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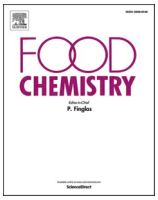

High hydrostatic pressure enhances the formation of oleocanthal and oleacein in 'Arbequina' olive fruit

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ABSTRACT

During olive oil production, the activity of endogenous enzymes plays a crucial role in determining the oil's phenolic composition. β-Glucosidase contributes to the formation of secoiridoids, while polyphenol oxidase (PPO) and peroxidase (POX) are involved in their oxidation. This study investigated whether high hydrostatic pressure (HHP), known to cause cell disruption and modify enzymatic activity and food texture, could reduce PPO and POX activity. HHP was applied to 'Arbequina' olives at different settings (300 and 600 MPa, 3 and 6 min) before olive oil extraction. The tested HHP conditions were not effective in reducing the activity of PPO and POX in olives, resulting in oils with a lower phenolic content. However, HHP increased the secoiridoid content of olives, particularly oleocanthal and oleacein (>50%). The pigments in oils produced from HHP-treated olives were higher compared to the control, whereas squalene and α-tocopherol levels and the fatty acid profile remained the same.

1. Introduction

Extra virgin olive oil (EVOO) is highly appreciated for its organoleptic attributes and beneficial health properties, primarily attributed to bioactive components of the minor unsaponifiable fraction, especially phenolic compounds (Rodríguez-López et al., 2021). Consequently, there is a strong interest in producing EVOOs with high phenolic content, leading to substantial research focused on factors that can enhance it, including the agronomic conditions and oil production parameters (Temime & Manaî, 2017). By modulating these factors, it is possible to modify the activity of olive's endogenous enzymes, which play a critical role in both the anabolism and catabolism of phenolic compounds (Peres et al., 2017). While some secoiridoids are enzymatically formed during the oil production process, their oxidation and degradation also occur concurrently due to the presence of oxidative enzymes (García-Rodríguez et al., 2011; Velázquez-Palmero et al., 2017). In particular, β-glucosidase, esterases, polyphenol oxidase (PPO), and peroxidase (POX) are the primary endogenous enzymes responsible for shaping the phenolic composition of EVOO. β-Glucosidase hydrolyses the glucose moiety of oleuropein and ligstroside to form their aglycones (Velázquez-Palmero et al., 2017), and esterases catalyze the generation of oleacein and oleocanthal from these two aglycones, respectively (Volk et al., 2019), thereby increasing their content. In contrast, PPO and POX oxidize phenolic compounds, especially those derived from hydroxytyrosol (oleuropein derivatives) (García-Rodríguez et al., 2011), during the oil extraction process as they are exposed to oxygen. As a result, the content of phenolic compounds is decreased.

High hydrostatic pressure (HHP) is a non-thermal technology that has been investigated mainly as a way of preserving food from spoilage, as it can inactivate microorganisms and minimize chemical reactions, thus maintaining or even improving food quality attributes (Aganovic et al., 2021). Depending on the pressure levels, the specific enzyme, and

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the food matrix, HHP can lead to either protein unfolding and denaturation, which may result in enzyme inactivation (Aganovic et al., 2021; Roobab, Abida, et al., 2022), or it can induce changes in protein conformation and active sites that facilitate interactions with substrates, thereby boosting enzyme activity (Eisenmenger & Reyes-De-Corcuera, 2009; Roobab, Abida, et al., 2022). Furthermore, HHP can modify the textural properties of foods. For instance, it improves the separation of meat from the shells of shellfish by altering the quaternary and tertiary protein structures (Roobab, Fidalgo, et al., 2022), while it disrupts cellular integrity, enhances cell membrane permeability, and allows the free movement of water and metabolites through plant tissue (Gokul Nath et al., 2023), softening the tissues of plant-based products and destroying their intracellular structures (Aganovic et al., 2021). Consequently, the compartmentalized enzymes and substrates within intact cells are released, triggering their reaction.

HHP has been applied to various fruits and vegetables, including apples, pears, oranges, pineapples, strawberries, celery, green peppers, red peppers, and asparagus, as well as fruit juices, showing some positive results (Eisenmenger & Reyes-De-Corcuera, 2009; Gokul Nath et al., 2023; Roobab, Abida, et al., 2022; Zawawi et al., 2022). Nevertheless, there is limited literature on the use of HHP in olives. Previous research has explored the use of HHP for preserving table olives during storage (Martín-Vertedor et al., 2022; Tokuşoğlu et al., 2010). However, to the best of our knowledge, only one previous study applied HHP to olive fruit before oil production, with the aim of determining its effect on oil yield and shelf-life (Andreou et al., 2017).

The current study aimed to address two key objectives. First, it explored whether the textural changes induced by HHP in olive fruit could facilitate the separation of the mesocarp from the stone. Some studies have indicated that a significant amount of POX activity is associated with the olive seed (García-Rodríguez et al., 2011; Luaces et al., 2007), and hence, the production of olive oil using destoned olives enhances the phenolic content of EVOO (Amirante et al., 2010; Frangipane et al., 2022). And second, it examined whether HHP could inactivate or reduce the activity of the oxidative enzymes PPO and POX, or enhance the activity of β-glucosidase in olive fruit, with the goal of determining whether HHP treatment could lead to the production of an EVOO with higher content of secoiridoids. Since the HHP pressure used with food usually ranges from 200 to 600 MPa and is applied at ambient temperature for a duration rarely longer than 5 min (Aganovic et al., 2021), in the present work, 'Arbequina' olives were treated with mild (300 MPa) or high (600 MPa) pressure for a short (3 min) or long (6 min) time. Additionally, this is the first study to employ industrial HHP equipment to investigate its impact on the activity of olive's endogenous enzymes and its potential to enhance the phenolic content of olive fruit and oil.

2. Materials and methods

2.1. Reagents

n-Hexane, 0.5 N sodium methoxide, 14% boron trifluoride–methanol, sodium phosphate monobasic hydrate, sodium phosphate dibasic, catechol, *p*-phenylenediamine, hydrogen peroxide, citric acid, trisodium citrate dihydrate, *p*-nitrophenyl-β-D-glucoside (pNPG), polyvinylpyrrolidone (PVP), sodium tetraborate decahydrate and Triton X-100 were purchased from Sigma-Aldrich (St. Louis, MO, USA); cyclohexane and 0.1 N sodium thiosulfate ($Na_2S_2O_3$) was purchased from Carlo Erba Reagents (Val-de-Reuil, France); potassium iodide (KI) from Honeywell Fluka (Buchs, Switzerland); ethanol 96% from VWR Chemicals (Fontenay-sous-Bois, France); acetic acid, formic acid, chloroform, methanol, acetonitrile (ACN), *N,N*-dimethylformamide (DMF), and tertbutylmethyleter (TBME) from Sigma-Aldrich (Madrid, Spain); and sodium chloride (NaCl), sodium carbonate (Na_2CO_3), sodium hydroxide pellets (NaOH), starch 1% and phenolphthalein from Panreac Química SLU (Castellar del Vallès, Spain). Ultrapure water was obtained using a Milli-Q purification system (Millipore, Bedford, MA, USA).

Regarding the standards (≥90% purity), oleocanthal was purchased from Merck (Darmstadt, Germany), and oleacein, oleuropein aglycone, and elenolic acid from Toronto Research Chemical Inc. (ON, Canada). Oleuropein, ligstroside, pinoresinol, squalene, and (α)-tocopherol were acquired from Sigma-Aldrich; apigenin and *p*-coumaric from Fluka, and hydroxytyrosol from Extrasynthese (Genay, France). Methyl tridecanoate (C13:0), used as a standard for the analysis of fatty acids (FAs), was acquired from Sigma-Aldrich.

2.2. Olive samples

The olive cultivar used for this study was 'Arbequina'. Olives were collected on October 26, 2021, at the Institute of Agrifood Research and Technology (IRTA) Mas Bové in Constantí (Tarragona, Spain), which is located at latitude 41.172 N and longitude 1.169 E at 100 m altitude and 15 km from the Mediterranean coast, and kindly provided to our research group by the same Institute. The ripening index was 1.4 ± 0.09. The olives were transported to the IRTA Monells (Girona, Spain) where the HHP treatment and olive oil production were carried out the following day. Olives were stored at 4 °C until the HHP treatment.

2.3. HHP treatments

Olives were divided into five biological samples, including one control and four treatment groups. The four HHP treatments were conducted using two different pressures (300 and 600 MPa) and two durations (3 and 6 min) (Fig. 1). Each HHP treatment was performed three times, resulting in three independent olive replicates for each HHP treatment. The HHP equipment used was a 120-Liter Wave 6000 industrial apparatus (Hiperbaric, Burgos, Spain). The average pressure come-up rate was 150 MPa/min, while the release was immediate. The olives treated with HHP were vacuum packed inside a plastic bag. After each treatment, some olives were stored directly at − 80 °C for further analysis, while the rest were used for oil production (Fig. 1).

2.4. Olive oil production

Olive oil was produced with an Abencor system (Abengoa S.A., Seville, Spain) (Fig. 1) following the methodology described in a previous study (Olmo-Cunillera et al., 2021); the malaxation conditions found to be optimal for oil bioactive content (20 °C and 30 min), especially for tocopherols, squalene, and total phenolic compounds, were applied. Olive oil production from HHP-treated olives was conducted once for each of the three replicates, resulting in three olive oil replicates for each HHP treatment. To ensure and equivalent number of control olive oil replicates, the oil extraction from untreated olives was performed three times. Therefore, both the control and the HHP treatments had three olive oil replicates. The olive oil samples were stored at − 20 °C until the chemical analyses.

2.5. Appearance of the olive samples and oil content

A visual inspection of the olives was performed to determine any changes in physical appearance after applying HHP. Random samples from each treatment group and the control were cut with a scalpel to photograph the equatorial section in a standardized photo light box with a Canon EOS 50D camera and a 120 mm lens (Canon, Tokyo, Japan). The percentage of olives with mesocarp detachment was determined by examining the images of 10 olives for each treatment and their three replicates (n = 30).

The oil content of the olives was determined by nuclear magnetic resonance (NMR), using a Maran Ultra 23 MHz NMR Analyzer (Oxford Instruments, Abingdon, UK), after desiccating the olive fruit in an oven at 105 °C for 42 h.

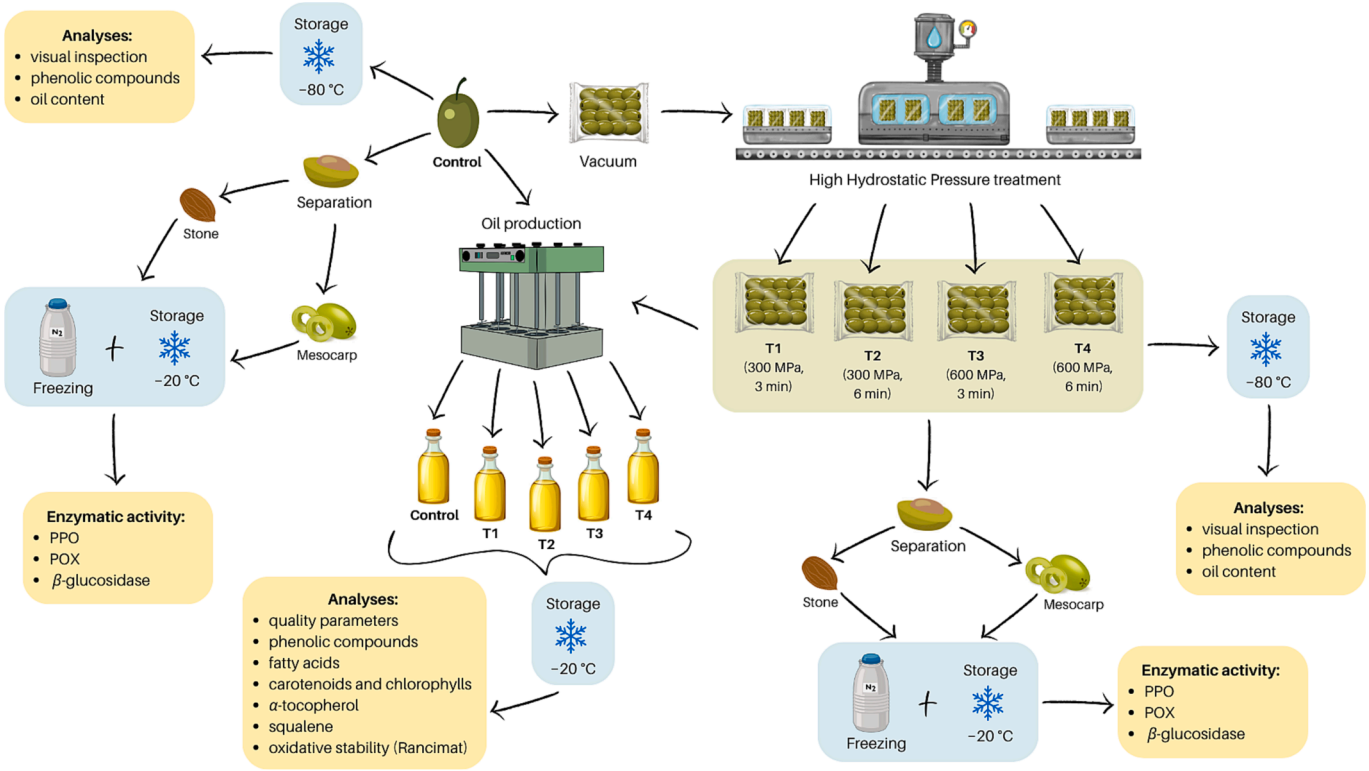

Fig. 1. Diagram of the procedure. Each HHP treatment was performed in triplicate. PPO: polyphenol oxidase; POX: peroxidase.

2.6. Determination of olive oil quality parameters

K_{232}, K_{270}, and ΔK, were determined by UV spectrophotometric examination as described in International Olive Council (2019). Briefly, 0.05 g and 0.10 g of olive oil were dissolved in cyclohexane up to a final volume of 10 mL to measure K_{232} and K_{270}, respectively. Rectangular quartz cuvettes with an optical path-length of 10 mm were used. ΔK was calculated applying the equation given in International Olive Council (2019).

The peroxide value and acidity were determined by titration as described in Firestone & Yurawecz (2023) and following the modifications of Olmo-Cunillera et al. (2021). For the peroxide value, a total of 30 mL of a solution of acetic acid and chloroform (3:2) and 0.5 mL of saturated KI were added to 5 g of olive oil. After mixing, 30 mL of water was added. The titration was performed with 0.1 M $Na_2S_2O_3$ until the olive oil solution turned yellow. Immediately, 0.5 mL of starch 1% was added, and the solution was titrated until the blue/purple color vanished. For the acidity, a total of 45 mL of ethanol was added to 7.05 g of olive oil, followed by 50 μL of phenolphthalein. This solution was titrated with 0.025 M NaOH until the color changed slightly to light pink.

2.7. Extraction and quantification of olive fruit and olive oil phenolic compounds

The extraction and quantification of the phenolic compounds in the olive oil and olive fruit were performed using the methodology described in Olmo-Cunillera et al. (2021, 2023), respectively, using liquid–liquid extraction and liquid chromatography coupled to mass spectrometry in tandem mode (LC-MS/MS). An Acquity TM UPLC (Waters; Milford, MA, USA) coupled to an API 3000 triple-quadrupole mass spectrometer (PE Sciex, Concord, ON, Canada) with a turbo ion spray source was used, employing an Acquity UPLC® BEH C18 column (2.1 × 50 mm, i.d., 1.7 μm particle size) and Acquity UPLC® BEH C18 Pre-Column (2.1 × 5 mm, i.d., 1.7 μm particle size) (Waters Corporation®, Wexford, Ireland). The chromatographic and mass spectrometric parameters are detailed elsewhere (Olmo-Cunillera et al., 2021, 2023).

The quantification was done with an external calibration curve using the following standards in a refined olive oil with no phenolic content: apigenin, hydroxytyrosol, *p*-coumaric acid, pinoresinol, oleuropein, ligstroside, oleocanthal, oleacein, oleuropein aglycone, and elenolic acid. Compounds without a corresponding commercial standard were quantified using standards of phenolic compounds with a similar chemical structure. Results from the olive fruits are expressed on a fresh weight basis.

2.8. Enzyme extraction and activity assay of polyphenol oxidase (PPO), peroxidase (POX) and β-glucosidase of the olive fruit

Immediately after the HHP treatments, the olive stones and mesocarps were separated, quickly frozen with liquid nitrogen, and stored at − 20 °C until they were submitted to cryogenic grinding with liquid nitrogen to obtain a fine frozen powder (6870 freezer/mill, SPEX, Metuchen, NJ, USA). In the case of mesocarp, the frozen powders were further processed into acetone powders. Briefly, 10 g of mesocarp powder was blended with 150 mL cold acetone (− 20 °C) using an Ultra-Turrax homogenizer (IKA, Staufen im Breisgau, Germany) and filtered. The residue was reextracted twice with acetone, finally washed with diethyl ether, dried, and stored at − 80 °C until enzyme extraction.

PPO and POX were extracted as described by Marszałek et al. (2015) with modifications. The extraction buffer consisted of 0.2 M phosphate buffer (pH 6.5) containing 4% (w/v) PVP, 1% (w/v) Triton X-100, and 1 M NaCl. Samples were mixed with the extraction buffer for 1 h at 4 °C, centrifuged at 13,000 g for 30 min at 4 °C, and the supernatants were kept as enzyme extracts. For the PPO activity assay, an aliquot of enzyme extract was incubated in 0.05 M catechol – 0.05 M phosphate buffer (pH 6.5) at 25 °C in a microplate spectrophotometer (Varioskan Flash, Thermo Fisher Scientific, Waltham, MA, USA). The formation of *o*-quinone from catechol was monitored at 390 nm for 15 min and used

to determine PPO activity. One unit of PPO activity was expressed as the amount of enzyme that catalyzed the formation of 1 μmol *O*-quinone/min at pH 6.5 and 25 °C. For the POX activity assay, an aliquot of enzyme extract was incubated in 0.07% (w/v) *p*-phenylenediamine – phosphate buffer (pH 6.5) and 0.05 % hydrogen peroxide at 25 °C in a microplate spectrophotometer. Formation of Bandrowski's base from *p*-phenylenediamine and oxygen peroxide (Zhang et al., 2017) was monitored at 500 nm for 15 min and used to determine POX activity. One unit of POX activity was expressed as the amount of enzyme that catalyzed the formation of 1 μmol Bandrowski's base/min at pH 6.5 and 25 °C.

β-glucosidase activity was determined as in Ribas-Agustí et al. (2017). Samples were mixed with 0.1 M sodium borate (pH 9.0) – 1.2 M NaCl – 1% (v/v) Triton X-100 – 1% (w/v) PVP for 2 h at 4 °C, centrifuged at 13,000 g for 30 min at 4 °C, and the supernatants were kept as enzyme extracts. An aliquot of the enzyme extract was incubated in 1 mM pNPG in 50 mM citrate buffer (pH 4.4) at 37 °C in a microplate spectrophotometer. Formation of *p*-nitrophenol from pNPG was monitored at 400 nm for 1 h and used to determine *β*-glucosidase activity. One unit of *β*-glucosidase activity was expressed as the amount of enzyme that catalyzed the formation of 1 μmol *p*-nitrophenol/min at pH 4.4 and 37 °C.

2.9. Determination of the olive oil fatty acid profile

Fatty acids (FAs) were extracted using the method for FA methyl esters (FAME) described in Olmo-Cunillera et al. (2022). Fast gas chromatography (GC) analyses were performed on a Shimadzu GC-2010 Gas Chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a Shimadzu AOC-20i Autoinjector. Separation of FAME was carried out on a capillary column (40 cm × 0.18 mm i.d. × 0.1 μm film thickness) coated with an RTX-2330 stationary phase of 10% cyanopropyl phenyl – 90% biscyanopropyl polysiloxane from Restek (Bellefonte, USA). The operating conditions of the GC and FA quantification are detailed elsewhere (Olmo-Cunillera et al., 2022).

2.10. Determination of olive oil carotenoids, chlorophylls, α-tocopherol, and squalene

Carotenoids and chlorophylls were determined by spectrophotometry, following the methodology described in Olmo-Cunillera et al. (2021). Absorbance was measured at 450 and 670 nm for carotenoids and chlorophylls, respectively, using an UV-3600, UV–VIS-NIR spectrophotometer (Shimadzu Corporation, Japan). The concentration was calculated applying the following equation:

$$\text{Concentration (mg/kg)} = (A \times DF) / (E \times M_S) \times 10000 \quad (1)$$

where A is the absorbance at 450 nm for the carotenoids and 670 nm for the chlorophylls, the dilution factor (DF) is 5, the extinction coefficient (E) is 613 for pheophytin (as a major component in the chlorophyll fraction) and 2000 for lutein (as a major component in the carotenoid fraction), and M_S is the mass of the sample in grams.

To determine *α*-tocopherol and squalene, a dilution of 200 μL of oil in 800 μL of TBME was analyzed by liquid chromatography with an Acquity UPLC coupled to a photodiode array detector (PDA) (Waters Corporation®, Milford, MA, USA). The column was a YMCTM C30 (250 × 4.6 mm, i.d., 5 μm particle size) (Waters Corporation®, Milford, MA, USA). The chromatographic parameters are detailed elsewhere (Olmo-Cunillera et al., 2023). For the quantification of each compound, a calibration curve of the corresponding commercial standard was employed.

2.11. Determination of the olive oil oxidative stability by Rancimat

The oxidative stability of the olive oil was evaluated with the Rancimat method (Gutiérrez-Rosales, 1989), which measures the oxidative stability of fats in accelerated conditions and is based on the induction of sample oxidation by exposure to high temperatures and air flow. Therefore, the longer the induction time, the more stable the sample. A 3 g oil sample was heated at 120 °C with a constant air flow of 20 L/h. The results were expressed as the induction time of oxidation (in hours) measured with the Rancimat 743 apparatus (Metrohm Co., Basilea, Suiza). The induction time of oxidation is the time required to cause a sudden change in the conductivity of an aqueous solution where the volatile compounds formed by the oil oxidation are collected.

2.12. Statistical analysis and multivariate analysis

All the analyses of the three olive oil replicates of the control and HHP treatments were done in triplicate, resulting in nine analyzed samples (n = 9). Statgraphics Centurion 18 software, version 18.1.13, and RStudio, version 2022.12.0 Build 353 (R Project for Statistical Computing version 4.2.2), were used to perform the analysis of variance. First, the normality of data and the homogeneity of variance were tested by the Saphiro-Wilk test and Levene's test, respectively. An analysis of variance of two factors (two-way ANOVA) with a Tukey test was applied to evaluate the effect of the HHP treatments on the oil samples (Control, T1, T2, T3 and T4), when the assumptions of normality and homogeneity of variance were met ($p \geq 0.05$). If any of these assumptions were not met ($p < 0.05$), a nonparametric statistical test was applied (Kruskal-Wallis with a pairwise Mann–Whitney U as a post-hoc test).

A multivariate analysis of the oil samples was carried out using the software SIMCA 13.0.3.0 (Umetrics, Umeå, Sweden). The following data were included: content of phenolic compounds, carotenoids, chlorophylls, *α*-tocopherol, and squalene, quality parameters (K_{232}, K_{270}, ΔK, peroxide value and acidity), enzymatic activity, and Rancimat values. An unsupervised approach, specifically a principal component analysis (PCA), was performed. The data were standardized with UV-scaling and mean-centering. The model had four PC with an explained variation (R^2X) of 0.821 and a predicted variation (Q^2X) of 0.646. Hotelling's T2 and DModX were used to identify strong and moderate outliers.

3. Results and discussion

3.1. Olive fruit appearance

The visual inspection showed that HHP-treated olives generally had a more unstructured mesocarp, more vesicles within the tissue, and greater detachment from the stone (Fig. 2).

The cut surface of treated olives was shinier with free oil (Fig. 2B). The application of HHP caused mesocarp softening and the release of water, as reported in the literature (Aganovic et al., 2021). The overall condition of the treated olives facilitated the separation of the mesocarp from the stone, which could suppose an advantage for the destoning process.

3.2. Olive oil quality parameters

The Commission Delegated Regulation (EU) 2022/2104 (European Commission, 2022) classifies olive oil in different categories according to the values of quality parameters (K_{232}, K_{270}, ΔK, peroxide value and acidity). To be categorized as EVOO, the values must be as follows: $K_{232} \leq 2.50$, $K_{270} \leq 0.22$, $\Delta K \leq 0.01$, acidity ≤ 0.8 g oleic acid/100 g, and peroxide value ≤ 20 mEq O_2/kg. According to our results (Table 1), all the oils obtained in the study met the EVOO criteria, although statistical differences were found for some treatments.

The peroxide value and K_{232} provide information about the primary oxidation of lipids, especially polyunsaturated FA (PUFA), the former measuring the conjugated hydroperoxides formed, and the latter, the diene conjugated products. The peroxide value increased when HHP was applied, reaching the highest value at 600 MPa, with similar values

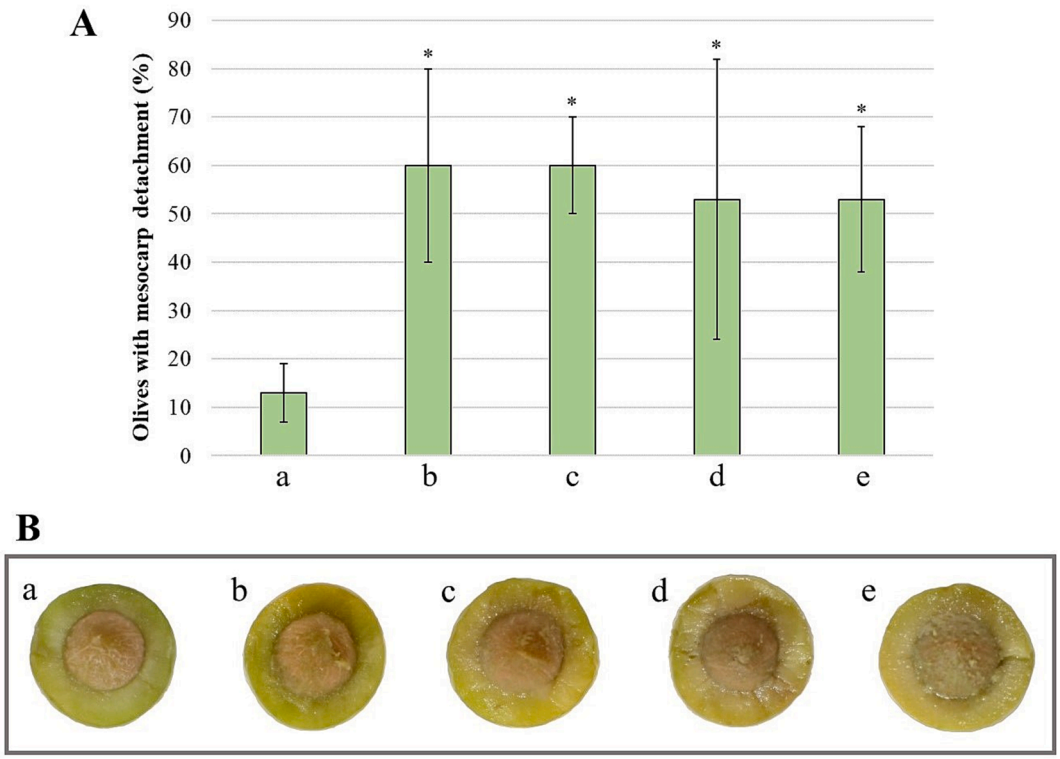

Fig. 2. Appearance of control and HHP-treated olives (equatorial sections) (**B**), showing the degree of mesocarp detachment from the stone (%) (**A**). The percentage of detachment (**A**) is, from left to right: 13 ± 6 % in control conditions (a), 60 ± 20 % at 300 MPa for 3 min (b), 60 ± 10 % at 600 MPa for 3 min (c), 53 ± 29 % at 300 MPa for 6 min (d), and 53 ± 15 % at 600 MPa for 6 min (e). Control was significantly different ($p < 0.05$) from HHP-treated samples. No significant differences were found between HHP treatments. Results are expressed as mean ± standard deviation, n = 30; asterisks denote statistical significance (*).

achieved by the application of 300 MPa for 6 min. At 300 MPa, the parameter K_{232} did not change compared to the control, whereas at 600 MPa it was lower, also decreasing significantly when HHP treatment was extended from 3 to 6 min. These results indicate that the application of HHP at 600 MPa may enhance the primary oxidation of lipids, favoring the generation of conjugated hydroperoxides but not diene conjugated products, which decreased. An increase in the peroxide value with increasing pressure levels has also been observed in meat and fish (Medina-Meza et al., 2014). It was suggested that the initiation of lipid oxidation was primarily attributed to membrane damage caused by HPP, which liberates radicals or precursors of radicals while also increasing the exposure of lipids. K_{270}, which measures the triene conjugated systems formed by the secondary oxidation of lipids, was practically the same for all treatments, but significantly lower at 600 MPa, indicating that secondary oxidation was reduced at this pressure. This aligns with K_{232} values, as oxidation progresses into secondary stages where more of the double bonds have been conjugated (Schaich, 2020), corresponding to the control and 300 MPa samples.

The acidity, which is used to determine oil deterioration due to the hydrolysis of triacylglycerols, was barely altered by the treatments, but slightly higher after HHP treatment, suggesting that lipase could be resistant to pressure. Previous studies have shown that the pressure stability of lipase can vary, but it can remain stable at pressures up to 600 MPa (Eisenmenger & Reyes-De-Corcuera, 2009; Medina-Meza et al., 2014). Finally, ΔK, which correlates with the oxidation state of the oils, was only significantly higher for T4, indicating a higher oxidation of oils produced from olives treated at 600 MPa for 6 min.

Accordingly, the main quality parameter affected by HHP seems to be the peroxide value. To the best of our knowledge, this is only the second time that the quality parameters of olive oils produced with HHP-treated olives have been determined. In the previous study, Andreou et al. (2017) did not find any significant effect on these parameters.

Notably, the oils produced with HHP-treated olives did not have the characteristic aroma of EVOOs, unlike the control, indicating the HHP treatment negatively affected the enzymes involved in the synthesis of aromatic volatile compounds. In particular, lipoxygenase is more susceptible to pressure than other plant enzymes, with substantial inactivation reported at 500 MPa or above (Houška et al., 2022). In tomatoes, the decrease in LOX activity induced by HHP was associated with a reduction in key contributors to the 'fresh' tomato flavor (Tangwongchai et al., 2000).

3.3. Olive and olive oil phenolic compounds

The application of HHP affected the phenolic content of olive fruit and olive oil differently. While the concentration of the phenolic compounds in the olive oil was significantly reduced (Table 1), variable tendencies were observed in olive fruit (Table 2). The negative effect on the phenolic content of olive oil was highest at 600 MPa, especially when applied for 6 min (Table 1). Thus, compared to the control, in T4 olive oil, pinoresinol was reduced by 56.59%, *p*-coumaric acid 50.65%, apigenin 88.91%, luteolin 87.09%, oleuropein aglycone 60.37%, ligstroside aglycone 74.39%, oleacein 54.74%, and oleocanthal 62.04%. The fact that the oxidative products of secoiridoids such as elenolic acid, hydroxyelenolic acid, oleaceinic acid, and hydroxyoleuropein aglycone were also reduced supports the hypothesis that phenolic degradation is increased by the application of HHP.

To better understand these changes in the olive oil, the phenolic content of the olive fruit was also analyzed (Table 2). Overall, applying 300 MPa had an enhancing effect, especially on oleuropein aglycone, oleocanthal, oleacein, hydroxytyrosol, and hydroxytyrosol acetate, which increased by more than 50%. Pressure of 600 MPa was also favorable for oleuropein aglycone, oleocanthal, oleacein, and hydroxytyrosol acetate, which increased by 50% or more. However, extending the duration of HHP from 3 to 6 min reduced the majority of compounds.

Table 1
Olive oil quality parameters (K_{232}, K_{270}, ΔK, peroxide value (mEq O_2/kg) and acidity (g oleic acid/100 g)), phenolic compounds (mg/kg), oxidative stability assessed with the Rancimat method (h), and content of pigments, α-tocopherol, and squalene (mg/kg) of the control sample and the four HHP treatments (T1, T2, T3 and T4). Results are expressed as mean ± standard deviation, n = 9. Different letters mean significant differences ($p < 0.05$).

HHP conditions	Control	T1	T2	T3	T4
Pressure (MPa)	–	300	300	600	600
Duration (min)	–	3	6	3	6
Quality parameters					
K_{232}	1.35 ± 0.01^{c}	1.36 ± 0.02^{c}	1.37 ± 0.01^{c}	1.29 ± 0.03^{b}	1.24 ± 0.02 ^{a}
K_{270}	0.10 ± 0.01^{b}	0.08 ± 0.01 ^{a}	0.10 ± 0.00^{b}	0.07 ± 0.00 ^{a}	0.08 ± 0.01 ^{a}
ΔK	0.0006 ± 0.0005 ^{ab}	0.0006 ± 0.0005 ^{a}	0.0005 ± 0.0004 ^{a}	0.0008 ± 0.0006 ^{ab}	0.0014 ± 0.0004^{b}
Peroxide value (mEq O_2/kg)	2.77 ± 0.08 ^{a}	4.07 ± 0.10^{b}	4.20 ± 0.13 ^{bc}	4.30 ± 0.11^{c}	4.24 ± 0.15^{c}
Acidity (g oleic acid/100 g)	0.11 ± 0.00 ^{a}	0.12 ± 0.01 ^{ab}	0.12 ± 0.01 ^{ab}	0.11 ± 0.01 ^{a}	0.12 ± 0.01^{b}
Phenolic compounds (mg/kg)					
Sum of phenolics	262.70 ± 34.48^{c}	144.08 ± 19.08^{b}	122.97 ± 13.73 ^{ab}	113.19 ± 8.26 ^{ab}	95.17 ± 9.54 ^{a}
Secoiridoids					
Ligstroside aglycone	66.85 ± 16.48^{c}	33.48 ± 8.33^{b}	28.57 ± 2.98 ^{ab}	22.68 ± 2.72 ^{ab}	17.12 ± 2.11 ^{a}
Oleuropein aglycone	40.12 ± 9.57^{b}	22.50 ± 4.41 ^{a}	19.05 ± 2.08 ^{a}	18.29 ± 2.03 ^{a}	15.90 ± 1.73 ^{a}
Oleocanthal	34.48 ± 2.23 ^{d}	19.90 ± 2.53^{c}	17.46 ± 2.38 ^{bc}	14.35 ± 1.10 ^{ab}	13.09 ± 2.17 ^{a}
Oleacein	84.69 ± 4.22^{c}	47.98 ± 8.65^{b}	38.57 ± 7.16 ^{ab}	41.79 ± 4.04 ^{ab}	38.33 ± 5.37 ^{a}
Secoiridoid derivatives					
Oleaceinic acid	0.60 ± 0.05^{b}	0.51 ± 0.06 ^{a}	0.49 ± 0.04 ^{a}	0.48 ± 0.02 ^{a}	0.48 ± 0.02 ^{a}
Hydroxyoleuropein aglycone	0.55 ± 0.02^{c}	0.55 ± 0.02^{c}	0.50 ± 0.02 ^{bc}	0.47 ± 0.02 ^{ab}	0.46 ± 0.01 ^{a}
Elenolic acid *	19.50 ± 2.57^{c}	12.60 ± 2.82^{b}	6.59 ± 1.55 ^{a}	5.70 ± 1.04 ^{a}	5.41 ± 1.91 ^{a}
Hydroxyelenolic acid *	0.98 ± 0.02^{c}	0.86 ± 0.05^{b}	0.82 ± 0.04^{b}	0.74 ± 0.00 ^{a}	0.74 ± 0.00 ^{a}
Flavonoids					
Apigenin	4.42 ± 0.58^{c}	4.34 ± 0.32^{c}	2.45 ± 0.25^{b}	3.04 ± 0.47^{b}	0.49 ± 0.28 ^{a}
Luteolin	3.02 ± 0.37 ^{d}	2.28 ± 0.47^{c}	1.70 ± 0.53 ^{bc}	1.45 ± 0.38^{b}	0.39 ± 0.29 ^{a}
Phenolic acids					
p-Coumaric acid	6.18 ± 0.42 ^{d}	5.28 ± 0.30^{c}	4.23 ± 0.37^{b}	3.70 ± 0.36^{b}	3.05 ± 0.35 ^{a}
Lignans					
Pinoresinol	13.20 ± 1.92 ^{d}	11.67 ± 2.44 ^{cd}	9.93 ± 1.75 ^{bc}	8.17 ± 1.11 ^{ab}	5.73 ± 1.02 ^{a}
Rancimat (h)	8.59 ± 0.11^{b}	7.50 ± 0.15 ^{a}	7.30 ± 0.30 ^{a}	8.33 ± 0.26^{b}	8.24 ± 0.32^{b}
Pigments, α-tocopherol, and squalene (mg/kg)					
Carotenoids	5.21 ± 0.20 ^{a}	8.37 ± 0.93 ^{bc}	10.39 ± 0.57 ^{d}	7.90 ± 0.15^{b}	8.91 ± 0.74^{c}
Chlorophylls	2.40 ± 0.35 ^{a}	2.59 ± 0.31 ^{a}	3.54 ± 0.14^{b}	3.98 ± 0.11^{b}	7.77 ± 0.84^{c}
α-Tocopherol	227.92 ± 3.42 ^{a}	221.37 ± 7.82 ^{a}	233.12 ± 10.12 ^{a}	227.29 ± 4.98 ^{a}	227.40 ± 10.80 ^{a}
Squalene	1506.50 ± 12.42 ^{a}	1458.92 ± 54.63 ^{a}	1508.41 ± 75.10 ^{a}	1472.79 ± 30.34 ^{a}	1454.82 ± 61.32 ^{a}

* Elenolic acid and hydroxyelenolic acid were not included in the sum of phenolics, as they are not phenolic compounds, but degradation products.

An increase in the phenolic content of table olives has also been reported after the application of HHP to improve preservation during storage (Martín-Vertedor et al., 2022; Tokuşoğlu et al., 2010).

The effect of HHP on the phenolic content is controversial. On the one hand, cell disruption can cause the release of phenolic compounds attached to the polymeric structure of the cell wall or present in cellular organelles, which results in a higher concentration. But, on the other hand, these free phenolic compounds are more susceptible to enzymatic or oxidative degradation (Aganovic et al., 2021).

These variable results indicate that although the application of HHP modifies the composition and physiology of olives, the changes are not necessarily translated to the olive oil, as other chemical reactions take place during the extraction procedure with significant effects on the final oil composition.

3.4. Enzymatic activity of PPO, POX, and β-glucosidase in olive fruit

Based on the observed changes in olive fruit and olive oil phenolic composition, it was hypothesized that the activity of enzymes involved in the phenolic pathway had been altered by HHP. Accordingly, the activity of enzymes involved in hydrolysis (β-glucosidase) and oxidation (PPO and POX) was analyzed in the olives, studying the mesocarp and stone separately (Table 2). The activity of PPO extracted from the mesocarp was reduced by the application of 300 MPa, the duration of treatment having no effect; it did not differ from the control when 600 MPa was applied for 3 min, but increased after 6 min. In contrast, the activity of PPO extracted from the stone was significantly reduced by all HHP treatments compared to the control, without differences between treatments. The activity of POX extracted from the mesocarp was also reduced by all the HHP treatments, although it was significantly higher after the application of 600 MPa *versus* 300 MPa. Conversely, HHP increased the activity of POX from the stone, especially at 600 MPa.

Table 2
Oil content (%), phenolic compounds (mg/kg fresh fruit), and enzymatic activity (UA/g) of polyphenol oxidase (PPO), peroxidase (POX), and β-glucosidase (GLUC) in control olives and the four HHP-treated olive samples (T1, T2, T3 and T4). The enzymatic activity was measured in the olive mesocarp '-m' and stone '-s' separately. Results are expressed as mean ± standard deviation, n = 9. Different letters mean significant differences ($p < 0.05$).

HHP conditions	Control	T1	T2	T3	T4
Pressure (MPa)	–	300	300	600	600
Duration (min)	–	3	6	3	6
Oil content (%)	40.26 ± 1.10	39.37 ± 0.46	41.05 ± 0.75	40.23 ± 0.65	40.00 ± 1.30
Phenolic compounds (mg/kg fresh fruit)					
Sum of phenolics (mg/kg)	11.53 ± 2.33^{b}	15.09 ± 2.21^{c}	12.26 ± 2.19^{bc}	10.25 ± 1.47^{ab}	7.86 ± 1.27^{a}
Secoiridoids					
Ligstroside aglycone	0.15 ± 0.02^{a}	0.16 ± 0.04^{a}	0.15 ± 0.01^{a}	0.16 ± 0.03^{a}	0.12 ± 0.04^{a}
Oleuropein aglycone	0.84 ± 0.37^{a}	2.33 ± 0.94^{bc}	1.69 ± 0.50^{ab}	3.10 ± 0.84^{c}	2.44 ± 0.92^{bc}
Oleocanthal	0.08 ± 0.05^{a}	0.22 ± 0.06^{b}	0.21 ± 0.11^{ab}	0.16 ± 0.04^{ab}	0.21 ± 0.18^{ab}
Oleacein	0.66 ± 0.31^{a}	2.51 ± 0.52^{b}	2.42 ± 1.44^{b}	2.87 ± 0.61^{b}	0.89 ± 0.36^{a}
Secoiridoid derivatives					
Elenolic acid *	1.13 ± 0.40^{ab}	2.38 ± 0.74^{c}	1.73 ± 0.57^{bc}	0.84 ± 0.21^{a}	0.67 ± 0.51^{a}
Flavonoids					
Apigenin	8.20 ± 1.16^{c}	8.04 ± 1.18^{c}	4.98 ± 1.04^{b}	2.70 ± 0.73^{a}	1.35 ± 0.42^{a}
Luteolin	1.36 ± 0.15^{c}	1.48 ± 0.27^{c}	0.93 ± 0.18^{b}	0.66 ± 0.16^{ab}	0.37 ± 0.08^{a}
Phenolic acids					
p-Coumaric acid	0.12 ± 0.00^{a}	0.16 ± 0.01^{c}	0.14 ± 0.02^{b}	0.13 ± 0.01^{ab}	0.12 ± 0.01^{ab}
Phenolic alcohols					
Hydroxytyrosol	0.17 ± 0.04^{a}	0.38 ± 0.07^{b}	0.31 ± 0.11^{b}	0.24 ± 0.06^{ab}	0.19 ± 0.04^{a}
Hydroxytyrosol acetate	0.30 ± 0.07^{a}	0.65 ± 0.14^{abc}	0.53 ± 0.17^{ab}	0.91 ± 0.31^{bc}	1.06 ± 0.56^{c}
Enzymatic activity (UA/g)					
PPO-m	3.89 ± 0.18^{b}	1.91 ± 0.05^{a}	1.57 ± 0.11^{a}	3.70 ± 0.83^{b}	5.76 ± 0.32^{c}
PPO-s	2.59 ± 0.57^{b}	1.21 ± 0.17^{a}	1.45 ± 0.07^{a}	1.20 ± 0.23^{a}	1.40 ± 0.13^{a}
POX-m	5.12 ± 0.48^{c}	3.31 ± 0.39^{a}	3.25 ± 0.61^{a}	3.57 ± 0.43^{ab}	4.29 ± 0.55^{b}
POX-s	1.94 ± 0.37^{a}	2.79 ± 1.85^{a}	4.74 ± 0.31^{b}	4.56 ± 0.51^{b}	4.76 ± 0.48^{b}
GLUC-m	0.68 ± 0.09^{b}	0.54 ± 0.08^{a}	0.52 ± 0.08^{a}	0.52 ± 0.07^{a}	0.66 ± 0.09^{b}
GLUC-s	0.15 ± 0.05^{b}	0.09 ± 0.02^{a}	0.09 ± 0.01^{a}	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}

* Elenolic acid was not included in the total phenolic content, as it is not a phenolic compound, but a degradation product.

Finally, the activity of β-glucosidase was reduced in both the mesocarp and stone, although in the former it did not differ from the control after the application of 600 MPa for 6 min. The differences in enzymatic activity in these two different tissues could be explained by the different matrix composition, the existence of isoenzymes with similar structures but different resistance to pressure (García-Vico et al., 2021; Sánchez et al., 2023; Zawawi et al., 2022), and different level of gene expression (Sánchez et al., 2023; Velázquez-Palmero et al., 2017). For example, very low expression of β-glucosidase was detected in stones of 'Arbequina' and 'Picual' olives, whereas in green mesocarp the expression was high (Velázquez-Palmero et al., 2017), which can be related to the very low β-glucosidase activity found in the stone compared to the mesocarp.

PPO and POX are one of the most pressure-resistant enzymes, although their susceptibility to inactivation by HHP varies depending on the species or cultivar, isoenzymes, processing conditions, and the food matrix (e.g., pH and sugar content) (Eisenmenger & Reyes-De-Corcuera, 2009; Roobab, Abida, et al., 2022). In some cases, they require pressures above 600 MPa for inactivation at room temperature within a reasonable treatment time (<15 min) (Houška et al., 2022). For example, in apple, pear, and carrot, 900 MPa was needed to inactivate them, while strong enzyme activity persisted in pear and carrot at 500 MPa (Anese et al., 1994; Asaka & Hayashi, 1991), aligning with the observed increase in PPO activity from olive mesocarp and POX from the stone at 600 MPa. In carrot juice, 300 MPa was also more effective in reducing PPO activity than 600 MPa (Stinco et al., 2019), but still different fruit juices have shown different behaviors due to the divergent pressure resistance of their enzymes (Roobab, Abida, et al., 2022). The effectiveness of HHP-induced inactivation can be enhanced when combined with temperature (Eisenmenger & Reyes-De-Corcuera, 2009; Marszałek et al., 2015; Roobab, Abida, et al., 2022; Zawawi et al., 2022), and differences in the food matrix, like pH and sugar content, can also influence (Anese et al., 1994; Roobab, Abida, et al., 2022).

Considering that the only enzymes in the olive fruit that can act on phenolic compounds are the ones located in the mesocarp, the enzymatic activity in the stone is not considered here. In intact untreated olives, β-glucosidase has a limited or negligible activity because of their compartmentalization. Therefore, it can actively perform its reactions when it comes in contact with phenolic compounds upon cell disruption (such as during olive oil production) (García-Rodríguez et al., 2011; Pourcel et al., 2007). The application of HHP causes cell disruption, resulting in the release and interaction of phenolic compounds and enzymes. Therefore, even though the activity of β-glucosidase was slightly lower in treated olives compared to the control, its contact with oleuropein could explain the higher concentration of oleuropein aglycone. The same could occur with the methylesterase involved in the formation of oleacein and oleocanthal, and the enzymes responsible for the hydrolysis of secoiridoids to form hydroxytyrosol and elenolic acid. Additionally, β-glucosidase and other esterases can produce more secoiridoids as plant defense mechanism (García-Vico et al., 2021; Koudounas et al., 2015), for example, as a response to the stress caused by HHP.

Regarding the oxidative enzymes PPO and POX, their activity in the fruit seems limited not only by the cell location but also by the lack of oxygen and H_2O_2 necessary for their reactions, which can become more available under stress conditions or after tissue damage (Hossain et al., 2015). However, the physical disruption caused by HHP seemed insufficient to trigger the oxidation of secoiridoids, because their concentration was not reduced by the treatment. Nevertheless, the decrease in elenolic acid and hydroxytrosol when pressure was increased from 300 to 600 MPa could be due to higher PPO and POX activities. Furthermore, the substantial reduction in oleacein when the 600 MPa treatment was extended from 3 to 6 min correlates with the increase of PPO and POX activities in the mesocarp. Besides, longer duration means greater exposure to phenolic compounds and, consequently, increased oxidation. In the case of flavonoids, their oxidation is associated with plant defense against biotic and abiotic stresses (Pourcel et al., 2007), and therefore, their reduced concentration after applying HHP, especially at 600 MPa, is likely attributable to their oxidation by PPO and POX, whose activity was also highest at 600 MPa. This could suggest that under HHP stress PPO and POX have a higher affinity for flavonoids than secoiridoids, probably due to the activation of chemical reactions involved in plant defense.

The different results we obtained from olive oil and olive fruit are due to the mechanical process of olive oil production, during which enzymes and substrates are released by cell breakdown, allowing them to interact. The activity of PPO and POX is further favored by exposure to oxygen and H_2O_2. In olive oils, PPO and POX from both the mesocarp and stone can contribute to the oxidation of phenolic compounds, resulting in a significant loss. According to Zawawi et al. (2022), $\geq$ 80% inactivation of PPO is required to control or slow down the enzymatic reaction in fruit products, and the inactivation reached in this study was $<$ 60%, which could explain why the content of phenolic compounds dropped at 300 MPa, even though PPO activity was reduced. In addition, the activity of POX from the stone was significantly increased by HHP, suggesting it could play an important role in phenolic oxidation, as reported in other studies (García-Rodríguez et al., 2011; Luaces et al., 2007). Besides, this depletion can be further increased by the reduction in β-glucosidase activity induced by HHP treatment, as less oleuropein aglycone, ligstroside aglycone, oleacein and oleocanthal are formed.

Therefore, it seems that the phenolic content in olive fruits, especially that of secoiridoids, can be enhanced by the application of HHP, whereas in the olive oil produced from the treated olives it is drastically reduced because of PPO and POX activity and lower β-glucosidase activity. If these two oxidative enzymes could be effectively inactivated, the extracted olive oils would probably have a higher content of phenolic compounds, especially secoiridoids, transferred from the olive fruit without oxidation. Future research should evaluate whether it might be possible to inactivate them by applying other HHP conditions, such as pressure higher than 600 MPa or in combination with temperature. Furthermore, olives with different ripening indices could be tested, as the sugar content decreases during fruit maturation (Marsilio et al., 2001).

3.5. Olive oil FA composition

Although the statistical analysis revealed that the percentage of some FAs in olive oil was significantly different between certain treatments (Table S1), the differences were minimal ($<$0.01 to 0.18%), suggesting they were probably due to the natural variability between samples rather than the HHP treatment. Similarly, the oil content of the olive samples used to produce each olive oil also varied slightly (Table 2). Such small differences are unlikely to have significant effects on the health benefits and oxidative stability of the oil. In fact, the oleic/linoleic ratio, which is related to the oxidative stability of the oil, was not substantially different (Table S1). It can therefore be concluded that the percentage of each FA of the triacylglycerols was not altered by the application of HHP. Andreou et al. (2017) also found quite similar FA values between the control and oil produced from HHP-treated olives (200 MPa for 1 min), although a statistical analysis was not described.

3.6. Olive oil carotenoids, chlorophylls, α-tocopherol, and squalene

The application of HHP led to olive oils with a greener appearance due to an increase in chlorophyll content, particularly at 600 MPa (Table 1) and when the treatment was increased from 3 to 6 min. The same behavior was observed for carotenoids (Table 1). These pigments have also been reported to increase after the application of HHP in other plant-based foods (Aganovic et al., 2021). This increase is attributable to the release of pigments in olive fruit by HHP treatment, which can be transferred to the oil phase during production. This could be a desirable attribute for those consumers that prefer olive oil with a strong green color.

On the contrary, neither α-tocopherol nor squalene levels were affected by HHP (Table 1). A literature search failed to find information about the effect of HHP on these two compounds in olive fruit. However, in a study applying HHP to olive paste during oil extraction, an increase in α-tocopherol concentration was observed, attributed to an improvement in its extractability caused by cell disruption (Andreou et al., 2022). Considering that cell disruption also occurred in the olive fruit, the absence of an increase in α-tocopherol content in our study could be related to its role in protecting the oil from the oxidation induced by HHP. This hypothesis is supported by the values of K_{232} and K_{270}, which give information about lipid oxidation, as they either did not differ or were improved compared to the control (Table 1), as well as the FA composition, which was not affected by HHP (Table S1).

3.7. Oxidative stability of the olive oil

The oxidative stability of the oil was only negatively affected by HHP when applied at 300 MPa (Table 1). Although at 600 MPa the Rancimat values were lower compared to the control, the differences were not statistically significant. This is an interesting result, as the phenolic content, which is one of the major contributors to the antioxidant capacity of olive oil, was considerably reduced by the HHP treatments. The levels of α-tocopherol, another important antioxidant of olive oil responsible for protecting PUFAs from oxidation, were unaltered by HHP. However, the higher oxidative stability observed at 600 MPa than at 300 MPa cannot be explained by the α-tocopherol or phenolic content, as the former was the same in both conditions, and the latter was lower at 600 MPa. Therefore, although the loss of oxidative stability at 300 MPa can be explained by the loss of phenolic compounds, the minimal reduction at 600 MPa suggests other factors are involved. Possible explanations for this behavior are that HHP induces the formation of other compounds with antioxidant capacity, causes chemical changes that protect the oil from oxidation, or stops or minimizes oxidative reactions. In a study on the shelf-life of olive oils, Andreou et al. (2017) found that oils produced with HHP-treated olives had better oxidative stability than the untreated oils, and they obtained the same result when applying HHP to olive paste (Andreou et al., 2022). Considering these results, HHP seems a promising technology to improve the oxidative stability of olive oils.

3.8. Multivariate analysis

The score scatter plot of the PCA shows a clear separation between the control olive oils and those obtained from HHP-treated olives according to the level of pressure (Fig. 3). Additionally, a separation according to the duration of HHP treatment (3 and 6 min) is noticeable at both pressures (300 and 600 MPa) (Fig. 3), 6 min being more to the right. This clearly indicates that HHP caused great changes in the olive oil, mainly due to the pressure applied. Although the length of treatment had a lower effect, it also contributed to the changes observed.

The loading plot in the biplot (Fig. 3) shows that the control olive oil was characterized mainly by a high content of phenolic compounds, as previously discussed, whereas the oils obtained from HHP-treated olives were richer in carotenoids and chlorophylls. Furthermore, the peroxide value was higher in HHP samples. On the other hand, squalene and α-tocopherol are located close to the center of the coordinates, meaning they have little influence on the sample distribution. These results correlate well with the findings discussed previously, and indicate that among the quality parameters, the peroxide value was the most affected by HHP. On the other hand, the variables that most influenced the sample distribution according to their contribution scores in PC1 were, in the control (left side), the secoiridoids (ligstroside aglycone, oleuropein aglycone, oleocanthal and oleacein), whereas the peroxide value, carotenoids, the activity of POX from the olive stone, and chlorophylls were characteristic of HHP samples (right side). Interestingly, acidity is also located in the right side of the plot, meaning that HHP samples are likely to have a higher value. As regards the enzymatic activity, even though all oxidative enzymes contributed to the loss of the phenolic content in HHP-treated oils, POX from the stone could have played a more important role, as suggested by the contribution scores in PC1.

A summary of the positive and negative effects found with the HHP treatments applied in this study is presented in Table 3.

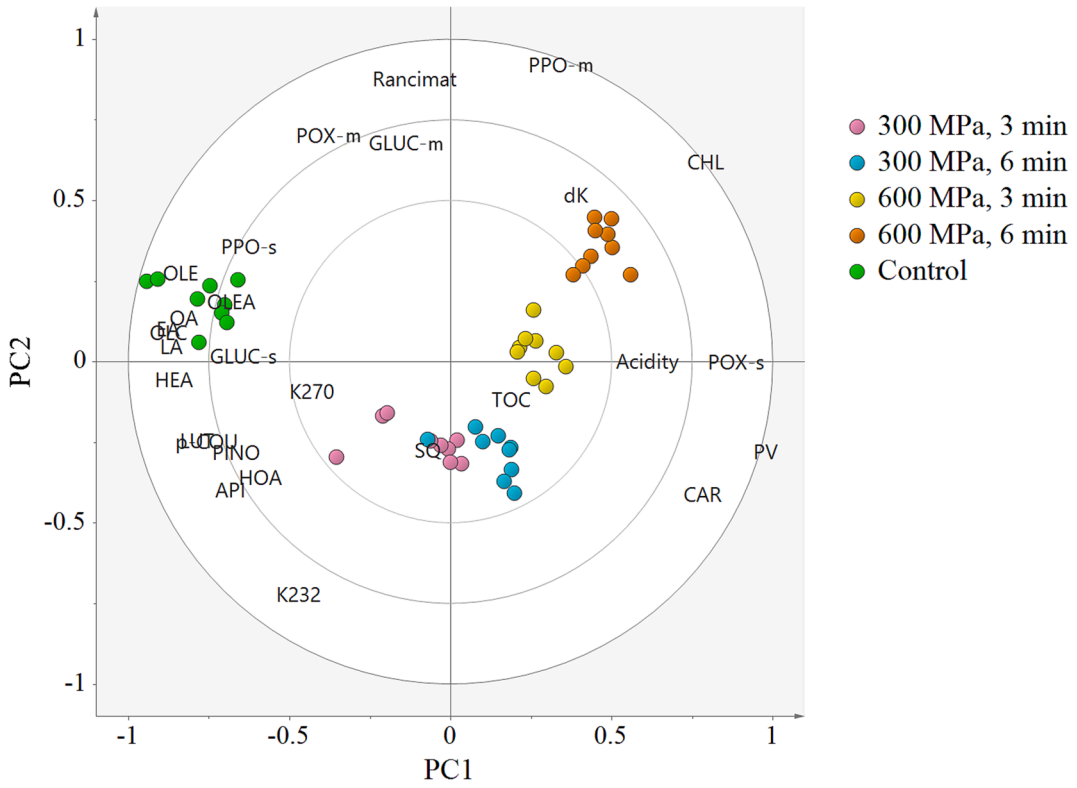

Fig. 3. Biplot of the loadings and scores of the PCA for PC1 and PC2, with an explained variation (R^2X) of 0.508 and 0.175, respectively. Variables are abbreviated as follows: OLE, oleacein; OLEA, oleaceinic acid; OA, oleuropein aglycone; LA, ligstroside aglycone; OLC, oleocanthal; EA, elenolic acid; HEA, hydroxyelenolic acid; LUT, luteolin; *p*-COUM, *p*-coumaric acid; PINO, pinoresinol; HOA, hydroxyoleuropein aglycone; API, apigenin; SQ, squalene; TOC, α-tocopherol; CAR, carotenoids; CHL, chlorophylls; PV, peroxide value; dK, ΔK. The enzymatic activity is referred to by the name of the enzyme and the tissue: GLUC, β-glucosidase; PPO, polyphenol oxidase; POX, peroxidase; -m and -s stand for mesocarp and stone, respectively.

4. Conclusions

When HHP was applied to olive fruit, the resulting cell disruption favored the enzymatic generation of the secoiridoids oleuropein aglycone, oleocanthal and oleacein. However, the treatment also favored the activity of the oxidative enzymes PPO and POX, and reduced the activity of β-glucosidase, leading to a significant loss of phenolic compounds in the olive oils produced from the treated olives. In contrast, HHP treatment resulted in greener oils due to an increase in pigments (carotenoids and chlorophylls). The squalene and α-tocopherol content were not altered, and the FA composition was maintained in all oil samples. Finally, oils produced with olives treated at 300 MPa had the lowest oxidative stability, whereas those produced using 600 MPa did not differ from the control. Therefore, although the oils produced from olives subjected to 600 MPa had the lowest phenolic content, they had good oxidative stability, suggesting that the application of HHP could have other impacts not analyzed in this study that could improve oxidative stability, such as the formation of other compounds with antioxidant capacity, changes in the food matrix that protect the oil from oxidation, or inhibition or reduction of oxidative reactions. Furthermore, the application of HHP successfully increased the detachment of the olive mesocarp from the stone, which can suppose an advantageous condition for oil production with destoned olives.

In conclusion, HHP treatment has a favorable effect on 'Arbequina' olives by enhancing the production of secoiridoids with health-promoting properties, such as oleocanthal and oleacein. However, to prevent the loss of these phenolic compounds in the oil, the inactivation of the oxidative enzymes PPO and POX is indispensable, especially POX from the stone, which appears to be the most influential. Compared to the control, olive oils produced from HHP-treated olives had a higher content of carotenoids and chlorophylls, the same levels of squalene and α-tocopherol, and the same FA composition. Therefore, if the inactivation of PPO and POX was achieved, (a) the oils would also have a high phenolic content and better oxidative stability, and (b) HHP technology would be a means of eliminating or minimizing the oxidation problem of phenolic compounds during oil extraction. Future studies should be conducted to test other HHP pressures, combined with temperatures slightly higher than 20–25 °C (room temperature), and with olives of different ripening indices to find the conditions that can inactivate PPO and POX. Furthermore, investigating the impact on different olive cultivars is essential to determine whether these findings remain consistent with or differ from 'Arbequina', given the potential variations in enzyme activity and the presence of isoenzymes among cultivars.

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CRediT authorship contribution statement

Alexandra Olmo-Cunillera: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Albert Ribas-Agustí:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing. **Julián Lozano-Castellón:** Investigation, Writing – review & editing. **Maria Pérez:** Writing – review & editing,

Table 3
Summary of the positive and negative effects of the HHP treatments applied on 'Arbequina' olives. FA, fatty acid; GLUC, β-glucosidase; PPO, polyphenol oxidase; POX, peroxidase; -m and -s stand for mesocarp and stone, respectively.

HHP conditions	Positive effects	Negative effects
300 MPa for 3 min	(1) Greater detachment of the mesocarp from the stone. (2) Increase in oleuropein aglycone, oleacein, and oleocanthal in olive fruit. (3) Increase in pigment content (carotenoids and chlorophylls): greener oil. (4) No impairment of squalene and α-tocopherol content, nor FA composition. (5) Decrease in PPO and POX-m activity.	(1) Increase in the peroxide value: formation of hydroperoxides (primary oxidation products). (2) Loss of the characteristic EVOO aroma. (3) Decrease in olive oil phenolic content. (4) Decrease in oxidative stability. (5) Decrease in GLUC activity.
300 MPa for 6 min	Same positive effects as 300 MPa for 3 min.	Same negative effects as 300 MPa for 3 min, plus: (6) Increase in POX-s activity.
600 MPa for 3 min	Points 1, 2, 3, and 4, plus: (6) Decrease in K_{232} and K_{270} values: formation of diene and triene conjugated products (primary and secondary oxidation products). (7) No impairment of oxidative stability. (8) Decrease in PPO-s and POX-m activity.	Points 1, 2, 3, 5, and 6.
600 MPa for 6 min	Same positive effects as 600 MPa for 3 min, plus: (9) No effect on GLUC-m activity.	Points 1, 2, 3, and 6, plus: (7) Increase of the global oxidation state (ΔK). (8) Decrease in GLUC-s activity. (9) Increase in PPO-m activity.

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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

The data that has been used is confidential.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137902.

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