



Review

Starter Cultures for the Production of Fermented Table Olives: Current Status and Future Perspectives

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Abstract: Table olives are widely produced and consumed in the Mediterranean area. The production of table olives is mainly based on spontaneous fermentations, which may have several drawbacks (e.g., the development of undesirable microorganisms; organoleptic defects) compared to fermentative processes driven by starter cultures (typically lactic acid bacteria, yeasts, or their combinations). Studies on the effect of starter cultures have been mainly focused on some technological traits (e.g., acidifying capability, the degradation of phenolic compounds, metabolite production) and, to a lesser extent, on the dynamics of olive microbiota during fermentation. Recently, the application of Ampli-con Targeted—High-Throughput Sequencing (AT-HTS) has enabled improvement of the knowledge on the composition and evolution of microbial communities during fermentations, including the role of starter cultures. The AT-HTS approaches used so far, however, have several constraints (e.g., poor investigation of mycobiota and metabolically active microorganisms) that do not allow a full understanding of the complex microbial interactions occurring in fermented olives. The aim of this review is to provide insights into the role of starter cultures in fermented olives and highlight the need to apply, as for other fermented foods, integrated “omics” approaches to predict and exploit their metabolic potential to improve the final properties of products.

Keywords: starters; lactic acid bacteria; yeasts; spontaneous fermentation; driven fermentation; olive microbiota; omics approaches



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1. Introduction

Fermented table olives are a traditional food widely consumed in the Mediterranean area, with a significant economic impact. The worldwide production of table olives for the season 2023/24 was about 2.65 million tons, 23% of which was produced and consumed in several European countries, such as Spain, Greece, Italy, and Portugal, with an average per capita consumption in the EU of approximately 2.0 kg/year [1].

According to trade standards [2], table olives can be processed and classified as summarized in Figure 1: (a) treated table olives (lye-treated olives; often referred to as *Spanish-style* olives), de-bittered with an alkaline solution (1.5–4.5% *w/v* of NaOH), rinsed with tap water, packed in brine (10–11% *w/v* of NaCl), and fermented for 3–7 months, mainly by lactic acid bacteria (LAB); (b) natural table olives (also called *Greek-style* olives), directly placed in brine (6–10% *w/v* of NaCl) and fermented for 8–12 months by yeasts and/or LAB (depending on the salt concentration); (c) dehydrated and/or shrivelled table olives, exposed (or not) to a mild alkaline treatment, and then stored in brine or partially dehydrated in dry salt (15% *w/w* of NaCl) and/or by heating or other technological process; (d) olives darkened by oxidation (*Californian-style* olives), preserved in brine, fermented or not, darkened by oxidation in an alkaline medium, stored in hermetically sealed containers, and sterilized; (e) specialties, i.e., olives that can be prepared with processes other than those listed above (e.g., Castelvetrano Sicilian-style green olives, Taggiasca Ligurian-style

black olives, Maiatica di Ferrandina-oven-dried black olives produced in Italy [3]; and “alcaparras” produced in Portugal [4]).

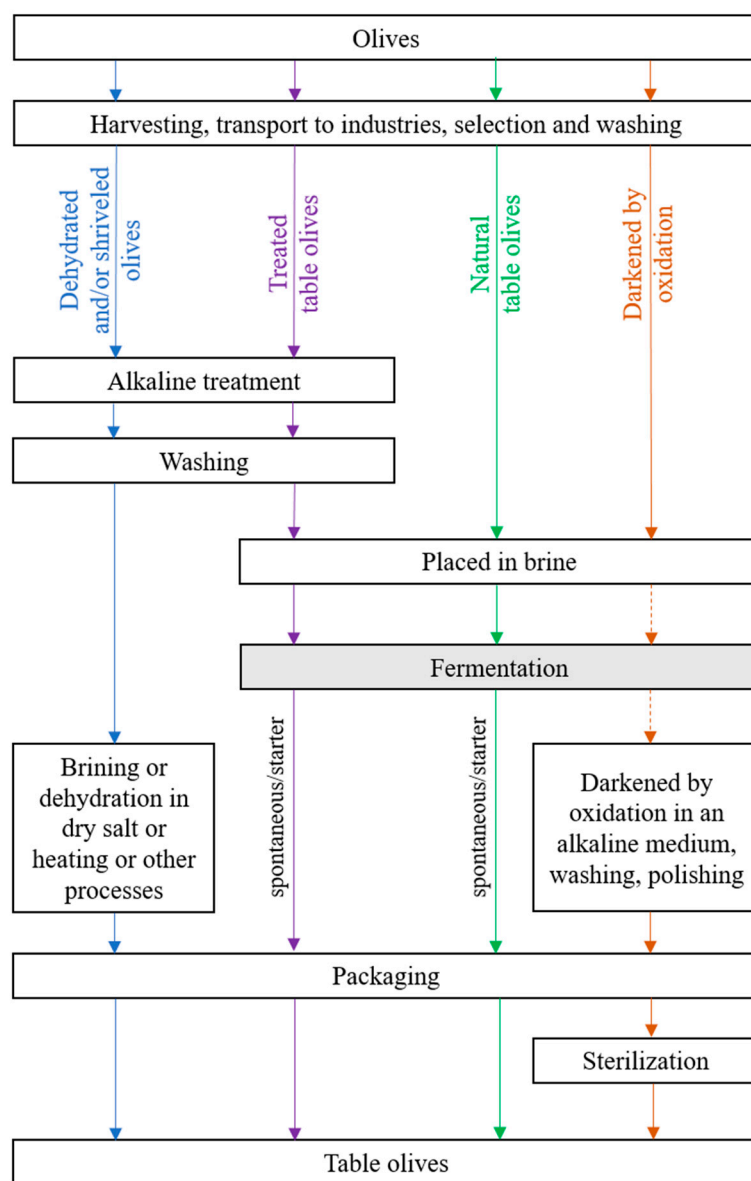


Figure 1. Schematic representation of the main steps in table olive production ([5], modified). Dotted lines indicated optional steps.

In fermented olives, the fermentative process is almost always spontaneous and mainly depends on the drupes-associated microbiota.

2. The Microbiota of Spontaneous Fermentation

The microbial communities of fermented table olives partially reflect that of unprocessed fruits, and the environment, equipment, and operative conditions (e.g., alkaline treatment, temperature, salt content, and fermentation) during production may strongly affect the composition and dynamism of the olives-associated microbiota.

The microbiota of fresh drupes is complex and mainly consists of yeasts and bacteria [6–12], whose composition in species and strains depends on the geographical area, olive cultivars, agronomic practices, degree of ripening, harvesting time, and techniques and storage conditions before processing [13–15].

The microbiota of unprocessed fruits may lead to spontaneous fermentation, and the effects on the final properties of products (e.g., quality improvement, or alteration and deterioration phenomena) depend on the ability of microbial groups to survive and cope with factors associated with the production process and thrive under the selective conditions of fermentation.

LAB and/or yeasts (Y), generally, dominate the spontaneous fermentation process. Among LAB, *Lactiplantibacillus plantarum* and *Lpb. pentosus* are the most abundant isolated species, although other lactobacilli (*Lpb. paraplantarum*, *Companilactobacillus alimentarius*, *Comp. farciminis*, *Levilactobacillus brevis*, *Loigolactobacillus coryniformis*, *Limosilactobacillus fermentum*, *Lacticaseibacillus casei*, *Lcb. paracasei*) were also found in several fermented products of Mediterranean countries [5,16,17]. Members of the genera *Leuconostoc* (mainly *Leuc. mesenteroides/paramesenteroides*), *Pediococcus* (mainly *Pc. ethanolidurans*, *Pc. acidilactici*, *Pc. pentosaceus*), *Enterococcus* (mainly *E. durans*, *E. faecalis*, *E. faecium*) and *Lactococcus* (*Lc. lactis*) have also been found in fermented table olives.

The yeast microbiota mainly consists of species belonging to the genera *Candida* (*C. diddensiae*, *C. boidinii*, *C. tropicalis*, and to a lesser extent *C. aaseri*, *C. apicola*, *C. oleophila*, *C. olivae*, *C. parapsilosis*, *C. quersitrusa*, *C. sorbose*, *C. tartarivorans*), *Pichia* (*P. membranifaciens*, and to a lesser extent *P. galeiformis*, *P. guilliermondii*, *P. kluyveri*, *P. kudriavzevii*, *P. manshurica*), *Saccharomyces* (*S. cerevisiae*), *Debaryomyces* (*D. hansenii*) and *Wickerhamomyces* (*W. anomalous*); however, other non-dominant species contribute to the yeast diversity of fermented table olives (*Aureobasidium pullulans*, *Metschnikowia pulcherrima*, *Rhodotorula glutinis*, *R. graminis*, *R. diobovatum*, *R. mucilaginoso*, *Schwanniomyces ethcellsii*, *Zygowilliopsis californica*, *Zygosaccharomyces mrakii*; [17–21]).

The use of High-Throughput Sequencing (HTS) technologies for the assessment of table olive microbiota [12–22], has revealed a wider diversity among microbial communities, and several other genera (also including halophilic and alkaliphilic LAB) have been recognised as important members of table olive microbiota (i.e., *Marinilactibacillus* spp. [23–26]; *Alkalibacterium* spp. [25,26]; *Celerinatantimonas* spp. [19,20,24,26]; *Halomonas* spp. [23,25]).

Moreover, spoilage bacteria (e.g., *Enterobacteriaceae*, *Clostridium* spp., *Marinomonas* spp. [27], *Pseudomonas* spp., *Staphylococcus* spp., *Vibrio* spp.) and occasionally moulds (e.g., *Penicillium* spp., *Aureobasidium* spp., *Geotrichum* spp.) can be found in the early stage of fermentation (slow and acidification) or in low-quality table olives [5,7,12,22,28].

During the olive fermentation, bacteria and yeasts coexist, but population dynamics and balance depend on several factors, such as type of process (NaOH-treated vs. natural), as well as on the fermentation starting parameters (salt concentration, pH, temperature, etc.).

In the first stage of the lye-treated process (reduced content of polyphenols; pH ranging from 8.0 to 9.0), members of *Enterobacteriaceae* and halophilic/alkaliphilic LAB are dominant. In the middle stage of the process, when the brine pH decreases (up to 6.0–7.0), *Leuconostoc* ssp. and *Pediococcus* spp. can be detected, and the genus *Lactiplantibacillus* gradually increases in abundance, becoming foremost at the end of fermentation (10^7 – 10^8 cfu/mL), leading to a significant lowering of the brine pH (down to 4.0) and, consequently, a reduction in the abundance of other microbial groups [23,29]. Yeast populations may reach 10^4 – 10^6 cfu/mL at the end of the process [12,13,30].

The composition of microbiota present in Greek-style processes is slightly more complex. In the first step of fermentation, several drupes-associated bacteria (e.g., *Enterobacteriaceae*, *Pseudomonas* spp., *Vibrio* spp. and *Clostridium* spp.) as well as halophilic/halotolerant bacteria (mainly from brine, e.g., *Halomonas* spp., *Salinicola* spp., *Marinobacter* spp., *Aliidiomarina* spp.) are mainly found, while LAB generally do not exceed 10^4 – 10^5 cfu/mL [12]. Nutrient availability, microbial competition and phenolic compound content, in fact, may inhibit LAB populations and reduce their occurrence during fermentation. Yeasts, being more tolerant than LAB to high concentrations of salt and phenolic compounds, can become the dominant group (10^7 – 10^8 cfu/mL) at the end of fermentation. The composition and

the diversity of yeast biota, however, may differ from those found in a NaOH-treated process [29–32].

In spontaneous fermentations, regardless of the type of process, the right ratio between LAB and yeasts, as well as a low occurrence of undesirable microorganisms, is suitable for obtaining good-quality products [12,33–35]. In non-optimal fermentative conditions (e.g., reduced salt content; slow and insufficient acidification, pH above 4.0), in fact, the development of undesired microorganisms (e.g., proteolytic bacteria, heterofermentative LAB, pectinolytic yeasts) may lead to several organoleptic defects (e.g., off-flavours and off-odours, swelling, drupe softening) that can significantly affect the quality of the final product [34,36–39]. Moreover, although the occurrence of foodborne pathogens in table olives is low, several studies (challenge test-based data) confirmed their survival in conditions occurring in the early stages of fermentation [40–42].

Therefore, controlling spoilage microbiota and potential pathogens during the fermentative process is crucial to guarantee the stability, the organoleptic features and the safety of table olives, especially for those commercialised without heat treatments.

3. Starter Cultures in Driven Fermentations

To date, the production of table olives is mainly based on spontaneous fermentations, which may have several drawbacks (see Section 2) compared to fermentative processes driven by starter cultures. The use of appropriate LAB, yeasts or their combinations, in fact, may help prevent failures occurring in brine fermentations with indigenous microbiota and may contribute to the production of high-quality products.

The main species used as starter cultures for the production of table olives have been widely reviewed [5,7,12–14,16,28] and are reported in Tables 1 and 2.

Selection criteria (i.e., ability to cope with harsh conditions, biofilm formation, adhesion to olive surface, hydrolysis of phenolic compounds, debittering capability, brine acidification, production of antimicrobial compounds) also have been extensively addressed [5,7,12–14,16,28].

The effects of starter cultures, of course, depend on several factors, including the type of process (e.g., starters are mainly used for *Spanish-style* olives), the type of microorganisms (LAB and yeasts have different functionalities) and the type of inoculum (single strain, mixtures of LAB or yeasts or LAB/yeasts; Tables 1 and 2).

3.1. Effect of Starter Cultures on the Features of Fermented Table Olives

During the fermentative processes, LAB (mainly *Lpb. plantarum* and *Lpb. pentosus*) promote brine acidification through the production of lactic acid from fermentable sugars, lower the pH, and increase in titratable acidity, which may inhibit the growth of spoilage microorganisms, improving the microbiological stability and shelf-life of the final product. Additionally, several strains may synthesize antimicrobial compounds (e.g., bacteriocins active against some pathogens) that, further, contribute to the preservation and safety of table olives [5,7,12–14,28].

In natural table olives, LAB are involved in the hydrolysis of oleuropein (because of β -glucosidase activity), reducing the debittering time and increasing the formation of some phenolic compounds (e.g., hydroxytyrosol) that improve the nutritional value of the products [43,44].

Yeast starters (mainly belonging to *C. boidinii*, *C. diddensiae*, *D. hansenii*, *S. cerevisiae*, *W. anomalus*, alone or in combination with LAB) also contribute to the debittering and degradation of phenolic compounds. Some strains, moreover, prevent oxidation of unsaturated fatty acid and peroxide formation (due to catalase and peroxidase activities; [45,46]) and lead to the production of important compounds (e.g., vitamins, amino acids, purines, and hydrocarbons [7,28,33,47]) that stimulate the growth of LAB.

For a long time, studies on the effect of starter cultures were mainly focused on the changes in pH, titratable acidity, phenolic compounds (total concentration; sometimes the overall profile) and production of the main metabolites (e.g., lactic and acetic acids,

ethanol). Recent data based on chromatographic analyses (i.e., Headspace-Solid-Phase Micro-Extraction/Gas Chromatography-Mass Spectrometry, HS-SPME/GC-MS; High Performance Liquid Chromatography, HPLC; Liquid Chromatography with tandem Mass Spectrometry, LC-MS/MS), combined with sensory evaluation, confirmed that LAB and yeast metabolism positively affected the aroma profile (organic acids, volatile organic compounds), texture (brightness, crunchiness, colour) and sensory properties (high overall acceptability) of table olives. Table 1 shows the effects of starter cultures on the phenolic profile and organoleptic properties of different types of fermented olives. For a better understanding of data, we reported only the trials that included also the spontaneous fermentations as control.

3.2. Effect of Starter Cultures on the Microbiota of Fermented Table Olives

Metataxonomic studies have increased knowledge on the microbiota of fermented olives, revealing a complex microbial diversity that could not be detected using the conventional culture-dependent methods. Amplicon Targeted High-Throughput Sequencing (AT-HTS) techniques (16S rRNA- and ITS-based, respectively, for bacterial and fungal communities) have been widely used to investigate the microbial ecology of spontaneous fermentation [17,19,20,24–26,29,48–51] and to describe the microbiota of commercial table olives, [27,52–56], mainly belonging to the Italian and Spanish varieties (both with natural and lye-treated methods).

The first metataxonomic approach was applied by Cocolin et al. [23], to investigate the effect of NaOH treatment on the microbial ecology of *Nocellara Etnea* olives; since then, many other studies have been performed [12], allowing the discovery of dominant, emerging and spoilage species of fermented table olives.

However, only a few studies have been focused on the effect of starter cultures on the dynamics of olive microbiota during fermentation. Most of the available data are based on experimental evidence obtained with traditional culture-dependent methods (i.e., plate counting; [8,15,44,57–60]), which indicate how starter cultures affect the evolution of microbial groups (e.g., bacteria, yeasts) during fermentation, but do not give a detailed description of changes occurring in the composition of microbial communities of fermented olives.

De Angelis et al. [61], for the first time, applied a metataxonomic approach to investigate the effect of different starter cultures on the microbiota of *Bella di Cerignola* olives. Subsequently, some other studies (Table 2) have been carried-out, confirming the competitiveness of starters (mainly *Lpb. plantarum* and *Lpb. pentosus*) and the ability to reduce undesired bacteria (e.g., *Enterobacteriaceae*, some halophilic and alkalophilic bacteria). The effect of starters on other LAB species, on the contrary, depended on the type of cultivar and process. However, almost all studies reported in Table 2 (except data from Ruiz-Barba et al. [62]) referred only to the bacterial diversity (16S rRNA-based), but did not consider the effect of starter cultures on the changes in fungal communities. These data suggest that future metataxonomy and metagenomic studies should also be tuned to the study of mycobiota, to have a more faithful description of the evolution of microbial communities in fermented olives. In Table 2, we report only the trials that included spontaneous fermentations as control.

Table 1. Effect of starter cultures on metabolite production and phenolic compounds of fermented table olives.

Olive cultivar	Type of Process and Fermentative Conditions	Species and Strains Used as Starter Cultures	Effect on Metabolite Production and Phenolic Compounds	Analytical Method [†]	Ref.
Starter cultures: Lactic Acid Bacteria (*)					
Tonata di Cagliari (Sardinia, Italy)	Natural process: Greek-style. Brine: 7% (w/v) NaCl. Fermentation time and T °C: 156 days at 27 °C, up to pH steady-state, and then 24 °C.	LAB starter SSL: <i>Lpb. plantarum</i> S1T10A; SIE: undefined autochthonous mixed starter of <i>Lpb. pentosus</i> . NF: natural fermentation, control. Inoculum level: 6.82 (SSL) and 7.25 (SIE) log cfu/mL	Final-stage fermentation (156 days, olives)—NF: lowest levels of hydroxytyrosol and highest levels of oleuropein. SIE and SSL: higher concentration of hydroxytyrosol. SSL, SIE: completely debittering after 156 days; NF needed 12 months. NF sample had higher cohesiveness, springiness. SIE: more elasticity and cohesiveness than SSL samples.	HPLC	[63]
Kalamon (Peloponnese, Greece)	Natural process: Greek-style. Brine: 5% (w/v) NaCl. Fermentation time and T °C: 70 days at room T °C (22 °C).	LAB starter B1: spontaneous fermentation. B2: <i>Lpb. pentosus</i> DSM 16366; B3: <i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i> Lm139. Inoculum level: 6 log cfu/mL	Early-stage fermentation (7–10 days, brines)—B2, B3: faster glucose consumption (7 days) compared to B1 (10 days); B2, B3: mannitol consumption was observed. Final-stage fermentation (70 days, brines)—B2, B3: higher amount of lactic, acetic and succinic acids compared to B1.	HPLC	[60]
Nocellara Etna (Adriano, Sicily, Italy)	Natural process: Sicilian style. Brine: 8% (w/v) marine salt. Fermentation time and T °C: 120 days at room T °C (20 ± 2 °C)	LAB starter BC1: <i>Lpb. plantarum</i> UT2.1, <i>Lcb. paracasei</i> N24, <i>Lpb. pentosus</i> TH969; BC2: <i>Lpb. plantarum</i> UT2.1; BC3: <i>Lcb. paracasei</i> N24, <i>Lpb. pentosus</i> TH969; BC4: <i>Lpb. plantarum</i> UT2.1, <i>Lpb. pentosus</i> TH969; BC5: <i>Lpb. plantarum</i> UT2.1, <i>Lcb. paracasei</i> N24; BC6: <i>Lpb. pentosus</i> TH969. C: uninoculated samples. Inoculum level: 7 log cfu/mL	Early-stage fermentation (1 day, brines)—C, BC1, BC2: high aldehydes content. Correlation among aldehydes and alcohols (octanal, nonanal, decanal, 1-nonanol, 1-undecanol, 1-dodecanol, and 1-octanol) and presence of <i>Proteobacteria</i> . Middle-stage fermentation (60 days, brines)—All samples: aldehydes content decreased, whereas alcohols, acids, esters and phenols increased. Final-stage fermentation (120 days, brines)—BC3, C: highest VOC content; BC1: lowest VOC content. C: high ethanol content. Correlation among ester products (ethyl-acetate, ethyl-propanoate, ethyl-butanoate, ethyl-lactate, butanoic acid 2-methylester, butanoic-acid 3-methylester, ethyl decanoate, and ethyl-benzoate) and <i>Sib. paracollinoides</i> , <i>Pc. parvulus</i> , and <i>Lgb. acidipiscis</i> (BC1, BC3, BC4, BC6).	SPME/ GC-MS	[64]
Itrana (Fogliano, Rocca Massima, Latium, Italy)	Natural process: Greek-style. Brine: 6% (w/v) NaCl. Fermentation time and T °C: 30 days at room T °C.	LAB starter A: Fogliano, less irrigation, <i>Lpb. pentosus</i> C8 and C11; B: Fogliano, more irrigation, <i>Lpb. pentosus</i> C8 and C11; C: Rocca Massima, rainfed, <i>Lpb. pentosus</i> C8 and C11; D: Rocca Massima, irrigation, <i>Lpb. pentosus</i> C8 and C11. Ac, Bc, Cc, Dc: related spontaneous fermentation. Inoculum level: 8 log cfu/mL	Final-stage fermentation (120 days, olives pulp)—Inoculated samples provided a strong decrease in oleuropein, dimethyl oleuropein, and 3,4-DHPEA-EDA, and an increase in hydroxytyrosol.	HPLC	[15]

Table 1. Cont.

Olive cultivar	Type of Process and Fermentative Conditions	Species and Strains Used as Starter Cultures	Effect on Metabolite Production and Phenolic Compounds	Analytical Method [†]	Ref.
<i>Nocellara Etna</i> (Adrano, Sicily, Italy)	Natural process: Sicilian style. <u>Brine</u> : 4, 5, 6, 8% (<i>w/v</i>) NaCl. <u>Fermentation time and T °C</u> : 120 days at room T °C (18 ± 2 °C).	<u>LAB starter</u> <i>Lpb. plantarum</i> UT2.1 and <i>Lcb. paracasei</i> N21 (1:1 ratio). E1–E4 : inoculated fermentations with 4, 5, 6, 8% NaCl, respectively. C1–C4 : related spontaneous fermentations. <u>Inoculum level</u> : 7 log cfu/mL	<u>Final-stage fermentation (120 days, brines)</u> — E1–E4 : high content of hexanoic and propionic acids, low content of acetic acid, ethanol, isoamyl and phenyl-ethyl alcohol. E2 : highest content of esters (butanoic-acid-2-methylester), acids, alcohols, phenols, aldehydes. C : high acidity. E1–E4 : high acceptability score.	SPME/ GC-MS	[8]
<i>Picala</i> (whole and cracked olives; Nicosia, Cyprus)	Natural process: Greek-style. <u>Brine</u> : 7% (<i>w/v</i>) or 10% (<i>w/v</i>) NaCl and 3.3% (<i>v/v</i>) citric acid. <u>Fermentation time and T °C</u> : 120 days at room T °C (23 ± 2 °C)	<u>LAB starter</u> S1, S4 : spontaneous fermentations in cracked and whole olives, respectively. S2, S5 : commercial starter <i>Lpb. plantarum</i> (Vege-Start 600, Chr. Hansen A/S) in 10% NaCl and 3.3% citric acid, cracked and whole olives, respectively; S3, S6 : <i>Lpb. plantarum</i> (Vege-Start 600) in 7% NaCl and 3.3% citric acid, cracked and whole olives, respectively. <u>Inoculum level</u> : 5 log cfu/mL	<u>Middle-stage fermentation (60 days, brines)</u> — All inoculated samples : rapid glucose depletion (undetectable after 45 days of fermentation). <u>Final-stage fermentation (120 days, brines)</u> — All inoculated samples : highest lactic acid content, production of succinic acid and a faster degradation of oleuropein, resulting in the production of higher levels of hydroxytyrosol (especially in S3 and S6 samples) compared to the control ones (S1 and S4).	HPLC	[43]
<i>Tonida di Cagliari</i> (Sardina, Italy)	Natural process: Greek-style. <u>Brine</u> : 7% (<i>w/v</i>) NaCl. <u>Fermentation time and T °C</u> : 180 days at 25 °C	<u>LAB starter</u> SIE : undefined mixed culture of <i>Lpb. pentosus</i> strains isolated from previous fermentations; Double-strain starter (DSS): <i>Lpb. pentosus</i> D104 and D702 isolated from SIE. NF : spontaneous fermentation. <u>Inoculum level</u> : 6 log cfu/mL	<u>Final-stage fermentation (180 days, olive pulp)</u> — SIE : undetectable level of oleuropein and high content of hydroxytyrosol, followed by verbascoside, tyrosol, luteolin, luteolin 7-glucoside. DSS and NF : comparable level of tyrosol, luteolin, luteolin 7-glucoside.	HPLC	[65]
<i>Nocellara Etna</i> (Adrano and Paternò, Sicily, Italy)	Natural process: Sicilian style. <u>Brine</u> : 5% or 8% (<i>w/v</i>) NaCl. <u>Fermentation time and T °C</u> : 80 days at room T °C (18 ± 2 °C)	<u>LAB starter</u> O1 : <i>Lpb. plantarum</i> F1.16 and F3.5, in 5% NaCl. O2 : <i>Lpb. plantarum</i> C11C8, F1.16, and F3.5, in 5% NaCl. C5 and C8 : uninoculated samples with 5% or 8% NaCl, respectively. <u>Inoculum level</u> : 7 log cfu/mL	<u>Middle-stage fermentation (15 days, olives)</u> — O2 : highest content of 2-butanone-3-hydroxy (acetoin), ethyl acetate, and lactic acid ethyl ester. <u>Final-stage fermentation (80 days, olives)</u> — O2 : highest content of acetic acid, 3-methyl-1-butanol, esters (phenylethyl alcohol, acetic acid 2-methyl ester, 2-heptanal, benzene propanoic acid methyl ester (with pleasant flavours)). C5, C8 : higher content of 4-ethyl-phenol, 2-methoxy-phenol, associated with off-flavour.	HS-SPME/ GC-MS	[66]

Table 1. Cont.

Olive cultivar	Type of Process and Fermentative Conditions	Species and Strains Used as Starter Cultures	Effect on Metabolite Production and Phenolic Compounds	Analytical Method [†]	Ref.
Itrana (Latina province, Italy)	Natural process: Greek-style. Brine: 8% (w/v) NaCl (sea salt). Fermentation time and T °C: 240 days at room T °C.	LAB starter A: spontaneous fermentation. B: <i>Lpb. plantarum</i> B1; C: <i>Lpb. plantarum</i> B1, <i>Lpb. plantarum</i> B51, <i>Lpb. plantarum</i> B124 (1:1:1 ratio). Inoculum level: 6 log cfu/mL	Final-stage fermentation (240 days, olives)—B, C: reduced debittering time, faster degradation of secoiridoids and higher production of hydroxytyrosol (especially in C, probably due to the multiple-strain starter).	HPLC	[44]
Manzanilla (Seville province, Spain)	Treated process: 2.3% NaOH solution for 5.5 h. Brine: 12.0% (w/v) NaCl. Fermentation time and T °C: 7 months; T °C: n.a. (**)	LAB starter M1, M2: spontaneous fermentation. M3, M4: <i>Lpb. pentosus</i> LP99. Inoculum level: 8 log cfu/mL at day 5, 7 log cfu/mL at day 12.	Final-stage fermentation (7 months, olive pulp)—All samples: fermentation significantly increased the number and concentrations of VOCs. M3, M4: highest amounts of 1-heptanol, 6-methyl-5-hepten-2-ol, isoamyl acetate, methyl benzoate and of 4-ethylphenol. The latter compound was associated with off-flavour, but the sensory evaluation indicated that not-significant differences were found among driven and spontaneous fermentations.	HS-SPME/ GC-MS	[67]
Starter cultures: Lactic Acid Bacteria and Yeasts					
Arbequina (Tarragona, Spain)	Natural process: Greek-style. Brine: 8% NaCl. Fermentation time and T °C: 52 days at room T °C (20 °C).	LAB and Y starter A: Spontaneous fermentation. B: <i>C. diddensiae</i> C6B19; C: <i>Lpb. plantarum</i> V10A2; D: <i>Lpb. pentosus</i> FxMA1; E: <i>Lpb. pentosus</i> 5E3A18; F: <i>Lpb. pentosus</i> 5E3A18, <i>C. diddensiae</i> C6B19; G: <i>Lpb. pentosus</i> 5E3A18, <i>Lpb. plantarum</i> V10A2; H: <i>Lpb. pentosus</i> 5E3A18, <i>Lpb. pentosus</i> FxMA1. Inoculum level: 6 log cfu/mL	Final-stage fermentation (90 days)—Y and LAB/Y starter: higher content of citric acid, lower content of malic acid (B, F). LAB starter: G and H significant increase in lactic acid compared with single LAB starter (C, D, E fermentations). D: high content of acetic acid.	HPLC	[68]
Bella di Cerignola (Modugno, Apulia, Italy)	Natural process: Greek-style. Brine: 7% (w/v) NaCl. Fermentation time and T °C: 90 days at room T °C (18–25 °C).	LAB and Y starter S: commercial <i>Lpb. plantarum</i> (Sacco Srl company); SY: commercial <i>Lpb. plantarum</i> (Sacco Srl) and autochthon <i>Wickerhamomyces anomalus</i> DiSSPA73; SYL: commercial <i>Lpb. plantarum</i> (Sacco Srl) and autochthons <i>W. anomalus</i> DiSSPA73, <i>Lpb. plantarum</i> DiSSPA1A7, <i>Lpb. pentosus</i> DiSSPA7. Ctrl: spontaneous fermentation. Inoculum level: 7 log cfu/mL	Final-stage fermentation (90 days, brines)—SY: ethanol (↑); S, SY, SYL: acetic acid (↑), ethyl acetate (↑), 1-hexanol (↑), propionic acid (↓).	SPME/GC-MS	[61]

Table 1. Cont.

Olive cultivar	Type of Process and Fermentative Conditions	Species and Strains Used as Starter Cultures	Effect on Metabolite Production and Phenolic Compounds	Analytical Method †	Ref.
<i>Cellina di Nardò, Leccino, Kalamàta, Conservolea</i> (Salento, Apulia, Italy) (Salento, Apulia, Italy)	Natural process: Greek-style. Brine: 8% (w/v; for <i>Kalamàta</i> and <i>Conservolea</i> cv) or 12% (w/v; for <i>Cellina di Nardò</i> and <i>Leccino</i> cv) NaCl. Fermentation time and T °C: 90 days at room T °C.	LAB and Y starter (sequential inoculum) <i>Leccino</i> cv: <i>S. cerevisiae</i> LI 180-7 (DSMZ27800) and then <i>Lpb. plantarum</i> L 180-11 (DSMZ27925); <i>Cellina di Nardò</i> cv: <i>P. anomala</i> CL 30-29 and then <i>Lpb. plantarum</i> C180-34; <i>Kalamàta</i> cv: <i>S. cerevisiae</i> KI 30-16 (DSMZ27801) and then <i>Leuc. mesenteroides</i> K T5-1 (DSMZ27926); <i>Conservolea</i> cv: <i>D. hansenii</i> A15-44 and then <i>Lpb. plantarum</i> A135-5. Control spontaneous fermentation. Inoculum level: 6 log cfu/mL	Final-stage fermentation (90 days)— Inoculated samples: complete consumption of glucose (except for <i>Leccino</i> cv); high levels of lactic (<i>Leccino</i> and <i>Conservolea</i> cv) and acetic (<i>Cellina di Nardò</i> and <i>Kalamàta</i> cv) acids. Higher content of hydroxytyrosol and tyrosol. VOCs (mainly alcohols and esters) increased in starter-driven fermentations, especially when Y were used. The use of starter significantly reduced the time of fermentation process from 180 to 90 days.	HPLC SPME/ GC-MS	[39]
<i>Kalamàta</i> and <i>Conservolea</i> (Arta, Greece)	Natural process: Greek-style. Brine: 8% NaCl. Fermentation time and T °C: 105 days at room T °C (12–21 °C).	LAB and Y starter Sequential inoculum: <i>Kalamàta</i> cv— <i>Leuc. mesenteroides</i> KT5-1, then <i>S. cerevisiae</i> KI30-16 (LY) or viceversa (YL). <i>Conservolea</i> cv: <i>Lpb. plantarum</i> A135-5, then <i>D. hansenii</i> A15-44 (LY) or viceversa (YL). Mixtures (MIX): <i>Kalamàta</i> cv— <i>Leuc. mesenteroides</i> KT5-1 and <i>S. cerevisiae</i> KI30-16. <i>Conservolea</i> cv: <i>Lpb. plantarum</i> A135-5 and <i>D. hansenii</i> A15-44 (LY). Sp: spontaneous fermentation. Inoculum level: 8 log cfu/mL	Final-stage fermentation (105 days)— <i>Conservolea</i> olives: YL and LY: higher level of esters and alcohols; LY: high content of terpenes; MIX and YL: high amount of hydrocarbons. <i>Kalamàta</i> olives: MIX: higher levels of esters, alcohols, hydrocarbons and terpenes. Middle-stage fermentation (14 and 63 days, brines)— <i>Conservolea</i> brines: MIX and LY: presence of oleoside after 14 and 63 days of fermentation, respectively. Final-stage fermentation (105 days, brines)— <i>Conservolea</i> brines, YL: higher content of tyrosol and hydroxytyrosol. <i>Kalamàta</i> brines: YL: caffeic and coumaric acid were not detected; MIX: high oleuropein content; LY: higher content of decarboxymethyl elenolic acid linked to hydroxytyrosol.	SPME/ GC-MS LC-MS/MS	[69]

Table 1. Cont.

Olive cultivar	Type of Process and Fermentative Conditions	Species and Strains Used as Starter Cultures	Effect on Metabolite Production and Phenolic Compounds	Analytical Method [†]	Ref.
Manzanilla (Seville region, Spain)	Treated process: Spanish-style; treatment with 3.2% (w/v) NaOH solution containing 2.2% (w/v) NaCl and 0.89% (w/v) CaCl ₂ for 7 h. Brine: 12.0% (w/v) NaCl, 0.13% (w/v) CaCl ₂ , and 0.08% (v/v) HCl. Fermentation time and T °C: 65 days, at uncontrolled T °C (ranging from 29 °C to 16 °C).	LAB and Y starter T1: <i>Lpb. pentosus</i> LPG1; T2: <i>Lpb. pentosus</i> Lp13; T3: <i>Lpb. plantarum</i> Lp115; T4: <i>W. anomalus</i> Y12; sequential starter T5: <i>W. anomalus</i> Y12 followed by a combination of <i>Lpb. pentosus</i> LPG1, Lp13, <i>Lpb. plantarum</i> Lp115. T6: spontaneous fermentation. Inoculum level: 7 log cfu/mL for single strains (T1, T2, T3, T4 ; after 8th days of brining); 5 log cfu/mL for <i>W. anomalus</i> Y12 (1st day of brining) and 7 log cfu/mL for LAB mixture (after 8th days of brining) in T5 fermentation.	Final-stage fermentation (65 days, brines)— T1: reduced methanol, β-damascenone, but increased 2-phenylethyl acetate, 2-butanol, 1-butanol, 3-methyl-3-buten-1-ol, 2-methyl-3-hexanol; T2: high 1-butanol content; T3: presence of methanol, isoxyaldehyde and 4-ethylphenol, and reduced content of coumarin, 5-tert-butylpyrogallol and vanillin; T4: presence of 1-butanol, ethanol, methyl acetate, ethyl acetate, 2-phenylethyl acetate, or 2-methyl-1-butanol production, and lower levels of methanol, coumarin, and vanillin; T5: high total VOC content (except for ethanol, 1-heptanol, or cis-5-octen-1-ol).	SPME/ GC-MS	[70]
Starter cultures: Yeasts					
Kalamàta, Picual and Manzanilla (Marsa Matrouh, Egypt)	Natural process: Greek-style. Brine: 11% (w/v) NaCl. Fermentation time and T °C: 40 days at room T °C (19–27 °C).	Yeasts starter M1, P1, K1: Manzanilla, Picual, Kalamàta spontaneous fermentation with 1% v/v vinegar in brines at the start of fermentation. M2, P2: Manzanilla, Picual olives with <i>S. cerevisiae</i> LI-180-7 (DSMZ27800); K2: Kalamàta olives with <i>S. cerevisiae</i> KI30-16 (DSMZ27801); M3, P3, K3: Manzanilla, Picual, Kalamàta olives with commercial <i>S. cerevisiae</i> baker's yeast. Inoculum level: 7 log cfu/mL	Final-stage fermentation (40 days; olives)— All inoculated samples: oleuropein degradation and increased hydroxytyrosol content. Compared to spontaneous fermentation, inoculated samples had a more complex profile in esters (e.g., isoamyl acetate, ethyl lactate, ethyl hexanoate, ethyl octanoate, phenyl acetate), alcohols (e.g., 2,3-methyl-1-butanol, phenylethanol, hexanol, cis 3-hexen-1-ol, 1-heptanol., associated with positive flavour (e.g., fruity-green notes).	HPLC HS-SPME/ GC-MS	[71]
Kalamàta (Northern Greece)	Natural process: Greek-style. Brine: 7% (w/v) NaCl acidified with 0.5% (v/v) vinegar (ca. 6.0%, v/v, acetic acid). Fermentation time and T °C: 150 days at room T °C.	Yeasts starter A: <i>C. boidinii</i> Y27; B: <i>C. boidinii</i> Y28; C: <i>C. boidinii</i> Y30; D: <i>C. boidinii</i> Y31; E: <i>S. cerevisiae</i> Y34; F: spontaneous fermentation. Inoculum level: 6 log cfu/mL	Final-stage fermentation (150 days)—Y starter exhibited different behaviour in metabolite production. A: highest amount of lactic and succinic acids; C: highest level of acetic and citric acids; E: highest amount of ethanol. Y27 showed the highest survival rate within olive fermentation; on the contrary, Y34 had the lowest survival.	HPLC	[72]

(*) The current taxonomy for lactobacilli (ex-*Lactobacillus* genus) was used in this study, according to Zheng et al. [73]. Genus abbreviations for lactobacilli: *Lacticaseibacillus*, *Lcb.*; *Lactiplantibacillus*, *Lpb.*; *Lentilactobacillus*, *Lnb.*; *Ligilactobacillus*, *Lgb*; *Secundilactobacillus*, *Slb*. (**): not available. [†] Analytical method abbreviations: HPLC, High Performance Liquid Chromatography; HS-SPME/GC-MS: Headspace-Solid-Phase Micro-Extraction/Gas Chromatography-Mass Spectrometry; LC-MS/MS, Liquid Chromatography with tandem Mass Spectrometry. ↑: increase; ↓: decrease.

Table 2. Effect of starter cultures on the microbiota of fermented table olives.

Olive cultivar	Type of Process and Fermentative Conditions	Species and Strains Used as Starter Cultures	Effect on the Microbiota	Sequencing Method	Ref.
Starter cultures: Lactic Acid Bacteria (*)					
<i>Nocellara Etnica</i> (Adrano, Sicily, Italy)	Natural process: Sicilian style, without lye treatment. Brine: 8% (w/v) marine salt. Fermentation time and T °C: 120 days at room T °C (20 ± 2 °C).	LAB starter BC1: <i>Lpb. plantarum</i> UT2.1, <i>Lcb. paracasei</i> N24, <i>Lpb. pentosus</i> TH969; BC2: <i>Lpb. plantarum</i> UT2.1; BC3: <i>Lcb. paracasei</i> N24, <i>Lpb. pentosus</i> TH969; BC4: <i>Lpb. plantarum</i> UT2.1, <i>Lpb. pentosus</i> TH969; BC5: <i>Lpb. plantarum</i> UT2.1, <i>Lcb. paracasei</i> N24; BC6: <i>Lpb. pentosus</i> TH969. C: uninoculated samples. Inoculum level: 7 log cfu/mL	Early-stage fermentation (1-day, brines)—All samples: <i>Halomonas</i> spp., <i>Achromobacter</i> spp., <i>Marinobacter</i> spp., <i>Serratia</i> spp., <i>Bradyrhizobium</i> spp. were the most abundant genera in C samples; lactobacilli (ex- <i>Lactobacillus</i> genus) dominated all inoculated samples. Middle-stage fermentation (60 days, brines)—BC2, BC3: <i>Salinicola</i> spp. (↑). BC2, BC3, BC6: <i>Enterobacteriaceae</i> (↑). BC3, BC6: <i>Pediococcus parvulus</i> (↑), <i>Secundilactobacillus paracollinoides</i> (↑), <i>Ligilactobacillus acidipiscis</i> (↑), <i>Salinicola</i> spp. (↑). BC6: <i>Lactococcus lactis</i> (↑). BC3, BC6: <i>Lpb. plantarum</i> (↓). Final-stage fermentation (120 days, brines)—BC1, BC3, BC4, BC6: <i>Pediococcus parvulus</i> (↑), <i>Slb. paracollinoides</i> (↑), <i>Lgb. acidipiscis</i> (↑). BC1, BC2, BC3, BC4, BC5: <i>Salinicola</i> spp. (↑). BC2: <i>Marinilactibacillus</i> spp. (↑), <i>Halomonas</i> spp. (↑). BC5: <i>Lcb. casei/paracasei</i> group (↑). BC2, BC5, C: <i>Lpb. plantarum</i> (↑). BC1, BC3, BC4, BC6: <i>Lpb. plantarum</i> (↓), although remained the most abundant group. All inoculated samples: <i>Enterobacteriaceae</i> (↓).	Ion Torrent PGM (V3 region of 16S rRNA gene)	[64]
<i>Pitaval</i> (whole and cracked olives; Nicosia, Cyprus)	Natural process: Greek-style. Brine: 7% (w/v) or 10% (w/v) NaCl and 3.3% (v/v) citric acid. Fermentation time and T °C: 120 days at room T °C (23 ± 2 °C).	LAB starter S1, S4: spontaneous fermentations in cracked and whole olives, respectively. S2, S5: commercial starter <i>Lpb. plantarum</i> (Vege-Start 600, Chr. Hansen A/S) in 10% NaCl and 3.3% citric acid, cracked and whole olives, respectively. S3, S6: <i>Lpb. plantarum</i> (Vege-Start 600) in 7% NaCl and 3.3% citric acid, cracked and whole olives, respectively. Inoculum level: 5 log cfu/mL	Early-stage fermentation (1-day, olives)—All samples: <i>Thermogemmatispora onikobensis</i> , <i>Chitinophaga soli</i> (dominant species), <i>Thiomonas thermosulfata</i> , <i>Bradyrhizobium pachyrhizi</i> (co-dominant species), <i>Lewinella lutea</i> , <i>Brevibacterium casei</i> , <i>Lpb. plantarum</i> group (secondary species). Middle- and final-stage fermentation (60 and 120 days, brines)—S2, S5, S3, S6: <i>Lpb. plantarum</i> group (↑), <i>Lcb. manihotivorans</i> (↑). S1: <i>Lcb. brantae</i> (↑), <i>Lentilactobacillus parakefiri</i> (↑); <i>Lcb. plantarum</i> group (↓). S4: high diversity, <i>Lcb. plantarum</i> group was the most abundant species, followed by <i>Lcb. manihotivorans</i> , <i>T. onikobensis</i> , <i>T. thermosulfata</i> , <i>Lcb. brantae</i> , <i>Lnb. parafarraginis</i> , and <i>Lnb. parakefiri</i> .	Illumina MiSeq (V3–V4 region of 16S rRNA gene)	[43]
<i>Nocellara Etnica</i> (Adrano and Paternò, Sicily, Italy)	Natural process: Sicilian style, without lye treatment. Brine: 5% or 8% (w/v) NaCl. Fermentation time and T °C: 80 days at room T °C (18 ± 2 °C).	LAB starter O1: <i>Lpb. plantarum</i> F1.16 and F3.5, in 5% NaCl. O2: <i>Lpb. plantarum</i> C11C8, F1.16, and F3.5, in 5% NaCl. C5 and C8: uninoculated samples with 5% or 8% NaCl, respectively. Inoculum level: 7 log cfu/mL	Middle- and final-stage fermentation (15 and 80 days, olives)—O1, O2: lactobacilli (ex- <i>Lactobacillus</i> genus; including <i>Lpb. plantarum</i>) dominated all inoculated samples; low occurrence of <i>Enterobacter</i> spp. Middle- and final-stage fermentation (15 and 80 days, olives)—C5: <i>Enterobacter</i> spp. and <i>Weissella</i> spp. (↑) at 15 days; <i>Enterobacter</i> spp. and lactobacilli (ex- <i>Lactobacillus</i> genus) (↑) at 80 days. C8: <i>Weissella</i> spp. was dominant at 15 and 80 days; low abundance of <i>Bacteroides</i> spp., <i>Faecalibacterium</i> spp., <i>Klebsiella</i> spp., <i>Raoultella</i> spp. at 15 days.	Illumina MiSeq (V3 region of 16S rRNA gene)	[66]

Table 2. Cont.

Olive cultivar	Type of Process and Fermentative Conditions	Species and Strains Used as Starter Cultures	Effect on the Microbiota	Sequencing Method	Ref.
Kalamata (Greece)	Natural process: Greek-style. <u>Brine</u> : 6% (w/v) NaCl. <u>Fermentation time and T °C</u> : 150 days, at 20 °C.	<u>LAB starter</u> A: spontaneous fermentation. B: <i>Lcb. rhamnosus</i> GG ATCC53103; C: <i>Levilactobacillus brevis</i> ATCC8287 starter culture; D: <i>Lpb. plantarum</i> ATCC14917. <u>Inoculum level</u> : n.a. (**)	Final-stage fermentation (150 days, olives)— All samples : starter cultures did not significantly affect the microbiota composition. <i>Lactiplantibacillus</i> (mainly <i>Lpb. plantarum</i> , followed by <i>Lpb. pentosus</i> , <i>Lpb. plajomi</i> , <i>Lpb. paraplanctarum</i>) and <i>Leuconostoc</i> (mainly <i>Leuc. mesenteroides</i> , and then <i>Leuc. gelidum</i>) were the most abundant species.	Nanopore MinION™ (near full-length V1–V9 region of 16S rRNA gene)	[74]
Manzanilla (Seville region, Spain)	Treated process: Spanish-style; treatment with 2.3% (w/v) NaOH solution for 7 h. <u>Brine</u> : 11.0% (w/v) NaCl acidified with 37% HCl. <u>Fermentation time and T °C</u> : 83 days, at room T °C.	<u>LAB starter</u> I: commercial starter containing 3 <i>Lpb. pentosus</i> strains (Oleica Starter Advance, TAFIQS in FOODs, Seville, Spain). U: spontaneous fermentation. F: fruits; B: brines (for coding samples) <u>Inoculum level</u> : 5 log cfu/mL	Early-stage fermentation (0 days, olives and brines)—U: high diversity, <i>Vibrio</i> spp., <i>Salinivibrio</i> spp., <i>Marinilactobacillus</i> spp., <i>Alkalibacterium</i> spp., <i>Halolactibacillus</i> spp., <i>Aerococcus</i> spp.; I: mainly <i>Vibrio</i> spp., <i>Marinilactobacillus</i> spp., <i>Alkalibacterium</i> spp., <i>Halolactibacillus</i> spp. Middle-stage fermentation (24 days, olives and brines)—U samples: high diversity and variability; UF: <i>Vibrio</i> spp., <i>Marinilactobacillus</i> spp., <i>Alkalibacterium</i> spp., <i>Lactiplantibacillus</i> spp. (↑); UB: <i>Lactiplantibacillus</i> spp. was dominant (↑). IF, IB: <i>Lactiplantibacillus</i> spp. was dominant (↑), followed by <i>Vibrio</i> spp. and <i>Marinilactobacillus</i> spp. Final-stage fermentation (83 days, olives and brines)—U samples: high diversity and variability; UF, UB, IF, IB: <i>Lactiplantibacillus</i> spp. was dominant (↑), <i>Vibrio</i> spp. (↓) and <i>Marinilactobacillus</i> spp. (↓).	Illumina MiSeq (V3–V4 region of 16S rRNA gene)	[54]
Starter cultures: Lactic Acid Bacteria and Yeasts					
Bella di Cerignola (Modugno, Apulia, Italy)	Natural process: Greek-style. <u>Brine</u> : 7% (w/v) NaCl. <u>Fermentation time and T °C</u> : 90 days at room T °C (18–25 °C)	<u>LAB and Y starter</u> S: commercial <i>Lpb. plantarum</i> (Sacco Srl company); SY: commercial <i>Lpb. plantarum</i> (Sacco Srl) and autochthon <i>Wickerhamomyces anomalus</i> DiSSPA73; SYL: commercial <i>Lpb. plantarum</i> (Sacco Srl) and autochthons <i>W. anomalus</i> DiSSPA73, <i>Lpb. plantarum</i> DiSSPA1A7, <i>Lpb. pentosus</i> DiSSPA7. Ctrl: spontaneous fermentation. <u>Inoculum level</u> : 7 log cfu/mL	Un-processed olives: <i>Hafnia</i> spp. and <i>Methylobacterium</i> spp. were dominant. Early-stage fermentation (1 day, brines)—Ctrl: <i>Hafnia</i> spp. (<i>Hafnia alvei</i>) (↑) was dominant. S, SY: co-occurrence of <i>Hafnia</i> spp. and <i>Lpb. plantarum</i> (↑); SYL: <i>Lpb. plantarum</i> / <i>Lpb. pentosus</i> (↑) were dominant. Middle-stage fermentation (75 days, brines)—Ctrl: co-occurrence of <i>Lpb. plantarum</i> (↑) and <i>Lactococcus lactis</i> (↑), presence of other minor genera; <i>Lpb. plantarum</i> (↑) for S, SY or <i>Lpb. plantarum</i> / <i>Lpb. pentosus</i> (↑) for SYL were completely dominant. Final-stage fermentation (90 days, brines)—Ctrl: <i>Lpb. plantarum</i> was the most abundant and metabolically active, together with a low fraction of <i>Clostridium</i> spp.; <i>Lpb. plantarum</i> (↑) for S, SY or <i>Lpb. plantarum</i> / <i>Lpb. pentosus</i> (↑) for SYL were completely dominant and metabolically active. Final-stage fermentation (90 days, olives)—Ctrl: <i>Lpb. plantarum</i> was the most abundant and metabolically active, together with a low fraction of <i>Lc. lactis</i> spp., <i>Clostridium</i> spp. (↓); <i>Lpb. plantarum</i> (↑) for S, SY or <i>Lpb. plantarum</i> / <i>Lpb. pentosus</i> (↑) for SYL were completely dominant and metabolically active, together with a low fraction of <i>Methylobacterium</i> spp.	Bacterial tag-encoded FLX amplicon pyrosequencing (V1–V3 region of 16S rRNA gene—on both DNA and RNA)	[61]

Table 2. Cont.

Olive cultivar	Type of Process and Fermentative Conditions	Species and Strains Used as Starter Cultures	Effect on the Microbiota	Sequencing Method	Ref.
Manzanilla (Seville region, Spain)	<p>Treated process: Spanish-style; treatment with 3.2% (w/v) NaOH solution containing 2.2% (w/v) NaCl and 0.89% (w/v) CaCl₂ for 7 h. Brine: 12.0% (w/v) NaCl, 0.13% (w/v) CaCl₂, and 0.08% (v/v) HCl.</p> <p>Fermentation time and T °C: 65 days, at uncontrolled T °C (ranging from 29 °C to 16 °C).</p>	<p>LAB and Y starter T1: <i>Lpb. pentosus</i> LPG1; T2: <i>Lpb. pentosus</i> Lp13; T3: <i>Lpb. plantarum</i> Lp15; T4: <i>Wickerhamomyces anomalus</i> Y12; sequential starter T5: <i>W. anomalus</i> Y12 followed by a combination of <i>Lpb. pentosus</i> LPG1, Lp13, <i>Lpb. plantarum</i> Lp15. T6: spontaneous fermentation. Inoculum level: 7 log cfu/mL for single strains (T1, T2, T3, T4; after 8th days of brining); 5 log cfu/mL for <i>W. anomalus</i> Y12 (1st day of brining) and 7 log cfu/mL for LAB mixture (after 8th days of brining) in T5 fermentation.</p>	<p>Final-stage fermentation (65 days, olives)—All samples: <i>Marinilactibacillus</i> spp., <i>Halolactibacillus</i> spp., <i>Lactobacillus</i> spp. (<i>Lpb. plantarum</i> and/or <i>Lpb. pentosus</i>) and <i>Alkalibacterium</i> spp. were the most abundant genera, with a significant prevalence of <i>Marinilactibacillus</i> spp. (approximately from 40% to 60%). The highest %: <i>Marinilactibacillus</i> spp. in T1; <i>Lactobacillus</i> spp. (<i>Lpb. plantarum</i> and <i>Lpb. pentosus</i>) in T5; <i>Halolactibacillus</i> spp. in T2; <i>Alkalibacterium</i> spp. in T3 and T4. <i>Aerococcus</i> spp., <i>Halomonas</i> spp. and <i>Bacillaceae</i> family were also detected in all samples at very low occurrence.</p>	<p>Illumina MiSeq (V3–V4 region of 16S rRNA gene)</p>	<p>[70]</p>

(* The current taxonomy for lactobacilli (ex-*Lactobacillus* genus) was used in this study, according to Zheng et al. [73]. Genus abbreviations for lactobacilli: *Lacticaseibacillus*, *Lcb.*; *Lactiplantibacillus*, *Lpb.*; *Lentilactobacillus*, *Lnb.*; *Ligilactobacillus*, *Lgb.*; *Secundilactobacillus*, *Slb.* (**): not available. ↑: increase; ↓: decrease.

4. “Omics” Approaches to Investigate the Functions of Table Olive Microbiomes and the Role of Starter Cultures

As already highlighted, data related to the microbiomes of fermented olives are mainly based on the AT-HTS approaches which, in recent years, allowed an increase in knowledge on the composition, taxonomic diversity and dynamism of microbial communities during fermentation (although with a greater focus on bacteriome than mycobiome). Unlike other fermented foods, further “omics”, “meta-omics” and “multi-omics” approaches (including tools and bioinformatics [75,76]) have been scantily applied to fermented table olives.

Shotgun metagenomics, a more powerful tool in depicting the diversity and functionality of microbiomes, have not been applied to fermented olives. To our knowledge, only Soto-Giron et al. [77] used a metagenome-assembled genome (MAG) approach to investigate the microbial communities of green olives, focusing attention on the genomic diversity and functionalities of 5 LAB-like MAGs (n.1 *Loigolactobacillus coryniformis*, n.2 *Lentilactobacillus buchmeri*, n.2 *Lactobacillus acetotolerans*).

Available proteomic data are related to the proteome profile and functionalities of some LAB isolated from olives and/or brines [78–80], but, to date, no metaproteomic protocols have been exploited to study the in situ functionalities (during fermentation) of starter cultures and olives-associated microbiomes.

More recently, López-García et al. [81] investigated the transcriptome profile of *Lpb. pentosus* LPG1 during *Manzanilla* cv. fermentation (Spanish-style treatment, 60 days incubation), suggesting that many genes were differentially expressed in both brine- and biofilm-associated cells. As for metatranscriptome analysis (needed to investigate the metabolically active fraction of microbiomes), to date, no data are available for fermented table olives.

Although metabolomic approaches (i.e., HS-SPME/GC-MS, HPLC, LC-MS/MS) have been mostly used to evaluate the effect of the fermentative process in table olives (for both commercial and experimentally obtained products [22]; this study), the available information is poorly integrated with other “omic” data, not allowing adequate correlations between the composition, evolution and functionalities of microbiota and the final features of the products.

5. Conclusions

Starter cultures also play an important role in fermented table olives, preventing or reducing the growth of undesired microorganisms and improving the properties of final products. The effects of starter cultures on fermented olives have been investigated for several decades, mainly with a focus on the acidifying capability, antimicrobial activity, degradation of phenolic compounds, and production of some metabolites. Information on the genomic diversity of the strains, as well as on their metabolic interactions with other members of olive microbiota, is still limited.

The recent AT-HTS data improved the knowledge on the composition and evolution of microbial communities during olive fermentation and, in some cases, the effect of starter cultures on the indigenous microbiota. Much of the information, however, still describes the microbiota at the genus level, not providing a faithful picture of microbial dynamics during the fermentative process. Most of HTS approaches, moreover, have been exclusively targeted on hypervariable regions of the 16S rRNA gene, neglecting the fungal diversity of olives microbiota and using the genomic DNA as a template, overlooking the metabolically active microorganisms during fermentation.

Other “omics” techniques were scantily applied to the olive ecosystem, including the starter cultures, thus limiting the studies to a single or few technological traits, rather than to complex microbial interactions that occur during the fermentative process. Therefore, as for other fermented foods, future investigations based on the integration of “omics” or “meta-omics” data could be helpful to understand and predict the metabolic potential of starter cultures and indigenous microbiota, respectively, and improve the properties of table olives.

Additionally, although the use of starter cultures for olive fermentation can be advantageous and attractive for the food industry, further research and industrial-scale validation are needed to develop tailored formulations, place them on the market, and make them commercially available, as has already happened with other cultures exploited for other fermented foods.

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