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Compositional and Tissue Modifications Induced by the Natural Fermentation Process in Table Olives

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Olive fruits contain high concentrations of phenols that include phenolic acids, phenolic alcohols, flavonoids, and secoiridoids. The final concentration of phenols is strongly affected by brine conditions. The factors involved in modification by brine are still partially unknown and can include hydrolysis of secoiridoid glucosides and the release of hydrolyzed products. In this study olives from various Italian cultivars were processed by natural fermentation (e.g., without a preliminary treatment of olives with NaOH) using a selected *Lactobacillus* strain. Processed olives are characterized by a low phenolic concentration of phenols, consisting mainly of phenyl alcohols, verbascoside, and the dialdehydic form of decarboxymethylelenolic acid linked to (3,4-dihydroxyphenyl)ethanol (3,4-DHPEA−EDA), whereas a high level of phenols occurs in olive brine from all the cultivars studied. Olives of the Coratina cultivar, control and with fermentation by *Lactobacillus pentosus* 1MO, were analyzed in a frozen hydrated state by cryo scanning electron microscopy and energy-dispersive X-ray microanalysis, on both surface and transversal freeze-fracture planes. Structural modifications, found in olives after fermentation, may explain the phenol release in brine.

KEYWORDS: ***Olea europaea*** **L.; table olives; polyphenols; debittering; skin modification; cryo-SEM; EDXMA**

INTRODUCTION

Table olives are one of the most popular fermented foods in Mediterranean European countries. Spain, Italy, and Greece represent the most important producers (*1, 2*). The olive drupe contains high concentrations of phenolic compounds that can range between 1% and 3% of the fresh pulp weight (*3*). The main classes of phenols in olive fruit are phenolic acids, phenolic alcohols, flavonoids, and secoiridoids (*4*).

The phenolic compounds classified as secoiridoids, exclusively present in members of the family of Oleaceae including *Olea europaea* L., are characterized by the presence of either elenolic acid or elenolic acid derivatives in their molecular structures. Oleuropein, demethyloleuropein, ligstroside, and nüzhenide are the most abundant secoiridoid glucosides in olive fruit (*3, 4*).

Two main methods are used to prepare fermented table olives: the Spanish method for green olives and the Greek method for black olives (*1*). In both the cases the fruits become edible after a spontaneous, "natural" fermentation performed by a mixed population of indigenous microorganisms. Just for Spanish-style green olives, the fermentation step is preceded by a preliminary treatment with sodium hydroxide (lye) needed to hydrolyze the bitter-tasting secoiridoid glucosides such as oleuropein and demethyloleuropein (*5*). Otherwise the olives are debittered during a period of fermentation in brines (*1*).

The spontaneous fermentation of Spanish-style green olives mainly depends on *Lactobacillus plantarum*, while lactic acid bacteria (LAB) and yeasts dominate the brines of naturally fermented olives brined directly after harvesting (*6*). Nevertheless, it is commonly recognized that the natural process leads to unpredictable and longer fermentation as well as low-quality products (*7, 8*) with variable sensory characteristics. Several works have been aimed to standardize the quality of "naturally processed" products and to reduce the debittering times using selected starter cultures and/or the modification of physicochemical process parameters (*8–10*). As reported by Ruiz-Barba et al. (*11*), the basic characteristics of starter cultures for table olive fermentation include rapid and predominant growth, homofermentative metabolism, few growth factor requirements, and tolerance to salt, acid, and polyphenols (*12, 13*). Servili et

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Table 1. Phenolic Composition (mg/kg) of Fresh Olive Fruits Obtained for Different Italian Cultivars

compound	Frantoio		Carolea		Coratina		Leccino	
3,4-DHPEA[a]	292.6	(32.5) a	378.7	(28.2) a	702.1	(60.9) b	567.4	(50.3) c
p-HPEA	122.8	(7.2) a	121.0	(10.4) a	248.4	(15.4) b	164.7	(12.6) c
demethyloleuropein	1115.0	(91.7) a	507.2	(42.9) b	2265.8	(187.5) c	903.5	(75.5) a
verbascoside	886.3	(78.1) a	1461.7	(137.1) b	2355.6	(230.6) c	2296.8	(218.1) c
3,4-DHPEA-EDA	2643.9	(220.6) a	1752.2	(161.1) a	9222.8	(907.3) b	5839.9	(508) c
oleuropein	504.9	(46.8) a	1065.8	(103.9) b	1784.5	(162.7) c	1451.1	(132.6) d
p-HPEA−EDA	23.3	(1.9) a	nd	nd	nd	nd	106.2	(7.6) b

[a] Data are the mean values of three independent evaluations; the standard deviation is reported in parentheses. Values in each row followed by different letters (a−d) are significantly different from one another at $p < 0.01$.

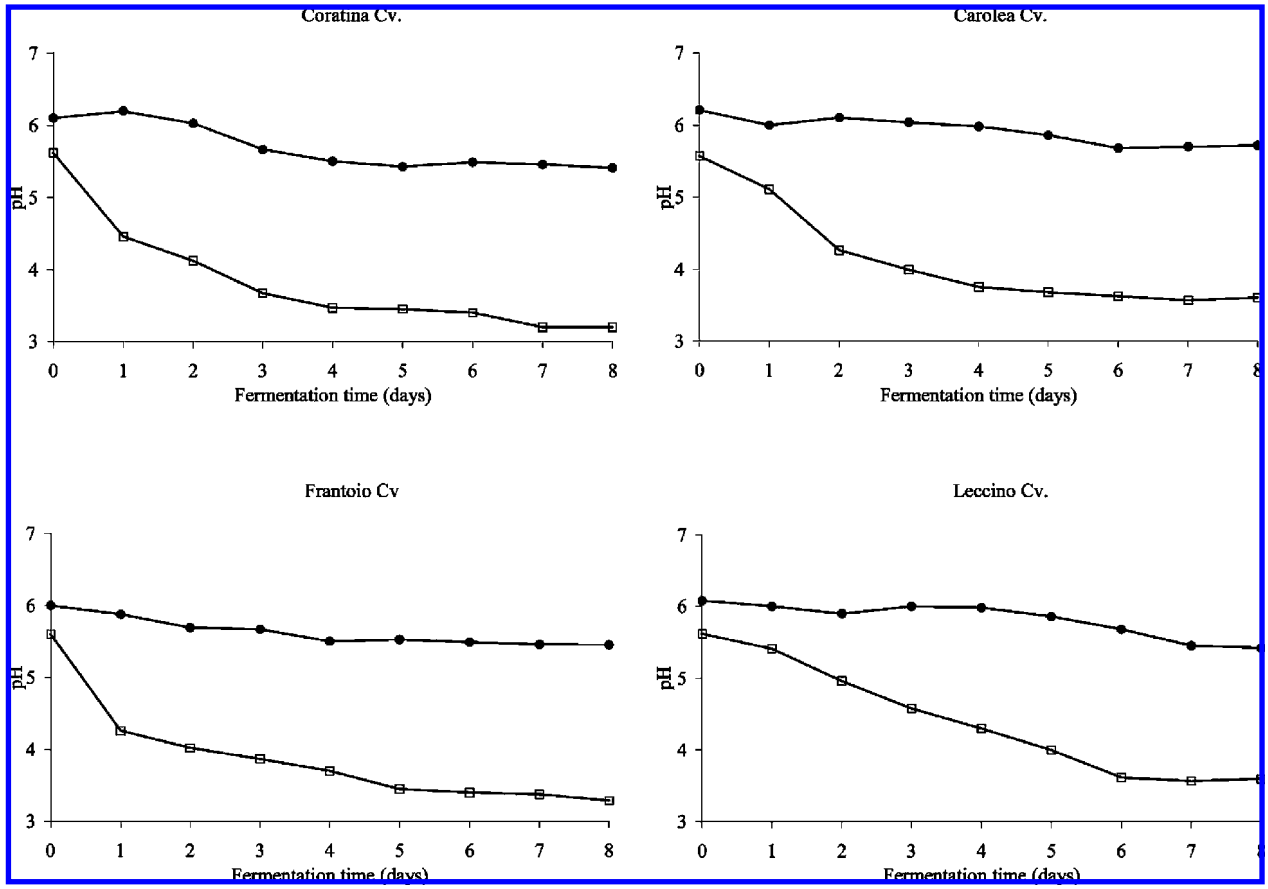

Figure 1. Change in the pH of brines. Legend: control (●); inoculated with *Lb. pentosus* 1MO (□).

al. (*9*) have previously demonstrated that the use of *Lactobacillus pentosus* 1MO was able to reduce the debittering time of cv. Itrana and Leccino olives.

During natural fermentation, the following hydrolyzed phenols are released in high levels into the brine (*9*): (3,4-dihydroxyphenyl)ethanol, (3,4-DHPEA), (*p*-hydroxyphenyl)-ethanol (*p*-HPEA), and aglycon derivatives of oleuropein and demethyloleuropein such as the dialdehydic form of decarboxymethylelenolic acid linked to (3,4-dihydroxyphenyl)ethanol (3,4-DHPEA−EDA). This process is probably due to the modifications of the olive cell structure caused by fermentation, but cell structural variations that may explain release of phenols in the brine after biological debittering are still unknown.

The aim of this work was to study the modifications of polyphenols in the olives and their relative brines fermented by *Lb. pentosus* 1MO in comparison to a control without inoculation. Moreover, to study the mechanisms involved in this release process, low-temperature scanning electron microscopy (cryo-SEM) and energy-dispersive X-ray microanalysis (EDXMA) of frozen hydrated olive samples were utilized to analyze olive skin and pulp structure modifications.

MATERIALS AND METHODS

Olives. Olive samples of the Frantoio, Carolea, Coratina, and Leccino cultivars from different areas of Italian production were harvested at the following ripening stages, expressed as the pigmentation index and evaluated as reported in a previous paper (*14*) Frantoio, 1.4 (60% of black olive peel); Carolea, 1.2 (50% of black olive peel); Coratina, 0.8 (30% of black olive peel); Leccino, 1.8 (80% of black olive peel).

Reference Compounds. Oleuropein was purchased from Extrasynthese (Genay, France), caffeic acid from Fluka (Milan, Italy), 3,4-DHPEA from Cayman Chemicals Ltd., and *p*-HPEA from Janssen Chemical Co. (Beerse, Belgium). The dialdehydic form of elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA−EDA and *p*-HPEA−EDA), demethyloleuropein, and verbascoside were isolated from the phenolic extract of olive fruit by semipreparative HPLC, according to the procedure previously reported by Servili et al. (*4*). The chemical structures were confirmed by NMR (*16*), and their level of purity (higher than 98%) was evaluated by HPLC.

Storage in Brine and Fermentation of Olives. To define the impact of olive brine storage and fermentation in the phenolic release from the fruit, for each cultivar 20 kg of olives was combined with 20 L (6% NaCl) of brine; after 5 days of storage one sample of 10 kg of those olives with 10 L of its own brine was inoculated, while the

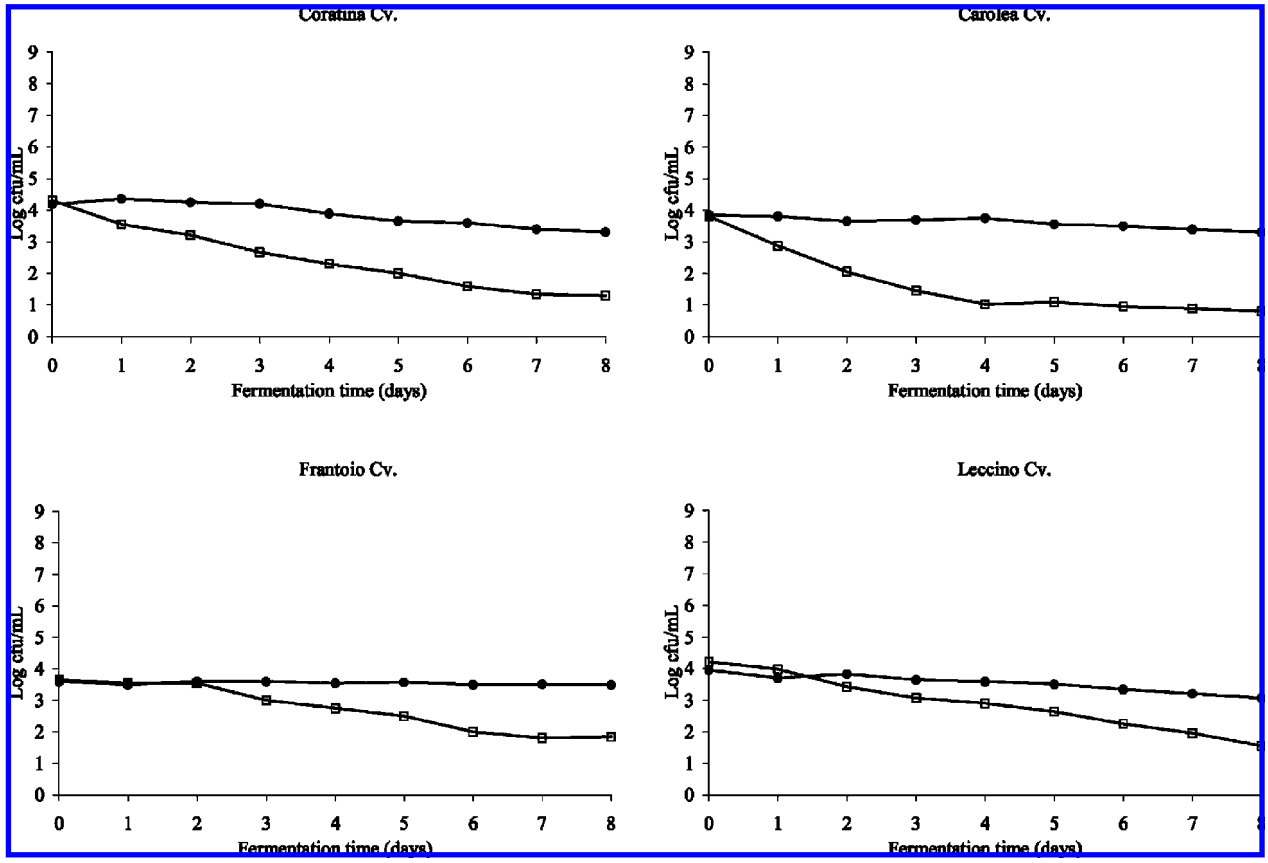

Figure 2. Change of coliforms in brines. Legend: control (●); inoculated with *Lb. pentosus* 1MO (□).

remaining sample was left in its brine without inoculation, as a control. The inoculum consisted of freeze-dried preparations of *Lb. pentosus* 1MO (cell concentration of 10^{11} cfu/mL), purchased from THT SA (Gembloux, Isnes, Belgium). *Lb. pentosus* 1MO was rehydrated by addition of 15% (w/v) water at 30 °C for 2 h and then inoculated in brine at an initial cell concentration of about 10^8 cfu/mL. The brine pH was corrected to a value of 5.60, and 3 g/L glucose (Oxoid, Milan, Italy) and 0.5 g/L yeast extract (Oxoid) were added.

Fermentations were performed at laboratory-scale at 25 °C for all the cultivars studied. The control sample was stored in brine (6% NaCl) at 25 °C, with neither inoculation nor glucose and yeast extract addition, until the end of the experiments.

After fermentation, the olives and brines of all the trials (control included) were analyzed for their microbiological, chemical, and tissues aspects as described below.

Microbiological Analysis. Viable counts of mesophilic lactobacilli (at 24 h intervals) occurred in MRS agar medium (Oxoid) containing cycloheximide (170 mg/L) under microaerophilic conditions at 30 °C for 48 h. Coliforms were counted every 24 h of fermentation by violet red bile agar (VRBA; Oxoid) at 37 °C for 24 h.

Chemical Analyses. Concomitantly with microbiological analysis, brine samples were chemically assayed. The pH was measured by a Corning 140 pH meter (Corning, Halstead, England). The concentrations of glucose and D- and L-lactic and acetic acids were estimated using enzymatic methods according to the manufacturer (Boehringer-Mannheim, Milan, Italy), and the phenolic compounds were analyzed by HPLC.

Phenolic compounds were evaluated from both olive and brine matrixes. Olive phenolic compound extraction and evaluation were carried out by HPLC as previously described by Servili et al. (*16*). The method was modified as follows: A 10 g sample of fresh olives was homogenized with 100 mL of 80% methanol containing 20 mg/L sodium diethyl dithiocarbamate (DIECA). The extraction was performed three times. After methanol removal, the aqueous extract was used for SPE phenol separation. The brines were first filtered through a 0.45 μm CA syringe filter (Whatman). The SPE procedure was followed for both olive extract and brine loading with 2 mL of sample and a 5 g/25 mL Extraclean high-load C_{18} cartridge (Alltech Italia Srl, Sedriano, Italy) using 200 mL of methanol as the eluting solvent. An Inertsil ODS-3 column (150 × 4.6 mm i.d.) (Alltech) was employed for HPLC analysis.

Cryo-SEM and EDXMA of Olive Fruits. These techniques have been applied because they may allow the structural and microanalytical modifications to be followed into the fruit cells and tissues, caused by the debittering process, maintaining the sample condition close to the natural one (*17*).

Portions of olives (Coratina cultivar), from the control and fermented by *Lb. pentosus* 1MO (five replicates) were excised and quickly frozen in liquid nitrogen, where they were stored until the analysis (*17*). A frozen hydrated (FH) sample was mounted under liquid nitrogen gas in an aluminum stub with Tissue-Tek, transferred to a dedicated cryo preparation chamber (SEM cryounit, SCU 020, Bal-Tech, Balzers, Liechtenstein), freeze-fractured by a motor-driven fracturing microtome at −120 °C, surface etched for 5 min at −80 °C under high vacuum ($P < 2 \times 10^{-4}$ Pa), and sputter-coated with 10 nm of gold in an argon atmosphere ($P < 2.2 \times 10^{-2}$ Pa) to produce an electrically conductive surface. FH specimens were then transferred to a cryostage (−180 °C) inside a scanning electron microscope (Philips SEM 515, Eindhoven, The Netherlands) equipped with an SEM cryounit, SCU 020 (Bal-Tech). EDXMA of FH specimens was performed with SEM using an acceleration voltage of 17 kV, a takeoff angle of 16.5°, and a working distance (sample to the final lens of the SEM instrument) of 12.0 mm. Spectra from 0 to 20 keV were collected at increments of 10 eV per channel with the electron beam focused on a spot area in the center of the selected cells. The background and element-specific peak spectra were analyzed using the program EDAX DX-4 2.0 (EDAX, San Francisco, CA), which fully deconvolutes the spectra and allows corrections for interference between elements. The results are presented as peak/background ratio percentages for Na, Cl, and K elements. In addition, the two-dimensional distribution pattern was recorded by scanning an area of the specimen repeatedly for 30 min. Counts were integrated for the most abundant elements—Na, Cl, and K—within their

Table 2. Effect of Fermentation on the Phenolic Composition (mg/kg) of Olives Inoculated with *Lb. pentosus* 1MO

	Frantoio		Carolea		Coratina		Leccino	
compound	control	inoculated	control	inoculated	control	inoculated	control	inoculated
3,4-DHPEA[a]	357.1 (29.1) a	490.3 (41.2) b	461.5 (41.7) c	833.1 (80.5) d	856.9 (80.2) df	2119.2 (201.4) e	692.9 (60.1) f	1169 (108.5) g
p-HPEA	121.2 (8.7) a	112.2 (9.3) b	119.4 (8.5) c	135.5 (10.7) d	245.0 (17.5) e	232.7 (22.3) e	162.5 (12.6) f	157.9 (14.1) g
demethyloleuropein	990.5 (85.3) a	214.5 (18.3) b	450.6 (38.1) c	98.0 (82.4) d	2012.9 (198.8) e	489.8 (42.1) f	802.7 (77.3) a	156.3 (13.2) g
verbascoside	787.4 (66.2)a	662.7 (67.3) b	1298.5 (119.8) c	870.6 (78.2) d	2092.6 (201.9) e	1094.8 (99.2) f	2040.4 (195.7) e	773.0 (75.2) g
3,4-DHPEA−EDA	2576.4 (248.1) a	1536.0 (149.7) b	1707.4 (162) c	802.5 (80.1) d	8987.3 (890.2) e	5816.7 (578.5) f	5690.8 (556.5) f	2935.7 (289.7) g
oleuropein	460.0 (31.4) a	nd	995.6 (90.1) b	nd	1639.5 (157.1) c	nd	1377.3 (128.4) c	229.6 (20.4) d
p-HPEA−EDA	21.0 (1.5) a	8.2 (0.6) a	nd	nd	nd	nd	103.0 (9.9) b	nd

[a] Data are the mean values of three independent evaluations; the standard deviation is reported in parentheses. Values in each row followed by different letters (a−g) are significantly different from one another at $p < 0.01$.

respective spectrum windows into dot maps. Therefore, the dot maps collected provide only qualitative information of element distribution and are not sensitive in depicting small variations.

Statistical Analysis. An a priori one-way ANOVA, using Tukey's honest significant differences test, was performed (*18*).

RESULTS AND DISCUSSION

Phenolic Modification in Olives and Brines by Biological Debittering. The olives used for this work were chosen according to their differences in phenolic composition. As reported in **Table 1**, the olives before brine contact and fermentation contain high levels of phenols. Differences were observed not only in terms of the absolute values of phenolic compounds but also in relation to the phenolic composition. In fact, high variability was observed particularly for the concentrations of oleuropein, demethyloleuropein, and verbascoside. Oleuropein ranged between 504.9 and 1784.5 mg/kg. Demethyloleuropein ranged between 507.2 and 2265.8 mg/kg, while verbascoside showed concentrations between 886.3 and 2355.6 mg/kg. This aspect is very interesting to show the ability of the *Lb. pentosus* strain to remain viable even in the presence of high concentrations of phenolic compounds. In fact, as reported in a previous paper (*9*), the concentration of verbascoside strongly affects the growth capability of *Lactobacillus* spp. In contrast, we found that all brines presented a stable number of lactobacilli of 10^8 cfu/mL during the whole fermentation period (data not shown) and the pH values dropped from 5.60 to 3.2−3.6 at the end of the fermentation period (8 days) in the presence of *Lb. pentosus* 1MO (**Figure 1**. Moreover, the total glucose consumption occurred after 3 days, and metabolite production was measured between 1.62 and 4.46 g/L for lactic acid and between 0.11 and 0.27 g/L for acetic acid after five days of fermentation. Coliforms showed an initial concentration of about 10^4 cfu/mL for all the brines. In the control fermentation their value remained rather constant during the 8 days of fermentation, while in inoculated brines their number decreased considerably after 48− 72 h (**Figure 2**). These results reflected the dominance of lactobacilli during the fermentation and their competition toward the indigenous microorganisms.

Moreover, those observations confirm the strong capability of *Lb. pentosus* 1MO to ferment in olive brines with high values of phenolic concentration. In all brines inoculated with *Lb. pentosus* 1MO, the number of these lactobacilli remained stable at 10^8 cfu/mL.

The effect of biological debittering produced by *Lb. pentosus* 1MO is a strong reduction of oleuropein, demethyloleuropein, and 3, 4-DHPEA-EDA in the olive pulp, confirming that the hydrolytic activity of this bacterial strain was not only in the secoiridoid glucosides but also in their aglycon derivatives such as 3,4-DHPEA−EDA. This effect was observed in all the olives studied and is reported in **Table 2**. The modifications of the verbascoside concentration in terms of the hydrolytic process were not observed. In fact, in agreement with results reported previously (*9*), no modifications of caffeic acid concentration were found in the olive fruit or in the brine after fermentation (data not shown). Moreover, the most important evidence was related to phenol release in the brine. The mechanism that explains this process is still unknown but may be linked to the structural modifications of olive peel and pulp tissues caused by the occurrence of salt in the brine and/or to the fermentation process. However, as seen by the resultant phenolic concentration in brine (**Table 3**), the olives' permanence in the brine without inoculation (control) shows a low effect of phenol release which occurs strongly after fermentation by *Lb. pentosus* 1MO. These results are observed in all the cultivars studied,

Table 3. Effect of Fermentation on the Phenolic Composition (mg/L) of Brines Inoculated with *Lb. pentosus* 1MO

	Frantoio		Carolea		Coratina		Leccino	
compound	control	inoculated	control	inoculated	control	inoculated	control	inoculated
3,4-DHPEA[a]	7.8 (0.6) a	560.1 (51.1) b	2.1 (0.2) c	610.9 (58.9) bg	4.9 (0.4) d	259.4 (20.3) e	7.2 (0.6) f	680.0 (60.6) g
p-HPEA	0.4 (0.03) a	29.1 (0.1) b	0.4 (0.03) c	31.1 (2.7) b	2.9 (0.2) d	71.6 (6.8) e	2.7 (0.2) f	67.6 (4.5) e
demethyloleuropein	20.8 (1.5) a	95.0 (8.9) b	5.6 (0.4) c	11.1 (0.7) d	15.7 (1.1) e	212.0 (20.1) f	3.2 (0.2) g	46.2 (3.1) h
verbascoside	2.3 (0.2) a	102.0 (9.2) b	0.5 (0.04) c	22.4 (1.5) d	27.6 (1.8) e	1203.4 (92.2) f	7.3 (0.6) g	318.9 (28.3) h
3,4-DHPEA−EDA	40.3 (2.9)a	491.4 (39.2) b	16.6 (1.2) c	579.0 (49.7) b	19.2 (1.4) d	1091.0 (8.1) e	16.2 (1.3) f	770.9 (70.5) g
oleuropein	15.4 (1.1) a	74.3 (6.1) b	10.1 (0.7) c	112.5 (10.5) d	20.3 (1.9) e	278.9 (18.6) f	21.9 (1.5) g	290.0 (22.3) f
p-HPEA−EDA	nd	nd	nd	nd	nd	nd	nd	nd

[a] Data are the mean values of three independent evaluations; the standard deviation is reported in parentheses. Values in each row followed by different letters (a−g) are significantly different from one another at $p < 0.01$.

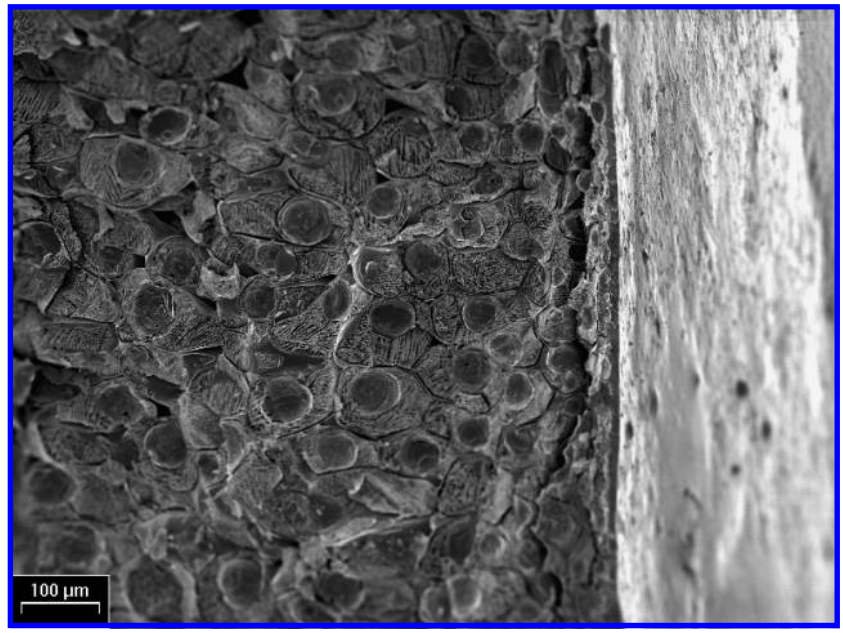

Figure 3. Cryo-SEM image of frozen hydrated olive of the Coratina cv. in the control. At the right is the olive external surface seen in perspective, while the transversal freeze-fracture surface is the in foreground at the left. Internal tissues below the surface are perfectly preserved; the parenchyma cells with oil droplets inside are visible.

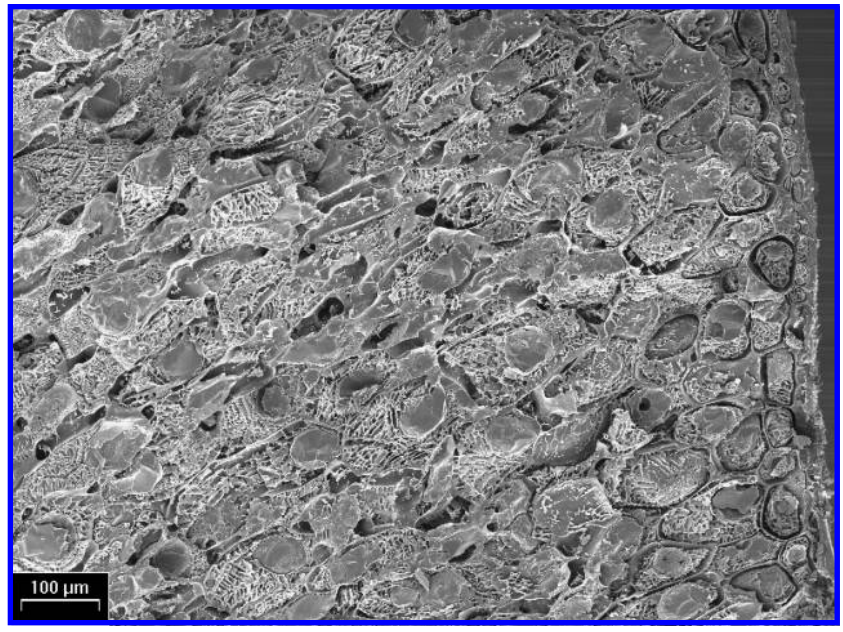

Figure 4. Cryo-SEM image of frozen hydrated olive of the Coratina cv. after fermentation by *Lb. pentosus* 1MO. Close to the right margin is the olive external surface border along the cuticle layer. In the foreground the transversal freeze-fracture surface is visible. Below the outer surface all the internal tissues appear damaged, from the epidermis to the content of the parenchyma cells, which appear coagulated and detached from the walls. Also the oil droplets inside the vacuoles appear degraded.

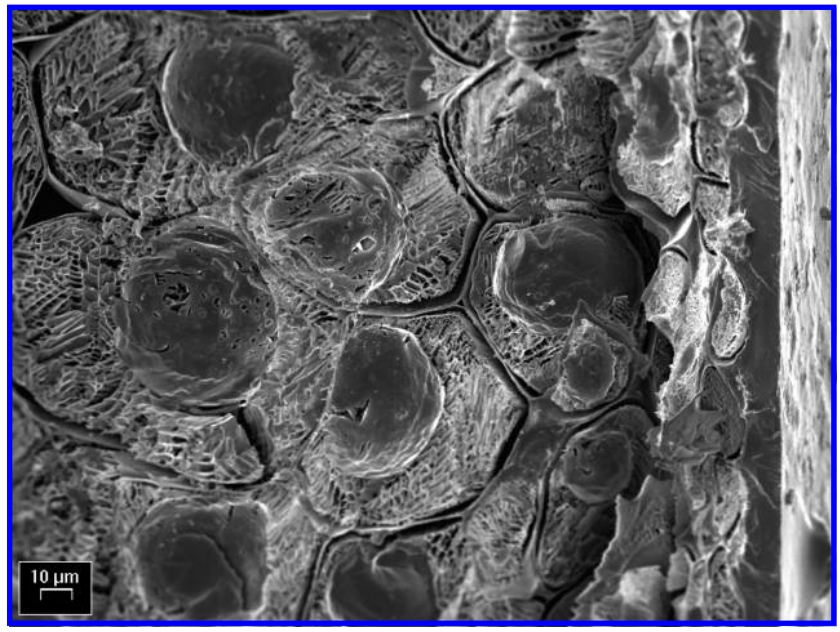

Figure 5. Cryo-SEM image of frozen hydrated olive of the Coratina cv. in the control. Details of **Figure 6**, showing close to the right border the fruit external surface in perspective. On the transversal freeze-fracture surface, beginning from the right side below the outer surface, a compact layer of cuticle is shown, and immediately below it, a single layer of epidermal cells and the first three layers of parenchyma cells (hypocarp) are shown.

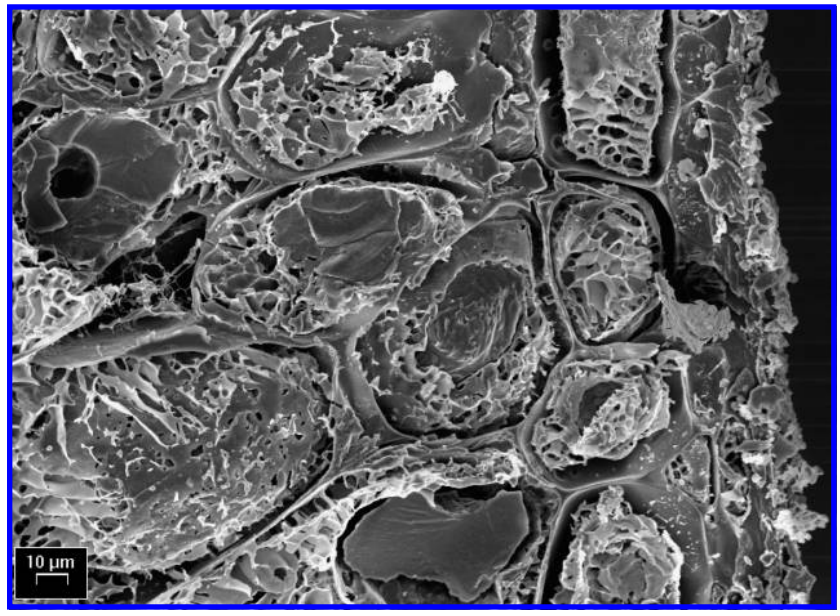

Figure 6. Cryo-SEM image of frozen hydrated olive of the Coratina cv. after the fermentation by *Lb. pentosus* 1MO. Details of **Figure 7**, showing on the right side the fruit epicarp and on the left the hypocarp. On the transversal freeze-fracture surface, beginning from the right, both the outer surface and cuticle layer (epicarp) appear destroyed and the content of the parenchyma cells appears coagulated and detached from the walls. Also the oil droplets inside the vacuoles appear degraded.

pointing to the conclusion that fermentation has a strong effect on phenolic release because it may modify the structure of olive tissues. To confirm this finding, the cryo-SEM analysis of FH olives of the Coratina cultivar stored in the brine, with and without *Lb. pentosus* 1MO inoculation, was performed. The choice of the Coratina cv. was based on the highest phenolic concentration of the olives and, consequently, on the strongest release of phenols in brines after fermentation.

Cryo-SEM and EDXMA of FH Olive Fruits. The cryo-SEM analysis of FH olives of the Coratina cv., from the control (**Figures 3**, **5**, and **7**, bottom right panel) and fermented by *Lb. pentosus* 1MO (**Figures 4**, **6**, and **8**, bottom right panel), showed external surface and internal tissues, perfectly preserved at the end of the preparation procedures.

In the control (**Figures 3** and **5**, right-hand side), the external surface of the olive (in perspective, close to the right border) and the rest of the epicarp appeared intact and compact. On the transversal freeze-fracture surface obtained, a compact layer of cuticle (11.3 ± 1.2 μm, **Figure 5**) is easily visible with a single layer of epidermal cells immediately below. The first layers of the parenchyma cells of the mesocarp were perfectly preserved (as were all the other layers more toward the endocarp, data not shown), showing compact oil droplets fully immersed in vacuoles (**Figure 5**). Moreover, the cells were tightly connected, and cell separation was not found.

In the olives analyzed after *Lb. pentosus* 1MO fermentation (**Figures 4** and **6**), the epicarp appeared completely degraded and all the internal tissues were damaged (**Figure 4**). Below

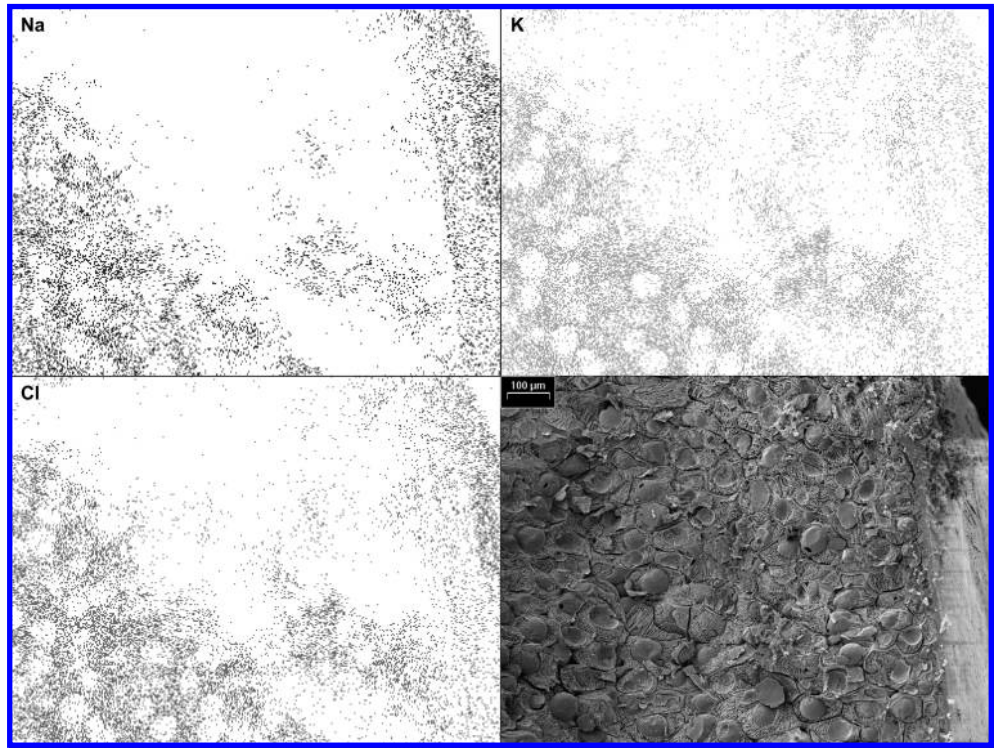

Figure 7. Na, Cl, and K EDXMA dot maps of the image in the bottom right panel, a freeze-fractured frozen hydrated olive of the Coratina cv. in the control. We point out that Na and Cl are distributed around oil droplets, while K distribution is uniform inside the mesocarp.

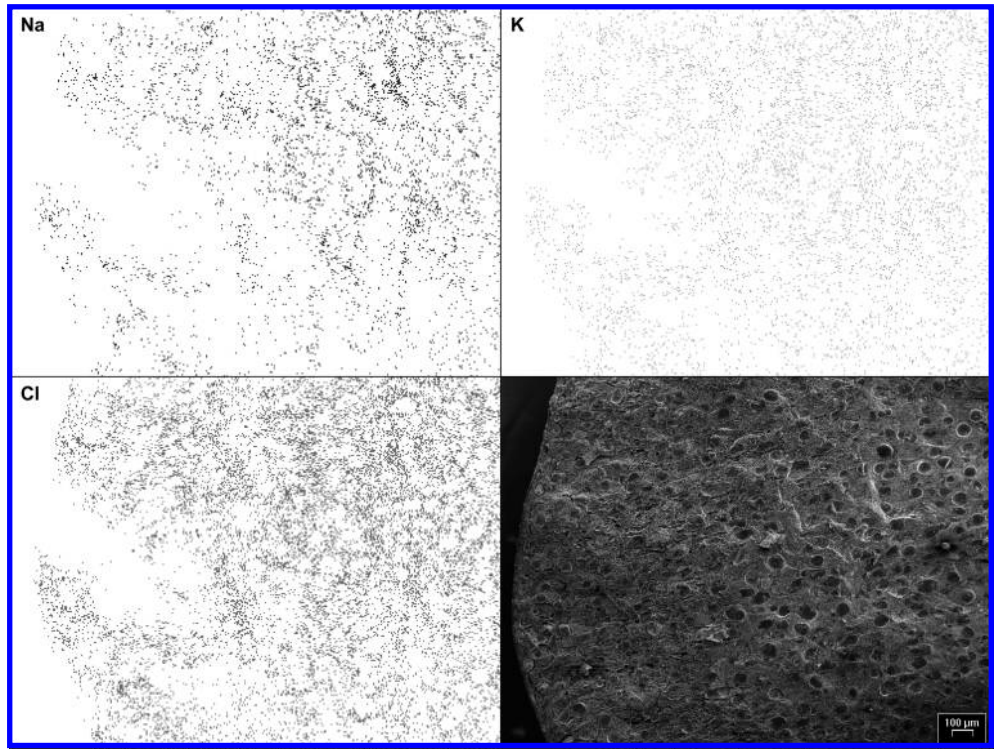

Figure 8. Na, Cl, and K EDXMA dot maps of the image in the bottom right panel, a freeze-fractured frozen hydrated olive of the Coratina cv. after fermentation by *Lb. pentosus* 1MO. The Na, Cl, and K distribution shows the same pattern found in the control.

the outer surface the content of both the epidermal and the parenchyma cells appeared coagulated and detached internally from the walls (**Figure 6**). Also the oil droplets inside the vacuoles were degraded. As with unfermented olives, cell separation was not visible between cells, both in the epicarp and in the mesocarp.

X-ray spectra obtained by EDXMA of freeze-fractured olive fruits showed that Na, Cl, and K elements were detected at levels significantly above the background and also at a certain depth from the fruit surface (data not shown). Any relevant differences in the preparation of the above indicated ions between the fermented olives and the corresponding control were observed. Differences were always found between Na and Cl uptake.

EDXMA dot maps of Na, Cl, and K showed the same pattern of penetration of Na and Cl through the skin into the fruit of the control and fermented by *Lb. pentosus* 1MO olives (**Figures**

7 and **8**). It was noted that Na and Cl were always distributed around oil droplets, never entering inside, while K was more uniformly distributed inside the mesocarp (**Figures 7** and **8**).

The use of cryo-SEM and EDXMA, for studying fruit surface and internal structure modifications during debittering, permitted the analysis of both structural and chemical characteristics of the olive fruit and minimized the number of preparation artifacts (*17*). In fact, the use of samples in chemical fixation and successive dehydration can cause aberrant results (*19*), with respect to the presence of olive cell separation during ripening. Cryo-SEM images of control and fermented by *Lb. pentosus* 1MO drupes, subjected to fast cryofixation and maintained frozen hydrated during the analysis, allowed us to better distinguish the occurrence of real structural modification occurring inside the tissues, retaining, at the same time, the original distributions of inorganic elements inside the cells. Nevertheless, in Coratina olives, no cell separation was found between the parenchyma cells, of both the epicarp and mesocarp, neither without nor even after the fermentation process. Rather, transversal freeze-fracture images showed strong modifications of both the cuticle and parenchyma cell content due to the *Lb. pentosus* 1MO fermentation process (**Figures 4** and **6**), in spite of the relatively short time of application. The similarities of Na and Cl pattern distribution in both samples, as shown by EDXMA dot maps (**Figures 7** and **8**), demonstrate that the skin degeneration phenomenon was not due to the salt uptake.

In conclusion, the modifications observed in the fermented olive can explain the release of the phenol in the brine after olive fermentation by *Lb. pentosus* 1MO. In fact, it is possible to suppose that the degradation of the skin cuticle reduces the capability of olive pulp cells to retain phenols. These structural modifications, in combination with the hydrolysis of the secoiridoid glucosides, which reduces the molecular weight of phenolic compounds, improving their potential release from the olive cell tissues, can explain the strong release of phenols in brines observed after biological debittering. Thus, the surface and parenchyma cell structure modifications seem to justify the verified, quick release of phenols in brines olive biological debittering.

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