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### **Review Paper/Derleme Makale**

### Microflora of table olive fermentation

### Sofralık zeytin fermantasyonunun mikroflorası

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#### Abstract

**Objective:** Olive consumption has been showing an increasing trend in recent years due to the increasing interest in healthy and balanced nutrition. Olives are consumed in different ways such as table olives and olive oil. Fermentation of table olives is very important to remove bitterness and to produce safe table olives with appropriate sensory and biochemical properties and long shelf life. Table olive fermentation is carried out under spontaneous or controlled conditions. There are many microorganisms in the natural microflora of olives. When the studies are examined, it is generally seen that the dominant species are lactic acid bacteria such as *Lactobacillus pentosus*, *Lactobacillus plantarum*, yeasts such as *Candida*, *Saccharomyces*, *Pichia* and mould species such as *Penicillium*, *Aspergillus*. Therefore, table olive production is a very complex process due to the rich microflora diversity.

**Conclusion:** In this review, general information about olive fruit and microorganisms involved in table olive fermentation are given. The effects of microorganisms on the organoleptic properties and biochemical properties of table olives during the fermentation period have been studied in detail. An important resource has been presented to researchers and industry working on table olives with this study.

Keywords: Olive; spontaneous fermentation; starter culture; microflora

Öz

**Amaç:** Son yıllarda sağlıklı ve dengeli beslenmeye artan ilgiden dolayı zeytin tüketimi artış eğilimi göstermektedir. Zeytin, sofralık zeytin ve zeytinyağı gibi farklı şekillerde tüketilmektedir. Sofralık zeytin fermantasyonu, acılığın giderilebilmesi, uygun duyusal ve biyokimyasal özelliklere sahip, raf ömrü uzun ve güvenli sofralık zeytin üretebilmek için oldukça önemlidir. Sofralık zeytin fermantasyonu, spontan veya kontrollü koşullarda gerçekleştirilmektedir. Zeytinin doğal mikroflorasında çok sayıda mikroorganizma bulunmaktadır. Yapılan çalışmalar incelendiğinde genel olarak baskın türlerin *Lactobacillus plantarum, Lactobacillus pentosus* gibi laktik asit bakterilerinden, *Candida, Saccharomyces, Pichia* gibi mayalardan ve *Penicillium, Aspergillus* gibi küf türlerinden oluştuğu görülmektedir. Dolayısıyla, sofralık zeytin üretimi sahip olduğu zengin mikroflora çeşitliliğinden dolayı oldukça kompleks bir süreçtir.

**Sonuç:** Bu derleme çalışmasında zeytin meyvesi ve sofralık zeytin fermantasyonunda rol oynayan mikroorganizmalar hakkında genel bilgiler verilmektedir. Fermantasyon süresi boyunca mikroorganizmaların, sofralık zeytinlerin organoleptik özellikleri ve biyokimyasal özellikleri üzerindeki etkileri detaylı bir şekilde incelenmiştir. Bu çalışma ile birlikte sofralık zeytin üzerine çalışan araştırmacılara ve endüstriye önemli bir kaynak sunulmuştur.

Anahtar Kelimeler: Zeytin; spontan fermantasyon; starter kültür; mikroflora

### 1. Introduction

Olive, which is traditionally cultivated in Mediterranean countries (Sozbilen and Baysal, 2016) and is a fruit of the Olea erupaea tree, is a drupe and fleshyfruit with a high fat content, low concentration and bitter component sugar (oleuropein) (Arroyo-López et al., 2012). It is believed that the olive plant first grew in the region encompassing Hatay, Mardin, Palestine, Syria, and the island of Cyprus (Anonymous, 2019). The spread of the olive to the world has been in the form of Southeastern Anatolia, Western Anatolia, Aegean Islands and Greece-Italy-France-Spain (Anonymous, 2016).

According to the data of the Food and Agriculture Organization (FAO), olive decile areas increased by 6.42% and olive production increased by 19.98% between 2012 and 2022 worldwide. In the 2021-22 season, olive production was realized on 10.95 million hectares of land worldwide and 21.45 million tons of crops were obtained. The countries with the highest olive production in 2021-22 were Spain, Greece, Türkiye, Italy, Morocco, Egypt, Portugal, Tunisia, Syria and Algeria, respectively. According to the International Oliveoil Council (IOC) data, the per capita table olive consumption by country in the 2019/20 production season is 5.6 kg in Syria, 6.2 kg in Algeria, 5.5 kg in Egypt, 3.9 kg in Türkiye, 3.5 kg in Spain and 2.5 kg in Peru (IOC, 2023).

Olive is a food containing high levels of fat (12-30%), low levels of sugar (2.6-6%), oleuropein, which is a bitter component, water, protein, anthocyanins, organic acids and mineral substances (Tokuşoğlu, 2016; Rokni et al., 2017). In recent years, olive consumption has increased due to the increasing interest of people in issues such as quality life, health and balanced nutrition (Özdek and Aybar, 2019). Olive consumption is considered under two main categories: table olives and olive oil.

Table olives are defined in the Turkish Food Codex Communiqué on Table Olives numbered 29097 and dated 23 August 2014 as "olives obtained from cultivated olive tree (*Olea europaea L.*) fruits by removing the bitterness in accordance with the technique, subjected to fermentation work or not kept, adding starter cultureand/or other additives, when necessary, with or without pasteurisation or sterilisation process" (Anonymous, 2014). Table olives are divided into 3 groups: green, different colors and black olives according to the maturity level of the fresh fruit (Rejano et al., 2010). In addition, according to customer demands and the way they are presented to the market, they are classified as whole olives, pitted olives, filled olives, half olives, quarter olives, divided olives, sliced olives, broken olives, crushed olives, scratched olives, saddle olives, etc. (Tokuşoğlu, 2016).

In this study, information about the importance of fermentation in table olive production, production problems related to the optimisation of fermentation conditions and microbial flora that play a role in fermentation are reviewed.

### 2. Natural flora of the olive fruit

Table olive fermentation usually occurs spontaneously. The natural microflora of olives plays an important role in spontaneous fermentations (SF). The microflora of olives varies depending on the type of olive, the geographical region in which it is located and the type of processing.

There are limited number of studies on the natural flora of raw olive fruit. In one of these studies, lactic acid bacteria (LAB), Bacillaceae, Enterobacteriaceae. Micrococcaceae, Pseudomonadaceae, mould and yeast species were detected from the fruits of Hurma and Erkence olive species grown in Karaburun district of Izmir, Türkiye (Sozbilen and Baysal, 2016). Bella di Cerignola olives harvested from Santo Stefano in the Apulian Region of Italy were fermented according to Spanish and natural fermentation methods and LAB, mesophilic bacteria. Pseudomonadaceae, Enterobacteriaceae, Micrococcaceae, Staphylococcus spp., and yeasts were detected. Bacillus species were dominant in mesophilic microorganism population of raw olives and Chryseobacterium spp., Enterobacter amnigenus and Enterobacter cloacae were isolated in the first phase of fermentation (Campaniello et al.. 2005). Enterobacteriaceae, Clostridium, Pseudomonas, Staphylococcus spp., LAB and some mold species were isolated from processed olives and their brines (Erten et al., 2015). In the early days of fermentation, LAB such as Pediococcus cerevisiae and Leuconostoc mesenteroides (Turantaş, 2021) and Gram negative bacteria (Enterobacter, Citrobacter, Aeromonas,

*Escherichia* and *Klebsiella*) grow. The growth of Gram negative bacteria continue until LAB grow and reduce the pH of the environment to a certain level. It has been reported that *Saccharomyces*, *Pichia, Candida* genera are frequently encountered in table olive fermentations. The most important microorganism group in olive fermentations is LAB. Especially, *Lactobacillus* spp. (*Lactobacillus plantarum*) are the most dominant species (Erten et al., 2015). In addition, *Rhizopus nigricans*, *Fusarium semiticum*, *Penicillium*, *Aspergillus* mould species were detected in the microbial flora of olive fruits. *Penicillium* species are the most dominant species are the most dominant species anong the moulds (Tokuşoğlu, 2016).

# **3.** Table olive fermentation and importance of fermentation

Olive is the only fruit that cannot be consumed directly after harvesting., The bitterness of olives must be removed in order to consume them. Several processes can be apply for removing bitterness in olive fruits. Bitterness in olives can be eliminated by alcali application, brining, salting, fermentation, acidification, enzyme treatment, sonication, freezing-thawing and drying methods (Kara and Özbaş, 2013; Yılmaz et al., 2022). Fermentation of table olives occurs under spontaneous or controlled conditions (with or without starter culture). The microorganisms involved in fermentation are yeasts and LAB (Arroyo-López, 2012; Bonatsou et al., 2017). It is stated that oleuropein, which is a phenolic compound and the cause of bitterness in olives, is degraded by the enzyme  $\beta$ -glucosidase produced by yeasts and LAB during fermentation and then converted into hydroxytrisol and elenolic acid by esterase enzyme (Kara and Özbaş, 2013; Boskou et al., 2015). LAB are very important for olive fermentation because they can survive in brine, tolerate high pH and sodium chloride (NaCl) values, produce high amounts of lactic acid, and have specific enzymes (Abouloifa et al., 2020b). LAB especially cause a decrease in pH with the lactic acid produced in table olive fermentation. When fermentation ends, the amount of salt used to preserve the product decreases due to the increase in the acidity of the environment (Özdemir, 2011), the microbial load of olives decreases and thus the shelf life prolonged. The metabolites and volatile components (alcohol, ethyl acetate and acetaldehyde) produced by yeasts involved in fermentation produce the desired taste and aroma in olives (Bonatsou et al., 2018). In addition, the odor, color, taste and texture such as properties of the final product are improved thanks to the lipase and esterase activities of the yeasts (Bonatsou et al., 2018; Perpetuini et al., 2020).

Table olive fermentation is very important for the growth of desirable sensory and biochemical properties in the end product (Kaltsa et al., 2014), inhibition of undesirable microorganisms (lactic acid, bacteriocin, etc.) (Hurtado et al., 2012), reduction of high salt concentration which is harmful for health and improves olive flavour (Özay et al., 1994; Özdemir, 2011), reduction of alcali use due to the natural removal of the bitterness cause of oleuropein from olives (Boskou et al., 2015) All these are very important in process in terms of producing a quality product with a long shelf life and food safety (Chranioti et al., 2018).

## 4. Production problems related to optimisation of fermentation conditions

It has been reported that the optimum salt concentration at which LAB can grow is maximum 9% (Erten et al., 2015). This ratio is in the range of 12-14% in olive fermentation produced by traditional method (Özay et al., 1994). Therefore, the use of high salt is disadvantageous for the growth of microorganisms involved in fermentation. In addition, halophilic Archaea, which can grow under high salt concentration and low acidity conditions and are spoilage factors in foods, can be observed (Abriouel et al., 2011).

Spontaneous fermentation takes place with the natural microflora on the surface of the olive fruit. Accordingly, spontaneous fermentation may lead to the production of low-quality products with variable sensory characteristics due to the long period under uncontrolled conditions (Boskou et al., 2015). In short, spontaneous fermentation has more disadvantages compared to fermentations using starter culture (Bonatsou et al., 2017).

During the alkaline treatment and washing stages in the bitterness removal process, the sugar content Therefore, in olive fruits decreases. the fermentation process proceeds outside the desired. Accordingly, acidity remains at low levels. As a spoilage result. factors and pathogenic microorganisms may grow in the environment and cause risky food production and product spoilage in terms of food safety. In addition, alkaline application causes a decrease in phenolic compounds that contribute to the texture, taste, colour and antioxidant capacity of olives. The fact that the amount of phenolic compounds in the environment decreases over time also leads to the inability to achieve the desired sensory properties in the final product (Chranioti et al., 2018).

In the spontaneous table olive fermentation performed in the tank, if the tank lid is left open or if air remains in the upper part of the tank, oxidative yeasts and moulds can accumulate on the brine surface and cause olive spoilage (Kailis and Kiritsakis, 2017). Mould growth in brine can cause the formation of mycotoxins, which is an important problem in terms of food safety. Aflatoxins, ochratoxin and citrinin are mycotoxins that cause problems in olives (Tokuşoğlu, 2016). In order to prevent or reduce the formation of moulds and also mycotoxins in table olives during the process from harvest to consumption, preventions are important such as applying good manufacturing procedures at the harvest stage, keeping storage conditions (temperature, packaging, salinity, etc.) under control (Bavaro et al., 2017).

In recent years, interest in the use of starter cultures in the industrial production of table olives has increased. However, the use of starter cultures in table olive production has not yet become widespread. This is because the optimization of microbial strains to be used as starter cultures in table olive fermentation has not been carried out. Therefore, microbiological controls of the fermentation process cannot be performed (Chranioti et al., 2018).

## 5. Spontaneous fermentation in table olive production

Spontaneous fermentation is carried out by the natural microflora present on the surface of the olive fruit. Table olive species can generally be processed through spontaneous fermentation (4-10% NaCl) at 25°C under anaerobic conditions (Kailis and Kiritsakis, 2017).

In a study bacterial identification of six different naturally fermented table olive species (*Nocellara de Belice, Itrana nera, Itrana Bianca, Bella di Cerignola Peranzana* and *Cellina Di Nardò*) supplied from three regions of Italy (Apulia, Sicily and Lazio) were performed and, *Lactobacillus fermentum, Lactobacillus pentosus, Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus casei* and *Enterococcus durans* species were identified and it was observed that *Lactobacillus pentosus* was the dominant species (Tofalo et al., 2014).

Leuconostoc pseudomesenteroides, Pediococcus parvulus and Lactobacillus pentosus, species were identified in the study to determine the characterisation of LAB (Lactobacillus, Pediococcus and Leuconostoc) isolated from Aloreña green table olives naturally fermented (fermentation in vats and large tanks at room temperature) in four plants from Málaga, Spain. Most of the isolates of Lactobacillus pentosus and Leuconostoc pseudomesenteroides showed high acidification capacity by lowering the pH up to 3.8 (Abriouel et al., 2012).

The yeast population and dynamics involved in the spontaneous fermentation of Negrinha de Freixo green table olive species performed at room temperature in brine with an initial salt content of 7% and a pH set to 4 with the addition of lactic acid were evaluated in both brine and olive fruit. The species Rhodotorula glutinis, veast Pichia guilliermondii, Rhodotorula graminis, Candida norvegica. Galactomyces reessii, Candida boindini, Debaromyces hansenii, Saccharomyces cerevisiae, Pichia membranifaciens and Candida tropicalis were isolated from the fruit during fermentation. The veast species Pichia manshurica. Galactomyces reessii, Candida boindini, Debaryomyces hansenii, Saccharomyces cerevisiae, Pichia membranifaciens and Candida tropicalis were isolated from the brine during fermentation. It was observed that the most frequently detected yeast species during fermentation was Saccharomyces cerevisiae, followed bv Candida tropicalis. Pichia membranifaciens and Candida boindini yeast species. (Pereira et al., 2015).

Table olives were produced by natural fermentation using Bella di Cerignola, Nocellara del Belice, Itrana bianca, Itrana nera, Cellina di Nardò and Peranzana olives grown by different producers in the Puglia, Sicily and Lazio regions of Studies have confirmed Italy. that the microorganisms responsible for fermentation are veasts, and Saccharomyces cerevisiae has been isolated from all olive varieties. It was determined that the yeasts found in the environment were Candida boidinii, Candida ishivadae. Wicheramomyces anolamus, Candida ishivadae and Pichia galeiformis species (Tofalo et al., 2013). In another study investigated the microbial dynamics of Manzanilla olive species processed by Spanish-style green olive fermentation without any starter culture, acidified with lactic acid and HCl, with a salt ratio of 10-11%, it was observed that the dominant species was Lactobacillus pentosus. Apart from Lactobacillus pentosus, a total of 15 isolated. LAB were Weissella paramesenteroides/hellenica, Enterococcus saccharolyticus, Sporolactobacillus inulis/terrae, Pediococcus parvulus and Lactobacillus rhamnosus identified in this study have not been previously identified in Spanish-style table olive fermentations. Sporolactobacillus inulinus/terrae and Enterococcus saccharolyticus have not been previously sampled in any olive preparation. Enterococcus casseliflavus and Enterococcus saccharolyticus were observed at the beginning of fermentation. It has been observed that Candida thaimueangensis and Saccharomyces cerevisiae were predominate among the yeasts. Rhodotorula mucilaginosa and Candida butyri/asseri species have been identified from fermented olives obtained according to Spanish traditional methods. As a result of the studies carried out, Saturnispora mendoncae was carried out in isolation (Lucena-Padrós et al., 2014).

It has been confirmed that alkaliphilic and halophilic bacteria in the natural microflora are involved in the fermentation of green olives according to obtained Spanish traditional production methods without the addition of any starter culture. At the end of the study, 13 bacterial species belonging to 11 genera including Aerococcus Alkalibacterium sp., sp., Amphibacillus tropicus, Catenococcus thiocycli, olivae, Halolactobacillus Enterococcus sp., Halomonas mongoliensis, Marinilactobacillus sp., Matronobacillus azotifigens, Streptohalobacillus salinus, Vibrio sp. were identified. Among the identified species, a high number of halophilic (Alkalibacterium, Marinilactobacillus and Halolactobacillus) and alkaliphilic LAB were observed (Lucena-Padrós and Ruiz-Barba, 2016).

Bleve et al. (2015), investigated the profiles of microorganisms involved in small scale fermentations of *Kalamata* and *Conservolea* table olive species. Olives were subjected to a 180-day fermentation at ambient temperature (8-30°C) at 8% salt concentration. As a result of the study, "Generally Recognised as Safe" (GRASS) yeast

species; Pichia anomala and Debaryomyces hansenii in Conservolea olives, Pichia anomala, Pichia membranifaciens *Saccharomyces* Debaryomyces cerevisiae, hansenii, and Guehomyces pullulans (non-GRASS) in Kalamata olives were identified. In addition, Lactobacillus plantarum and Acetobacter tropicalis bacterial species were detected in Conservolea olives, while Leuconostoc mesenteroides and Lactobacillus plantarum bacterial species were detected in Kalamata olives.

In the study carried out to determine the LAB strains found in the biofilms of Alorena, Manzanilla and Gordal type table olives obtained from Spanish style green olives and direct pickled (natural) olive production enterprises, and which may be used as starter culture in table olive production, 79 different genotypes were obtained from the isolates obtained. Among these genotypes, 16 genotypes were observed to be dominant. Of these genotypes, 13 of them were identified as Lactobacillus pentosus and 3 of them were identified as Lactobacillus plantarum strains. It was observed that Lactobacillus pentosus Lp13 was the most dominant genotype among all strains except Alorena green natural olives, followed by Lactobacillus pentosus Lp6. In addition, in this study, it was observed that the biodiversity indices of Manzanilla and Gordal olive cultivars produced by the Spanish-style production method were higher than those of olives produced according to the direct brine olive processing method. It has been observed that the dominant species found in Spanish table olive biofilms was Lactobacillus pentosus, but some genotypes of Lactobacillus plantarum were also detected (Benítez-Cabello et al., 2019).

In the study in which 72 yeast isolates identified from the brines of Bosana table black olives grown on the Italian island of Sardinia and produced by spontaneous fermentation method were identified, it was determined that the dominant yeast species were *Nakazawaea molendini-olei* and *Wickerhamomyces anomalus*. It was also observed that *Candida boidinii, Candida diddensiae, Saccharomyces cerevisiae* and *Zygotorulaspora mrakii* species were present at lower rates (Porru et al., 2018).

In a study, the predominant bacterial species in the spontaneous fermentation of *Gordal* and *Manzanilla* table olives produced industrially with

the Spanish-style production technique were identified. Vibrio vulgaricus, Lactobacillus parafarraginis and Lactobacillus plantarum spp. identified in both olive were varieties. Halolactobacillus halophilus and Lactobacillus sanfranciensis were identified only in Gordal olive samples, while Staphylococcus sp. was detected only in the initial stage of Manzanilla olive fermentations. Also, the bacterial species in the solid sea salts used in the preparation of the brine were also identified and the presence of Bacillus, Enterococcus and Staphylococcus species, which were not detected during fermentation, were detected (Benítez-Cabello et al., 2016).

In the study, Müjdeci et al. (2018), yeast isolation was performed as a result of natural fermentation of Gemlik type olives grown in Akhisar and Iznik regions of Türkiye and identified the yeast strains. At the end of the study, *Candida mycentangi*, *Candida famata*, *Candida hellenica*, *Candida pelliculosa*, *Candida membranifaciens*, *Zygosaccharomyces mrakii* and *Saccharomyces cerevisiae* yeast species were identified. They also reported that *Candida famata* and *Candida pelliculosa* were the most common yeast species in both regions.

The probiotic properties of antifungal Lactobacillus strains isolated from brine samples obtained from an enterprise operating in Oujda, Morocco and producing table green olives by traditional fermentation method without alcali treatment were characterised. Candida pelliculosa isolated from previously fermented green olive brine was selected as the target microorganism for the identification of antifungal Lactobacillus strains. 104 LAB isolates obtained. 14 strains were observed to form high inhibition zones against Candida pelliculosa and molecular identification, it was determined that five of these strains consisted of Lactobacillus brevis, two of them Lactobacillus pentosus and seven of them Lactobacillus plantarum (Abouloifa et al., 2020a).

The yeast biota associated with table olives produced by natural fermentation method from *Nocellara messinese* olive variety obtained from olive groves in Calabria and the growth of this yeast biota in different brines were investigated (Sidari et al., 2019). The fermentations were designed in four different ways and carried out at room temperature for 240 days. According to the results of the study, it was observed that the most isolated species was Pichia kudriavzevii and the other isolated yeast species were Candida boidinii, Candida tropicalis, Candida aaseri, Wickerhamomyces anomalus, Candida diddensiae, Saccharomyces cerevisiae, Zygoascus meyerae, Zygoascus hellenicus and Pichia mexicana. It was observed that increasing the salt concentration from 5% to 8% promoted the presence of Pichia kudriavzevii species continuously throughout the fermentation. At the end of fermentation, the presence of Wickerhamomyces anomalus species was detected in all brine samples. Pichia mexicana and Saccharomyces cerevisiae species were detected in brines acidified with lactic acid.

In a study in which the antibacterial activities of LAB isolated from fermented table olive samples produced in the market and at home were investigated, isolates showing antibacterial activity were identified. It was observed that these isolates showing antimicrobial activity belonged to *Lactobacillus plantarum*, *Enterococcus faecium* and *Lactobacillus brevis* species (Kıvanç and Erikçi, 2018).

The factors causing the formation of soft and whitish areas on the olive surface during the olive (Alorena de Malaga) fermentation period have been investigated. The olives were fermented in brine without any starter culture for 20 days using traditional methods and then packaged by a company in Guadalhorce Valley. The packages contained olives, spices and brine. The packs were kept at 23  $\pm$ 2 °C and sampled periodically. At the end of the study, a total of 20 yeast and 65 LAB isolations were obtained in the brine and fruits of commercially packaged olives at different sampling times. Enterobacteriaceae (Enterobacter gergoviae) were only detected in very low populations immediately after packaging (day 1). The dominant flora during the shelf life has been LAB and yeasts. Ten Lactobacillus bacteria were isolated from visibly spoilt fruit. All these isolated bacteria belonged to Lactobacillus pentosus species. The microbial species detected in the product after packaging were Lactobacillus plantarum and Enterobacter gergoviae and among bacteria, Candida parapsilosis, Lodderomyces elongisporus and Candida tropicalis, among yeasts. Lactobacillus pentosus was found to be the dominant species in spoilt packages (Romero-Gil et al., 2016).

In another study aimed to determine the effects of the use of KCl and CaCl<sub>2</sub> instead of NaCl as brine during fermentation on the microbiological quality criteria of table green olives of the genus Macanilha algarvia. Fermentation processes were carried out in 5 stages with different brine salt concentrations in October and April for 162 days at room temperature by direct brine olive production method. It was observed that the Enterobacteriaceae population decreased over time in all fermentations. The rate of decrease was found to be faster and greater in brines containing sodium. calcium and PseudomonasListeria monocytogenes, Escherichia coli, Staphylococcus aureus and Salmonella were not detected in the table olives produced. In addition, Saccharomyces cerevisiae, Pichia membranifaciens, Priceomyces carsonii, Candida boidinii, Wickerhamomyces anomalus, Zygosaccharomyces mrakii and yeastlike fungus Galactomyces geotrichum were identified (Mateus et al., 2016).

Table olives of the *Taggiasca* genus were left to spontaneous fermentation in a brine containing 10% NaCl without any pretreatment. During the fermentation period, *Wickerhamomyces anomalus*, *Candida diddensiae*, *Aureobasidium pullulans*, *Cyteromyces nyonsensis* and *Pichia* membranifaciens were isolated by traditional culture-related methods and it was observed that the flora responsible for fermentation consisted of yeasts (Traina et al., 2024).

The Spanish-style olive production and spontaneous fermentation of Manzanilla olives harvested at the mature-green stage from Seville, Spain, have been studied comparatively. While Pichia, Lactobacillus and Saccharomyces are the dominant flora in the production of Spanish-style olives, it has been understood that Saccharomyces, Halomonas, Allidiomarina, Nakazawaea and Pichia take an active part in fermentation in spontaneous fermentation. As a result of fermentation, a higher acetic acid content was found in Spanish-style olives, while it was determined that olives obtained as a result of spontaneous fermentation had a higher methyl ketone content (Ruiz-Barba et al., 2023).

Biochemical, physicochemical and microbiological characterization of table olives of the genus *Cobrançosa* were carried out during spoantan fermentation. During the fermentation period, all organic acids showed an inverse

parabolic development. Although yeasts were the dominant flora at the beginning of the fermentation, an increase occurred due to the presence of LAB in later times. It was determined that the dominant LAB flora during fermentation consisted of *Lactiplantibacillus paraplantarum*, *Lactiplantibacillus pentosus*, *Oenococcus kitaharae* and *Pediococcus parvulus* (Reis et al., 2022).

## 6. Fermentation under controlled conditions in table olive production

Olive fermentation is a process influenced by factors such as water activity, pH, presence of nutrients, salt concentration in brine and presence of phenolic substances. In an spontanous fermentation, the growth of microorganisms that cause product spoilage and economic losses are likely to occur because of product spoilage. For this reason, continuous adjustment of the salt concentration of the brine and acidification of the brine are industrial practices applied to control the process and prevent abnormal fermentation. Starter cultures can also be used in controlled fermentations (Grounta and Panagou, 2017). The use of starter cultures will increase the fermentation rate and ensure a rapid decrease in pH. In this way, unwanted microorganisms will be eliminated in the environment. LAB such as Lactobacillus pentosus, Lactobacillus plantarum and Lactobacillus paracasei are frequently used as starter cultures in olive fermentations. In addition, these LAB are potential probiotic bacteria (Rokni et al., 2017).

In a study conducted, the effect of bacterial community and dynamics on metabolome formation during fermentation of Nocellara Etnea table olives was investigated. The fermentation process lasted for 120 days at 20°C±2 during fermentation, the salt amount of the brine was kept constant to be 8%. 6 Different combinations of Lactobacillus plantarum UT2.1, Lactobacillus pentosus TH969 and Lactobacillus paracasei N24 bacteria were used in fermentation. The group that was left to spontaneous fermentation without adding starter was evaluated as a negative control. as a result of the analyses performed at the beginning of fermentation, it was determined that Lactobacillus plantarum was the dominant in the environment. Of fermentation 60. and 120. as a result of the analyses performed on the day, it was observed that there was the development of

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*Pediococcus parvulus, Lactobacillus acidipiscis* and *Lactobacillus paracollinoides*. When fermentation ended, a significant decrease in the Enterobacteriaceae population occurred in all samples except the negative control group (Randazzo et al., 2017).

In a study investigating the technological performance of two starter cultures in the processing of Tonda di Cagliari olives sourced from Sardinia, Italy, a single strain of Lactobacillus plantarum (SSL) and a mixture of Lactobacillus pentosus strain (SIE) were used as starting cultures and spontaneous fermentation (SF) were accepted as the control group. The salt concentration was kept constant at 7% during the process and fermentation was carried out in an air-conditioned room and the temperature of the room was adjusted to 27° C and then to 24° C until a constant pH was reached for all groups. Studies have shown that SIE is more effective in inhibiting the microflora that causes deterioration compared to SSL and NF methods. Microorganisms belonging to the Enterobacteriaceae were not found in the SIE samples from the 10 day of fermentation, in the SSL and NF samples from the 30 day of fermentation. Yeasts were detected throughout the fermentation, although they decreased during the first 7 days in brine. However, mould population could not be detected in any sample. It was observed that the starter cultures used in the study rapidly acidified the brine and decreased the pH value below 4 after the 12th day of fermentation. In SF samples, the pH value decreased to 4.3 after 45<sup>th</sup> day of fermentation. In addition, high levels of hydroxytyrosol were detected in the samples of both starter cultures at the end of the process compared to SF samples (Campus et al., 2015). This shows that starter cultures are very effective in removing the natural bitterness of olives.

In a study to control the spontaneous fermentation process (5% NaCl and 30°C) of Moroccan Picholine green olives without alcali treatment, they were inoculated with two Lactobacillus strains (Lactobacillus pentosus S100 and Lactobacillus plantarum S175 ). Physicochemical parameters (reducing sugar, pH, sodium chloride, free acidity, hydrolysis products and oleuropein) and microbiological parameters (LAB, mesophilic aerobic bacteria, Staphylococcus spp., coliforms, moulds and yeasts) were regularly examined during fermentation. According to the results of the research, lactic acid population increased in the first 15 days in inoculated samples and in the first 20 days in non-inoculated samples and then slightly decreased. Yeast, mould and mesophilic bacteria population increased in the first 15 days and then decreased and became stable. It was observed that the use of starter culture was effective on yeast and mould population. Especially Lactobacillus pentosus S100 strain was more effective on yeast and mould population at certain times of fermentation compared to the samples inoculated with Lactobacillus plantarum S175 and control group samples. Coliform bacteria showed a rapid increase in the first 5 days in the inoculated samples and in the first 10 days in the non-inoculated samples in the first 5 days, and then rapidly decreased and were eliminated. Staphylococcus population showed a rapid increase in the first 10 days in all samples and was eliminated after a drastic decrease in the 3<sup>rd</sup> week of fermentation. It was also observed that the added starter cultures hydrolysed the bittering agent oleuropein, improving the hygienic quality and sensory characteristics of the final product. In addition, it was concluded that LAB strains are dominant species against spoilage and pathogenic microorganisms in the environment (Ghabbour et al., 2016).

Chranioti et al. (2018) evaluated the efficacy of commercial (CM: Lactobacillus pentosus) and autochthonous starter cultures (OSC: belonging to the Lactobacillus plantarum group isolated from olives) on physicochemical and microbiological profile parameters during the fermentation of Conservolea green olives. SF was considered as a control group. Salt concentration was kept constant at 6% during fermentation. Olive samples were prepared in two groups. The first group (A) was prepared without any bitterness removal before fermentation and the second group (B) was prepared by removing bitterness with alkali. According to the results of the study, LAB population varied between the groups. In the first days of fermentation, a decrease in the amount of LAB o was observed depending on the treatment. While a decrease was observed in samples inoculated with CM in groups A (0.6 log cfu/ml) and B (2.5 log cfu/ml), no decrease was observed in samples inoculated with OSC in both groups. The numbers of LAB in both groups were determined as OSC > CM > SF According to the results of the analyses, autochthonous starter

cultures survived better than commercial starter cultures. Yeast population increased during fermentation and showed almost no change after the 45th day of fermentation. Enterobacteriaceae were present in all groups at the initial stage of fermentation. Although they showed a certain level of increase in the first stage of fermentation, their numbers decreased rapidly and disappeared in the following stages. They were not detected after 30 days of fermentation in SF samples and after 20 days in CM and OSC samples. The mould population remained below the detection limit during fermentation. It was also observed that autochthonous starter cultures were more effective than other treatments in removing bitterness in olives and increasing the acidity of the medium by rapidly decreasing the pH.

In a study investigating the yeast diversity of naturally fermented black olives produced from Gemlik-type olives grown in Serinyol, Tarsus and Bahçe districts of Türkiye, olives were subjected to fermentation for 180 days at different salt concentrations of 6%, 8% and 10%. The salt content of brines containing 10% and 8% NaCl was kept constant during the fermentation period. It has been observed that natural microflora Candida Wickerhamomyces boidnii. anomalus and Saccharomyces sp. are the dominant in Tarsus and Garden districts. It has been understood that Candida boidnii and Saccahromyces sp. are the dominant natural microflora in Serinyol district (Leventdurur et al., 2016).

Olives harvested from a garden in Cyprus were subjected to three different fermentation processes (a-spontaneous fermentation at 10% NaCl (Control): b-fermentation with Lactobacillus plantarum starter culture at 10% NaCl and cfermentation with Lactobacillus plantarum starter culture at 7% NaCl) in brines to which 0.33% w/v citric acid was added after breaking, and the microbial population was monitored for 365 days at 23±2°C. According to the results obtained; Enterobacteriaceae and coliforms were present in the brine at the beginning of fermentation, but rapidly decreased and could not be detected after the 15<sup>th</sup> day in the samples inoculated with starter culture and after the 22<sup>nd</sup> day in the control group. LAB population was observed to be the dominant microorganisms in the samples inoculated with starter culture. In the control group, LAB population increased until the 22<sup>nd</sup> day of fermentation and reached the maximum level and

after a slight decrease at this point, LAB population remained constant until the end of fermentation. In the samples inoculated with starter culture, it was observed that it increased after a slight decrease in the first 8 days and reached the highest level on the 120<sup>th</sup> day of fermentation. Yeast population reached the highest level in approximately 8 days in all treatments. While the yeast population maintained their numbers from the 8<sup>th</sup> day onwards in the control group, a rapid decrease was observed in the samples inoculated with starter culture and they maintained their numbers from the 60th day of fermentation. Staphylococcus could not be detected throughout the whole process. In the study, it was observed that olive samples became consumable from the 120<sup>th</sup> day (Anagnostopoulos et al., 2020).

In a study investigating the effect of sequential inoculation with β-glucosidase positive Lactobacillus plantarum F3.3 strain and probiotic Lactobacillus paracasei N24 strains as starter culture on brine fermentation to produce low salt Sicilian table olives, Nocellara Etnea variety olives obtained from a local company in Sicily were used. Olives were processed without any alcali treatment and fermented in brines containing 5% and 8% (w/v) NaCl for 120 days at room temperature (18  $\pm 2^{\circ}$ C). The experimental fermentation design was designed to consist of 8 treatments at 5% and 8% (w/v) NaCl with (F5A, F5B, F8A, F8B) and without (F5C, F5D, F8C, F8D) starter culture additions. On the 60th day of fermentation, F5B, F5D, F8B, F8D samples were inoculated with the potential probiotic Lactobacillus paracasei N24 strain. Uninoculated samples were considered as control group. Sea salt was added periodically to maintain the initial salt concentration in the samples. At certain stages of fermentation, 600 LAB and 200 yeasts were isolated and identified. In the species-level identification of LAB, strains Lactobacillus belonging to paracasei, Lactobacillus plantarum, Lactobacillus casei andLactobacillus pentosus species were detected. Pichia kluyveri, Candida boidinii Candida diddensiae, Meyerozyma guilliermondii and Wickerhamomyces anomalus species were identified in yeast isolates obtained during fermentation. It was observed that the dominant LAB and yeast species were Wickerhamomyces anomalusand Lactobacillus plantarum, respectively. It was also observed that the potential probiotic Lactobacillus paracasei N24 strain survived the fermentation process (Pino et al., 2019).

In a study, the effects of six selected yeast starter cultures (Wickerhamomyces anomalus 1960, Candida adriatica 1985, Nakazawaea molendiniolei 2004, Cyteromyces matritensis 2005, Candida diddensiae 2011 and Saccharomyces cerevisiae 2046) on the fermentation of black table olives produced from Taggiasca variety olives using the Greek method at different salt concentrations and the survival of these yeasts under different conditions fermentation were evaluated. Fermentation was carried out in three different brines under ambient conditions (15-20°C) for 120 days. Brines: A: Unacidified brines containing 8% (m/v) NaCl; B: Unacidified brines containing 12% (m/v) NaCl; C: Brines containing 12% NaCl acidified with 0.3% citric acid are prepared in such a way. In addition, an uninoculated control group was formed for each type of brine. Microbiological analyses were carried out at the beginning, 30<sup>th</sup> day and 120th day of fermentation. According to the results obtained, it was observed that the inoculated yeasts survived differently depending on the salt concentration during the first 30 days, but on the 120<sup>th</sup> day of fermentation, Candida adriatica 1985, Wickerhamomyces anomalus 1960 and Candida diddensiae 2011 were the starter yeasts with the best survival capacity under all conditions. The veast strain Nakazawaea molendini-olei 2004 was detected in brine containing 8% NaCl, but not in NaCl. Cyteromyces brine containing 12% matritensis 2005 was not detected in 12% NaCl brine acidified with citric acid. In addition, it has been observed that the yeast population of the Pichia manshurica species, which is included in the natural microflora, increases during fermentation (Ciafardini and Zullo, 2019).

In a study conducted by Pino et al. (2018), the effect of different salt concentrations on the formation of physicochemical, microbiological, sensory and volatile organic compounds in order to produce *Nocellara Ethnea* table olives has been investigated. *Lactobacillus paracasei N24*, which is thought to have probiotic properties and *Lactobacillus plantarum* UT2.1 were used as starter cultures. All fermentations were carried out at room temperature ( $18\pm2^{\circ}C$ ) and monitored over a period of 120 days. Sea salt was added to maintain the brine salt concentration at the initial level. In addition, fresh brine was periodically fed

so that the olives were completely immersed in the brine to prevent the growth of moulds on the brine surfaces. The results of the analyses showed the dominance of LAB from the 7th day of fermentation and the decrease of yeasts and Enterobacteriaceae species in the starter-inoculated samples during fermentation. At the end of the fermentation, Enterobacteriaceae species could be counted only in the control group samples. In addition, LAB were identified, and the identified species were Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus casei and Lactobacillus pentosus. At the end of fermentation, Lactobacillus plantarum and Lactobacillus pentosus species were detected in all samples. It was also observed that Lactobacillus paracasei N24 strain showed high survival performance in all samples.

Kazachstania humilis AG5, Kluyveromyces lactis L39, Nakazawaea molendinolei IG9, Candida adriatica L30 and Candida diddensiae IG12 yeasts were used during the fermentation of Gordal and Manzanilla green table olives under controlled conditions. The natural culture medium obtained from olives was used in the selection of yeasts, and the amount of phenolic matter, the amount of volatile matter and organoleptic properties such as bitterness, odor, acidity, salinity and hardness were monitored during the fermentation period.

The yeast that increased the amount of volatile compounds the most in *Gordal* olives was *Kluyveromyces lactis* L39, while the yeasts that increased the amount of volatile compounds the most in *Manzanilla* olives were *Candida adriatica* L30 and *Kluyveromyces lactis* L39. *Candida diddensiae* IG12, *Nakazawaea molendinolei* IG9 and *Candida adriatica* L30 were effective in reducing the amount of phenolic substances at the end of fermentation in both olive genera. It has been observed that there is a correlation between the total amount of phenolic substances and bitterness during fermentation. (Ruiz-Barba et al., 2024).

Three different fermentation processes were carried out from *Chalkidiki* olives under controlled conditions according to the composition of chloride salts of brine (8% NaCl, 4% NaCl-4% KCl and 4% NaCl-3% KCl-1% CaCl<sub>2</sub>). It has been observed that lactic acid bacteria are responsible for the fermentation of olives processed at low NaCl concentrations, and a radical increase in the amount of acetic acid in the advanced stages of

fermentation confirms the existence of heterofermentative LAB metabolisms. The presence of 3methyl-1-butanol in groups containing high NaCl indicates that yeasts are the dominant microflora (Alvanoudi et al., 2024).

In a study in which the spontaneous and controlled fermentation of Gemlik black olive was examined by comparison, Lactiplantibacillus plantarum and Lactiplantibacillus pentosus crimes were vaccinated into separate groups during fermnation under controlled conditions. Brine containing 10% NaCl was used in two types of fermentation and the amount of salt in the brine was kept constant during the fermentation period under controlled conditions. It was observed that the pH value of both olive meat and brine juice was lower in the olive groups to which starter culture was added compared to the group obtained at the end of spontaneous fermentation. During the fermentation period, it was understood that the olive groups to which starter culture was added increased the acidity more compared to the group obtained as a result of spontaneous fermentation. In addition, both the LAB number and the yeast number were found to be higher in the fermentation of olives to which starter culture was added than the numbers obtained as a result of spontaneous fermentation. It has been determined that Lactiplantibacillus pentosus causes an improvement in sensory characteristics at the end of fermentation and has a higher potential to reduce reduced sugar faster, acidify faster, and promote microbial growth (Koyuncu and Cabaroglu, 2024).

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### 7. Conclusion

Table olive production is a very complex process due to many factors such as the diversity of microbial flora, salt concentration, acidity, olive type, cultivation region, processing method, and temperature. Some studies have found that Gramnegative bacteria species such as Pseudomonas, Aeromonas, Flavobacterium, Clostridium, and Bacillus dominate in the early stages of fermentation. As acidity decreases, lactic acid bacteria species such as Lactococcus, Pediococcus, and Leuconostoc are observed to dominate. Pediococcus and Leuconostoc species are thought to play a role in the rapid decrease of pH. Yeast species (Candida spp., Saccharomyces spp., Pichia spp.) have been observed throughout the fermentation process. Additionally, some studies have detected halophilic Archaea in olives and brines, which degrade the quality of olives and cause spoilage.

In alkaline treatment and debittering processes, the growth rate of microorganisms decreases due to the reduction of sugar in the environment, allowing pathogenic microorganisms to proliferate. Therefore, in addition to adding sugar and lactic acid to the environment at the beginning of fermentation, oleuropeinolytic microorganisms should be used. In this way, the nutritional value of the olives is preserved, and a safe product with a long shelf life is produced. The use of controlled fermentation and starter cultures will reduce product spoilage and prevent economic losses. Therefore, research on the use of starter cultures in olive fermentation should be increased. These studies should focus on identifying strains that can be used as starter cultures in table olive production and optimizing these strains.

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