https://doi.org/10.3390/pr10050950

Selvaggini, R., Esposto, S., Taticchi, A., Urbani, S., Veneziani, G., Di Maio, I., Sordini, B., & Servili, M. (2014). Optimization of the temperature and oxygen concentration conditions in the malaxation during the oil mechanical extraction process of four Italian olive cultivars. *Journal of Agricultural and Food Chemistry, 62*, 3813–3822. https://doi.org/10.1021/jf405753c

Squeo, G., Difonzo, G., Summo, C., Crecchio, C., & Caponio, F. (2020). Study of the influence of technological coadjuvants on enzyme activities and phenolic and volatile compounds in virgin olive oil by a response surface methodology approach. *LWT - Food Science and Technology, 133*, Article 109887. https://doi.org/10.1016/j.lwt.2020.109887

Squeo, G., Tamborrino, A., Pasqualone, A., Leone, A., Paradiso, V. M., Summo, C., & Caponio, F. (2017). Assessment of the influence of the decanter set-up during continuous processing of olives at different pigmentation index. *Food and Bioprocess Technology, 10*(3), 592–602. https://doi.org/10.1007/s11947-016-1842-7

Tamborrino, A., Mescia, L., Taticchi, A., Berardi, A., Lamacchia, C. M., Leone, A., & Servili, M. (2022). Continuous pulsed electric field pilot plant for olive oil extraction process. *Innovative Food Science and Emerging Technologies, 82*, Article 103192. https://doi.org/10.1016/j.ifset.2022.103192

Tamborrino, A., Perone, C., Mojaed, H., Romaniello, R., Berardi, A., Catalano, P., & Leone, A. (2021). Combined continuous machine to condition olive paste: Rheological characterization of olive paste. *Chemical Engineering Transactions, 87*, 283–288. https://doi.org/10.3303/CET2187048

Tamborrino, A., Squeo, G., Leone, A., Paradiso, V. M., Romaniello, R., Summo, C., Pasqualone, A., Catalano, P., Bianchi, B., & Caponio, F. (2017). Industrial trials on coadjuvants in olive oil extraction process: Effect on rheological properties, energy consumption, oil yield and olive oil characteristics. *Journal of Food Engineering, 205*, 34–46. https://doi.org/10.1016/j.jfoodeng.2017.02.019

Taticchi, A., Esposto, S., Veneziani, G., Minnocci, A., Urbani, S., Selvaggini, R., Sordini, B., Daidone, L., Sebastiani, L., & Servili, M. (2021). High vacuum-assisted extraction affects virgin olive oil quality: Impact on phenolic and volatile compounds. *Food Chemistry, 342*, Article 128369. https://doi.org/10.1016/j.foodchem.2020.128369

Taticchi, A., Selvaggini, R., Esposto, S., Sordini, B., Veneziani, G., & Servili, M. (2019). Physicochemical characterization of virgin olive oil obtained using an ultrasound assisted extraction at an industrial scale: Influence of olive maturity index and malaxation time. *Food Chemistry, 289*, 7–15. https://doi.org/10.1016/j.foodchem.2019.03.04

Veneziani, G., Esposto, S., Taticchi, A., Urbani, S., Selvaggini, R., Sordini, B., & Servili, M. (2018). Characterization of phenolic and volatile composition of extra virgin olive oil extracted from six Italian cultivars using a cooling treatment of olive paste. *LWT - Food Science and Technology, 87*, 523–528. https://doi.org/10.1016/j.lwt.2017.09.034

Veneziani, G., García-González, D. L., Esposto, S., Nucciarelli, D., Taticchi, A., & Servili, M. (2023). Effect of controlled oxygen supply during crushing on volatile and phenol compounds and sensory characteristics in Coratina and Ogliarola virgin olive oils. *Foods, 12*, 612. https://doi.org/10.3390/foods12030612

Vidal, A. M., Alcalá, S., de Torres, A., Moya, M., & Espínola, F. (2020). Use of talc in oil mills: Influence on the quality and content of minor compounds in olive oils. *LWT - Food Science and Technology, 98*, 31–38. https://doi.org/10.1016/j.lwt.2018.08.001

Vierhuis, E., Servili, M., Baldioli, M., Schols, H. A., Voragen, A. G. J., & Montedoro, G. F. (2001). Effect of enzyme treatment during mechanical extraction of olive oil on phenolic compounds and polysaccharides. *Journal of Agricultural and Food Chemistry, 49*, 1218–1223. https://doi.org/10.1021/JF000578S